Postprandial effects of resistant starch corn porridges on blood glucose and satiety responses in non-overweight and overweight adults

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Postprandial effects of resistant starch corn porridges on blood glucose and satiety responses in non-overweight and overweight adults

By

Danielle Alexander

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Diet and Exercise

Program of Study Committee:
Suzanne Hendrich, Major Professor
Christina Campbell
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Ames, Iowa

2012

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ABSTRACT

Background: Diabetes and obesity are major health concerns in the United States. There are several lifestyle factors that contribute to the development of these conditions and diet plays a large role in both etiology and treatment of these diseases. Poor carbohydrate quality and excess caloric intake can contribute to obesity and the development of insulin resistance, eventually progressing into Type 2 diabetes (DM2) and its associated co-morbidities. Resistant starch (RS), a type of dietary fiber, is thought to be a tool for prevention and treatment of obesity and DM2 due to its slow release of glucose post prandially, low energy density, and colonic health benefits from fermentation in the colon.

Methods: Twenty healthy non-overweight/obese weight (n=10; BMI 18.5-24.9 kg/m²) and overweight/obese (n=10; BMI > 25.0 kg/m²) consumed, in random order, 3 breakfast corn porridges providing 25 g starch derived from corn lines varying in levels of resistant starch. The porridges contained 3.1%, 8.4% and 28.9% RS of total starch. Post-prandial blood glucose was measured using a glucometer at baseline, 15, 30, 60 and 120 minutes. Postprandial satiety using a 100 mm Visual Analog Scales (VAS) was measured at baseline, 30, 60, 120, and 180 minutes. Subjects recorded 24 h food intake and gastrointestinal symptoms upon completion of each visit.

Results: There were no differences in post-prandial blood glucose, satiety, or food intake responses between non-overweight/obese and overweight/obese participants with
treatments. After data from the 2 weight groups were combined, mean plasma glucose at peak time-point 30 minutes was significantly lower in subjects consuming 28.9% RS treatment compared to the other treatments. Baseline-adjusted plasma glucose AUC was also significantly lower in subjects consuming the 28.9% RS porridge compared to the other porridges. There were no differences in subjective satiety or 24-hour food intake. Minimal gastrointestinal symptoms were experienced in the 24 hours following all 3 test meals.

*Conclusions:* RS substitution improved acute and peak post-prandial glucose responses, but higher doses of RS (greater than approximately 30% or more of total starch, the maximum provided in our study) may be needed to increase satiety and decrease food-intake over 24 h after ingesting the RS.
CHAPTER I: INTRODUCTION

Background

Resistant Starch intake is thought to control glucose and insulin levels, increase satiety, and lower calorie intake preventing obesity and insulin resistance. The purpose of this study was to examine the effects of a RS corn-porridge on postprandial blood glucose and satiety responses in ideal and overweight human subjects. It was hypothesized that non-overweight/obese and overweight subject would have different glucose and satiety responses. Overweight subjects would have higher blood glucose and lower subjective satiety after all treatments. Plasma glucose was hypothesized to be lowest and satiety response would be highest in highest resistant starch treatment. Moreover, increased satiety with high resistant starch treatment would result in lower food intake over 24-hour period for all subjects.

Thesis Organization

This thesis will begin with a review of literature examining the role of carbohydrates in the development of obesity and DM2. The review will then shift its focus to resistant starch and its effect on post-prandial blood glucose, insulin, and satiety. The review will finish with general conclusions about resistant starch dosing and magnitude of effects. Following the review, the methods and results of the resistant starch human feeding study will be given. The thesis will conclude with discussion, appendices, references, and acknowledgements.
CHAPTER II: REVIEW OF LITERATURE

Introduction

Obesity and Type 2 Diabetes (DM2) are growing epidemics in the United States. Obesity has dramatically increased over the last 20 years, and this equates to about one third of the American population (CDC, 2011). Obesity is closely linked with Type 2 Diabetes as 80% of these individuals are obese and approximately 90-95% of diabetes cases in the United States are Type 2. There are 25 million people, 20 years of age and older, with diabetes, and 2 million new cases are diagnosed every year (CDC, 2011). These numbers are a cause for concern because of the negative health risks and early mortality associated with DM2. Long-term complications of chronic hyperglycemia from DM2 includes both macrovascular and microvascular complications (Nelms et al. 2007). Macrovascular complications can lead to heart disease and stroke. Microvascular damage can cause nephropathy leading to kidney failure, retinopathy resulting in blindness, and neuropathy eventually leading to amputation (Nelms et al. 2007). Several diet and lifestyle factors contribute to the development of obesity and DM2. In an observational study examining diet and lifestyles factors of 84,941 nurses over a 16-year period, incidence of DM2, physical inactivity, cigarette smoking, poor quality of fats and carbohydrates, and excessive calorie intake were significant risk factors for the development of DM2 independent of overweight or obese status (Hu, 2001). Although these lifestyle factors contribute to the development of this negative health condition, obesity is the single most important risk factor for DM2, and high abdominal obesity, (absolute waist circumference >102 cm (40 in) in men and >88 cm (35 in) in women) regardless of weight categorization, is another critical
risk factor in DM2 development (Hu, 2011). Dietary factors are a key component in disease development and examination of intake patterns and macronutrient distribution will provide insight into the contributors of disease progression. Carbohydrates role in obesity and DM2 development will be addressed. This review will focus on intake data in the United States, structure and metabolism of carbohydrates and resistant starch (RS), and literature focusing on acute effects and mechanisms of RS on blood glycemia, satiety, and weight control. The ability of these acute effects to provide long-term protection against and treatment of obesity and DM2 will then be examined and summarized.

Macronutrient intake patterns and Type 2 Diabetes development

Obesity is a critical factor in DM2 development. Consumption of energy intake greater than recommended needs can lead to weight gain and obesity. Obesity from greater intake and carbohydrates, more specifically RDS (rapidly digestible starch), contribute to manifestation and progression of DM2 (Schultze et al. 2004). This is supported by NHANES data comparing trends of energy and macronutrient intake in the United States between 1971-1974 and 1999-2000 (Wright, 2004).

Total energy and carbohydrate intake have increased while total fat, saturated fat, and protein have decreased. Mean intake of total kcals has increased 6% in men and 21% in females; carbohydrates supplying those kilocalories have increased 6% in both males and females. Total daily fat intake has declined 4% in men and 6% women, while saturated fat has declined 3% and 2% respectively (Wright, 2004). These are small percentages, but long-term implications need to be realized. For example, assuming 3,500 kcals equals 1 lb, a 6%
increase in kcals per day leads to a 12 lb weight gain in a one year and a 60 lb weight gain in 4 years.

Although hypothetical implications, these data provide evidence for the increase in overweight and obese individuals in the United States through increased total energy and carbohydrate consumption. Interestingly, the most recent NHANES data trends comparing data between 1999-2000 and 2007-2008 show no change in total energy or fat intake, and a very slight decline in carbohydrate intake (1%) while incidence of obesity and DM2 have increased by an estimated 10% and 2% respectively over the last 10 years (Wright, 2010). These trends suggest diet quality, primarily carbohydrate quality for the purpose of this review, may have a role in obesity and DM2 development as several observational studies have found association between glycemic index and glycemic load and incidence of DM2 (Halton et al. 2008; Krishnan et al. 2007; Villegas et. al 2007).

Quality of carbohydrates is commonly measured through glycemic index and glycemic load first developed by Jenkins and colleagues (1981). The glycemic index (GI) ranks carbohydrates according to their effect on postprandial glucose area under the curve. GI values are expressed as a percentage of the blood glucose area under the curve compared to the same quantity of available carbohydrate in a standard product such as white bread or glucose. Glycemic index of foods are classified as low (<55), medium (56-69), and high (≥70) GI foods. The glycemic load (GL) is calculated from GI and is used to estimate the rise in blood glucose from total amount of carbohydrate consumed. High GL per serving is > 20, medium GL per servings is 11-19, and low GL is ≤ 10. It appears GI and GL may have a role in long-term health. In a meta-analysis of 37 observational studies conducted by
Barclay and colleagues comparing highest and lowest quintiles of GI and GL from food frequency data collected from 1,950, 198 subjects, found participants eating foods in the highest quintile of GI (median value 58) and GL (median value 142) had a higher relative risk ratio of chronic disease development of Type 2 diabetes, heart disease, colorectal cancer, endometrial cancer, and gallbladder disease. Barclay also found low GI (median value 49) and GL (median value 92) diets provided protective effects against incidence of chronic diseases including DM2 (Barclay et al. 2008).

Similar results were found in a prospective cohort study examining dietary intake of 37,846 participants using validated food-frequency questionnaires and incidence of diabetes as determined by risk ratios for an average follow up of 10 years. They found diets high in GL, GI, and carbohydrate, and low in fiber increased the risk of diabetes (Slujis et al. 2010). Halton et al. (2008) examined carbohydrate quality and quantity using semi-qualitative food frequency questionnaires of 4,670 documented cases of type 2 diabetes of nurses followed for an average of 20 years and found a strong association (relative risk of 2.47 above “gold standard” 1 for relative risk) between glycemic load of carbohydrates and DM2 incidence. Low-carbohydrate diets were not associated with decreased risk, but a trend for increased risk with greater carbohydrate consumption was documented. In a study of similar design, 59,000 US black women were followed for an average of 8 years and high glycemic index was found to be positively associated (relative risk 1.91) with DM2 prevalence using relative risk of incidence, and fiber intake was found to be inversely related (relative risk of 0.41) with DM2 incidence (Krishnan et al. 2007). Relative risk for DM2 increased as GI and GL increased in a cohort study of 64,227 Chinese women followed
for 4.6 years and increasing rice quantity of diet was also related to DM2 risk (Villegas et al. 2007). These data support carbohydrate quantity and quality related to higher postprandial glycemia as one possible mechanism for disease progression and suggest that modifying type of carbohydrate in the diet can impact long term health.

Starch Structure and characterization

Starch is a major source of dietary carbohydrate that is an essential macronutrient for health. Starches are composed of polysaccharide chains consisting of two main structural components based on the α-linkages of the monosaccharides: amylopectin and amylose. Amylopectin is a highly branched structure of α-1,4 and α-1,6 glycosidic bonds that create both short and long chains. Amylose is a linear, less branched structure of α-1,4 bonds that can form different helices. Together they create different types of chemical and crystalline structures affecting the digestibility of starch (Englyst, 2006). Starch can be divided into three categories based on digestibility: rapidly digestible starch, slowly digestible starch, and resistant starch. Rapidly digestible starch (RDS) is starch that is converted into glucose within 20 minutes of digestion in the small intestine. Slowly digestible starch (SDS) is fully digested within 20-120 minutes in the small intestine (Sajilata et al. 2006).

Resistant starch (RS) is any starch that escapes digestion of the small intestine and passes into the large intestine to be metabolized. This indigestibility is caused by the crystalline structure that slows and prevents α-amylase from hydrolyzing starch into glucose to be absorbed by the small intestine (Perera et al. 2010). Resistant starch is classified into
five categories: RS1-RS5. RS1 is starch that is physically inaccessible to digestion such as partially milled or whole grains, legumes, seeds, or pasta that are entrapped in a non-digestible matrix. RS2 is raw or ungelatinized starch with a tightly packed crystalline structure reducing the accessibility of digestive enzymes and is found in foods such as raw potatoes, green bananas, and genetically modified high-amylose corn. RS3 is retrograded starch from cooking and cooling of granules. The amylose molecules that leak out of starch granules during gelatinization begin to reassociate as cooling begins increasing crystallinity of the starch structure, thereby increasing resistance to enzymatic digestion. RS4 is chemically modified starch that creates novel bonds between chains by cross-linking and is completely resistant to digestion (Fuentes-Zaragoza, 2010). RS5 is an amylose-lipid complex in starch that is resistant to digestion by retrograding amylase with free fatty acids to form a complex less susceptible to α-amylase. Most research examining metabolic benefits of resistant starch have used RS2 and RS3 starches, likely due to RS2’s abundance in commonly eaten foods and cooking and cooling of RS2 creates RS3 (Murphy, 2008). Digestibility of starch fractions is an important concept that determines starch functionality in the body and influences health benefits.
**Table 1: Description and sources of resistant starch**

<table>
<thead>
<tr>
<th>Type of Resistant Starch</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS1</td>
<td>Inaccessible starch trapped in a digestion resistant matrix</td>
<td>Partially milled or whole grains, legumes, seeds, or pasta</td>
</tr>
<tr>
<td>RS2</td>
<td>Raw or ungelatinized starch with a tightly packed crystalline structure</td>
<td>Raw potatoes, green bananas, and genetically modified high-amylose corn</td>
</tr>
<tr>
<td>RS3</td>
<td>Retrograded starch from recrystallization of amylose after cooking and cooling</td>
<td>Bread and/or starch products cooked and cooled</td>
</tr>
<tr>
<td>RS4</td>
<td>Chemically modified starch that creates novel bonds between chains by cross-linking</td>
<td>Added to beverages, bread, cakes, porridges</td>
</tr>
<tr>
<td>RS5</td>
<td>Amylose-lipid complex retrograded with free fatty acids</td>
<td>Bread and other starch food products</td>
</tr>
</tbody>
</table>

RS=resistant starch

**Methods of Measurement**

The measurement of RDS, SDS, and RS in vitro provides information on the rate and extent of starch digestion in the small intestine. Methods for determining RS in food products vary among several protocol steps such as enzyme concentrations, types of enzymes, pH, temperatures and incubation periods. The basis for this discussion will focus on the Megazyme® kit as this is a widely used assay in determining RS of food products and Englyst’s methods (1982,1992). Most recent protocols have been developed and modified from these proposed methods. Englyst and colleagues first developed a protocol for total RS...
measurement in 1982, but it was later realized this assay only quantified RS3 as the homogenization, boiling and 40°C incubation eliminated RS1 and RS2 from total RS contribution. Englyst and others later modified this procedure in 1992 to measure all starch fractions by collecting sequential aliquots of sample during enzymatic hydrolysis at 20 minutes and 120 minutes of digestion and measuring glucose contents of samples. The content of glucose at 20 minutes represents rapidly digestible starch (RDS). This glucose content is subtracted from glucose content at the 120 min aliquot from the beginning of the digestion and the remaining glucose concentration represents slowly digestible starch (SDS). Total RS is then measured indirectly from the following equation: $RS = TS − (RDS + SDS)$. RS1 and RS2 are accounted for in this protocol because sample preparation by milling and boiling was omitted and temperature was no greater than 37 °C to more closely mimic human conditions, allowing RS1 and RS2 to remain unhydrolyzed. Megazyme® kit AOAC method 2002.02 and AACC Method 32–40 are widely used protocols for RS determination (Megazyme, 2008). It is only used to directly measure RS and does not measure RDS and SDS. Samples are ground to pass a 1-mm sieve which allows RS1 to be accounted for in total RS measurement. An α-amylase (3 Ceralpha Units/mg of activity) and amylglucosidase (3300 U/mL) mixture is used to hydrolyze starch in raw or processed food samples. Hydrolysis is carried out for 16 h at 37 °C leaving RS2 in raw foods unhydrolyzed. Enzymes are inactivated after incubation with 99% ethanol, and glucose is removed by two washings with 50% ethanol. Remaining starch pellets are collected from the digestion and treated with 2 M potassium hydroxide to extract RS3 from the fiber-rich matrix. The dextrins produced from this extraction are hydrolyzed to glucose with amylglucosidase
incubated for 30 min at 50 °C. Final glucose concentration is determined through a glucose oxidase-peroxidase colorimetric (Megazyme, 2008). Quantifying starch fractions provides valuable information about in vivo digestion and metabolism that can be used for determining health benefits of RS consumption.

**Starch metabolism**

Digestion of starch first begins in the mouth where salivary α-amylase begins to hydrolyze α-1,4 linkages of amylose and amylopectin forming dextrins. As dextrins reach the stomach they remain unchanged as α-amylase is inactivated by the low pH of the stomach (Gropper, 2009). Digestion continues in the small intestine by secretion of pancreatic α-amylase that hydrolyzes α-1,4 linkages and dextrins into the disaccharide maltose. Brush border enzymes maltase and α-dextrinase form glucose from maltose and limit dextrins from the α-1,6 linkages of amylopectin respectively. Glucose is actively transported into the mucosal cell where it enters circulation through facilitated diffusion and activates transport. Glucose is then carried to the liver to be metabolized and distributed in the body. Glucose leaves the liver through facilitated diffusion and is circulated to different tissues in the body (Gropper et al. 2009). Glucose plays a key role in cellular homeostasis and metabolism and is needed as a continuous source of energy for most cells. Its concentration is closely regulated in the blood and it is distributed to tissues by glucose transporters. One transporter of particular importance is GLUT 4 which is widely distributed in muscle and adipose tissue. GLUT 4’s function of glucose uptake is insulin dependent, and insulin resistance plays a key role in the development of DM2. Adipocytes have been shown to
suppress expression of GLUT-4 with obesity and Type 2 Diabetes, and muscle cells have shown impairment in translocation of GLUT-4 to cell membrane in obesity and DM2 (Davies et al. 1994). Starch metabolism is essential to the understanding of DM2 development.

Role of Starch in Type 2 Diabetes

DM2 is caused by a complex interplay of both environmental and genetic factors that is caused by insulin resistance. Insulin resistance is the resistance of body cells to the action of insulin that results in impaired blood glucose metabolism (American Diabetes Association, 2009). Abdominal obesity, excess calories, and diets high in RDS play a part in the pathophysiology of insulin resistance. Excess calories lead to obesity and this causes an overabundance of adipose tissue creating deleterious effects on insulin function which in turn affects glucose and lipid metabolism through large fat mass secretions of circulating levels of proinflammatory cytokines, hormone-like molecules, and other inflammatory markers (Balistreri, 2010). These secretions interfere with normal insulin function. Insulin resistance is a key component that precedes the onset of glucose intolerance and results in hyperinsulinemia with normal and high blood glucose concentrations (Bray, 2010).

Moreover, high consumption of rapidly digestible starches cause rapid spikes in blood glucose concentration (Ells, 2005) that leads to hyperinsulinemia and over time may contribute to the decline of insulin function. Ells et al. (2005) compared postprandial blood glucose, insulin and non-esterifried fatty acids (NEFA) after consumption of a meal containing 75 g rapidly digestible starches to 75 g slowly digestible starch with 21g of fat in 10 healthy female volunteers. They found more rapid and higher peaks of plasma glucose
and insulin in the first 30-60 minutes after consumption of RDS meal and similar high and rapid peaks in NEFA 4-6 hours postprandial indicating starch type effects on carbohydrate and fat metabolism. In another study, RS produced comparable results in postprandial glucose, insulin, and NEFA after a meal tolerance test in 10 healthy subjects following 24 h consumption of 100g RS2 consisting of 60% RS distributed in 25g doses compared to 40g RDS delivered in 10g dose in a diet of 42% carbohydrate (Robertson et al., 2003). These results show starch type can acutely affect insulin and glucose homeostasis and that long-term intake of simple carbohydrates may affect carbohydrate and fat metabolism resulting in obesity and DM2. This is confirmed in research conducted by Pawlak et al. (2004) examining effects of dietary glycemic index on adiposity, glucose homoeostasis, and plasma lipids in rats fed high and low GI diets. Diets were of the same macronutrient content, (69% carbohydrate, 20% protein, and 11%) but rats fed a high GI diet were fed starch made of 60% amylose and rats fed a low GI diet were fed starch made of 100% amylopectin. Rats fed the high GI food had similar body weight compared to low GI but almost twice the amount of body fat and less lean body mass. Rats fed high GI diet had greater areas under the curve for blood glucose and plasma insulin after a glucose tolerance, lower plasma adiponectin concentrations (levels are inversely associated with obesity), higher plasma triglyceride concentrations, and severe disruption of pancreatic islet-cell architecture.

When Pawak and others (2004) conducted a similar experiment with high and low fat diets, they found no difference in body fat accumulation between treatments, but less glucose uptake and plasma insulin in rats fed the high-fat diet. These findings suggest both excess fat and carbohydrates play a role in the development of DM2. Although when wild-
type *Drosophila melanogaster* (fruit flies) were fed high-fat, high-sugar, and control diets, larvae fed high-sugar diets developed hyperglycemia, obesity and insulin resistance, while insulin resistance was not seen in the high-fat diet suggesting carbohydrates play a greater role in the decline of insulin function (Musselman et al. 2011).

As insulin function declines glucose intolerance ensues causing high blood glucose levels that are harmful to the macro and micro vascular function resulting in DM2 diagnosis.

According to the American Diabetes Association, Type 2 Diabetes is diagnosed by classic symptoms of the disease with one of three criteria: 1) casual plasma glucose concentration > 200mg/dL (11.1mmol/L) 2) Fasting plasma glucose > 126 mg/dL (7.0 mmol/L) 3) plasma glucose >200mg/dL 2-hours postprandial an oral glucose tolerance test. A positive test should be followed with a repeat testing on a different day (ADA, 2009). Better understanding of the physiological effects of carbohydrates on metabolism will help develop recommendations for intake of carbohydrates and resistant starch. These will be important for improving health and combating the globalization of obesity and DM2.

**Metabolic effects of resistant starch**

Several factors determine resistant starch formation and metabolic effects of RS. Chemical structure, processing of RS, endogenous influences, nutrient interactions, and meal factors will give certain physico-chemical characteristic of starch influencing gastrointestinal handling (Englyst, 2005). Inherent structure and physical properties of resistant starch such as increased crystallinity, granular size, amylose content, and amylose
retrogradation will increase resistance to digestion (Sajilata, 2006). Processing conditions and metabolism of starch determines the amount of starch that is available to be absorbed in the small intestine and is reflected in elevation and duration of rise in postprandial blood glucose. Metabolism, or lack thereof, also determines amount of resistant starch left in the large intestine through measurement of fermentation and production of short chain fatty acids (Englyst, 2006). Control of glucose levels, fermentation of hydrolyzed starch, and decreased energy intake from undigested starch are the basic principles behind the beneficial physiological effects of RS as it relates to obesity and DM2.

**Glucose Metabolism and Insulin responses of resistant starch**

Resistant starches affect postprandial glucose levels through three common mechanisms: inhibiting \( \alpha \)-amylase from digesting starch into glucose, increasing the viscosity of chyme in the small intestine which slows the rate of glucose uptake, and binding glucose which prevents its diffusion into the mucosal cells. These mechanisms were determined through in vitro comparison analysis of glucose diffusion, absorption/binding, and \( \alpha \)-amylase activity in glucose-fiber complexes of RS, water insoluble, and water soluble fibers (Ou et al. 2001).

Several studies have confirmed lowering of postprandial blood glucose and insulin after consumption of RS. When a test meal of mixed macronutrients containing 50g of RS3 was compared with 50g of fully digestible cornstarch in 8 subjects, the retrograded amylose meal lowered AUC in both glucose and insulin during absorptive state. Bloods samples were taken up to 27 hours after ingestion, but no difference was seen between glucose and
insulin in post absorptive state, suggesting acute effects for application and benefits of RS3 consumption (Achnor et al. 1997). In another study examining healthy adults for 10 weeks, 12 males consumed a diet containing 70% high amylose for 5 weeks and a diet containing 70% high amylpectin for 5 weeks compromising 66% of total carbohydrate for weekly meals (Behall et al. 1989). At the end of each 5 week diet, a glucose tolerance test and starch load test containing 16% RS2 (1g/kg of bodyweight) were administered and postprandial insulin and glucose test were measured accordingly. They found total insulin and glucose (AUC) responses were not significantly different between treatments. However, acute ingestion of high amylose resulted in significantly lower glucose levels during the first hour and higher at 3 hours after ingestion compared to amylose control, and insulin levels were lower in first 2 hours compared to control still providing protection against glucose and insulin spikes that are detrimental to long-term health (Behall et al., 1989). Vans amelsvoort and Westrate (1992) found peaks in postprandial glucose and insulin curves at 30 minutes for high and low amylose meals. Low-amylose meals (0%) induced higher postprandial insulin concentrations at 0.5 and 1 hours resulting in significant increases (55%) in absolute and net glucose AUC compared to high-amylose meal. High-amylose treatment had higher glucose levels 2 and 6 hours postprandial confirming slow release of in hot meals of mixed macronutrients. Peaks in glucose and insulin found were similar, at 30 and 45 minutes respectively for all treatments, in Li and colleagues (2010) experiment comparing postprandial insulin and glucose responses in 16 healthy subjects of ideal body weight after consumption of RS2 rice (8g in 40 carbohydrate load), white rice, and glucose. RS (GI of 48) and white rice (GI of 77) both had significantly lower glucose and insulin AUC
compared to glucose load. The glucose and insulin AUC of RS rice was still lower than white rice confirming the potential of GI. Kendall et al. (2010) had subjects consume a beverage and cereal bar of 0g, 5g, 15g, and 25g of RS3 and found no difference between glucose and insulin AUC. However, there was a trend for a decline in glucose after the 25g RS treatment compared at 90 minutes in the 15g RS treatment, and 90 and 120 minutes in 0g RS treatment. Insulin showed a similar pattern with the 25g RS cereal bar at 60 minutes compared with the 15g RS treatment and 90 and 120 minutes of 0g RS treatment (Kendall et al., 2010). Higher doses of RS3, 50 grams or more, may be needed to produce significant declines in postprandial plasma glucose and insulin.

It is clear percent of amylose and RS, and total RS play a role in the effect of postprandial glucose and insulin levels. Behall and others (2002) examined these effects in breads containing different levels of amylose (30%, 40%, 50%, 60%, and 70%) and percent RS2 (2%, 8%, 12% and 15%). Twenty-five normal and overweight men and women ate a 2-day controlled diet and underwent a tolerance test with either a glucose solution or a test bread containing an equal amount of total carbohydrate. Plasma glucose concentration was significantly higher at 0.5 and 1 hours and lower at 3 hours after the glucose than after any bread. Peak glucose concentrations after breads containing 50-70% amylose starches were significantly lower than peak concentration after the 30-40% amylose breads and insulin levels showed a similar pattern. Lower glucose and insulin responses were seen with increased RS2 percentage in test breads. It was concluded starch levels greater than 50% amylose are needed to produce biologically significant declines in plasma glucose and insulin levels. A combination of RS and soluble fiber (β-glucan) provide greater effects on
postprandial insulin and glucose levels than RS alone. Behall et al. (2006) had subjects consume 1 g carbohydrate per kg body weight as a glucose solution plus 100 g water or a test muffin containing an equal amount of total carbohydrate. High RS muffins contained 5.06 g resistant starch/100 g muffin, high soluble fiber muffins contained 2.3 g beta-glucan/100 g muffin and combined muffins contained 8 and 2 grams respectively. The greatest reduction occurred in meals containing both high β-glucan and resistant starch (33 and 59% lower AUC for glucose and insulin, respectively). Although high resistant starch muffins provided better control of glucose levels compared to high β-glucan muffins alone (Behall et al. 2006). This study also compared responses between ideal (n=10) and overweight (n=10) women according to BMI. Glucose and insulin responses were improved in both groups, but overweight women had higher insulin levels than their ideal counterparts concluding overweight women were somewhat more insulin resistant despite having similar glucose responses between treatments.

Hyperinsulinemia is a critical factor that precedes insulin resistance and Behall and others (1995, 2005) conducted two studies comparing normal and hyperinsulinemic (HI) subjects consuming low and high amylose diets. In their first study, hyperinsulinemia was determined by fasting insulin and an insulin/glucose tolerance test (Behall et al, 1995). Subjects (n=24) ate 10 weeks of self-selected foods containing high in amylose (70%) or amylopectin (70%) followed by 4 weeks of a control diet with 55% of carbohydrates containing 70% amylose. Starch load test were conducted at weeks 4, 8, and 13 of high amylopectin or high amylose diets measuring postprandial glucose and insulin. Both groups had a decline in blood glucose levels in high-amylose treatment compared to high
amylopectin treatment. HI subjects had a 43.1%, 0%, 32.2% decline at 4, 8, and 13 weeks respectively compared to high-amylopectin and control subjects and similar reductions. There was a trend for glucose levels to be higher in HI subjects (n=14) after high-amylose treatment, but this was not significant although insulin levels were significantly higher between the two groups. Insulin decreased in both control (39.7%, 33.6%, and 47.9%) and HI (35.6%, 25.4%, and 47.7%) subjects on high-amylose diet for all three measurements respectively. Furthermore, HI subjects had higher glucose and insulin levels compared to ideal subjects after consuming high-amylopectin diets which would be expected in HI individuals. In their second experiment (Behall & Schofield, 2005), subjects underwent a meal tolerance test containing high-amylose or amylopectin (70%) chips (8-12g of RS) and muffins (16-25 of RS). Both HI and normal subjects had lower glucose and insulin after high amylose meals at 30 minutes for glucose and 1 hour with insulin. Average plasma glucose and insulin area under the curves after high-amylose foods were approximately 50% those after high-amylopectin foods although hyperinsulinemic subjects had significantly higher insulin and glucose responses and AUC compared with the normal responders. From both studies, it can be concluded that high-amylose diets can help lower glucose and insulin levels in ideal and overweight, normal and hyperinsulinemic individuals.

Aside from reductions in postprandial glucose and insulin, RS has also been shown to improve insulin sensitivity. Robertson and colleagues (2003) examined the effect of 24-hour RS (50 grams 60% RS) consumption on insulin sensitivity. Glucose and insulin curves were lower following high-RS diet compared to control. Insulin sensitivity following high-RS diet increased using the minimal model approach but not using homeostasis model.
assessment. In a similar study comparing 4 weeks of high and low RS diet (100 grams 60% RS) insulin sensitivity was measured using the hyperinsulinemic-euglycemic clamp (Robertson et al. 2005). Resistant starch supplementation significantly increased insulin sensitivity and mean muscle glucose clearance per pmol/L insulin increased by 44% during meal tolerance test, and insulin sensitization of adipose tissue was also increased. These finding suggest higher doses of RS2 (greater than 50g) and long-term consumption is needed to ensure increase insulin sensitivity from RS supplementation. Furthermore, insulin sensitivity and RS consumption in persons with type 2 diabetes needs to be assessed to make better recommendations.

Table 2: Comparison of glucose and insulin responses from resistant starch supplementation in humans

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Methodology</th>
<th>Test Meal</th>
<th>Measurements</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchor et. Al 1997</td>
<td>n=8</td>
<td>Cross-over design of two 27 hour test meal of high RS and low RS cornstarch consisting of 632 kcals 32% fat, 56% carbohydrate, and 12% protein</td>
<td>RS3 (50g, 70% amylose) in porridge (Hylon VII; Cerestar)</td>
<td>Post prandial glucose and insulin, and satiety response in absorptive and post-absorptive states</td>
<td>Retrograded amylose lowered AUC in glucose and insulin during absorptive state. No difference between glucose and insulin found in post absorptive state or satiety</td>
</tr>
<tr>
<td>Study</td>
<td>n=</td>
<td>Age</td>
<td>Sex</td>
<td>Type of Adults</td>
<td>Design</td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
<tr>
<td>Behall et al. 1989</td>
<td>12</td>
<td>34 y.o.</td>
<td>M</td>
<td>Healthy adults of ideal body weight</td>
<td>Cross-over design measuring glucose tolerance and starch load test at end of each 5 week period of diets with 66% of carbohydrates containing 70% amylose or 70% amylopectin</td>
</tr>
<tr>
<td>Behall &amp; Howe 1995</td>
<td>24</td>
<td>41 y.o.</td>
<td>F, M</td>
<td>Ideal and overweight HI (14) and non-HI (10) adults</td>
<td>Starch load test at week 4, 8 and 13 in 14 week cross-over design of 10 weeks self-selected amylose or amylopectin diets and 4 week control diets of diets with 55% of carbohydrates containing 70% amylose or 70% amylopectin</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcome</td>
<td></td>
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<tr>
<td>Behall &amp; Hallfrisch 2002</td>
<td>n=25</td>
<td>2 Day controlled diet followed by tolerance test of glucose solution or breads containing 30%, 40%, 50%, 60%, 70% amylose breads</td>
<td>RS2 (2, 8, 12 and 15%) in breads (American Maize-Products Company, Hammond, IN)</td>
<td>Postprandial glucose and insulin tolerance</td>
<td>Glucose and insulin AUC with glucose solution concentrations than all breads 60-70% amylose breads lowered insulin and glucose response than all other breads &gt;50% amylose needed for significant change in postprandial blood glucose and insulin</td>
</tr>
<tr>
<td>Behall et al. 2006</td>
<td>n=20</td>
<td>Cross-over design of 2 day controlled diet followed by tolerance test of glucose solution and muffins containing low, medium and high RS</td>
<td>RS2 (0.71, 2.57, or 5.06 g/100 g muffin) and β-glucan (0.26, 0.68, or 2.3 g β-glucan/100 g muffin) in muffins (American Maize-Products Company, Hammond, IN)</td>
<td>Postprandial glucose and insulin responses</td>
<td>No differences between weight groups in glucose response but differences with insulin response. High RS treatment ↓ glucose (59%) and insulin (38%) AUC compared and low RS treatment Insulin and glucose responses ↓ as RS increased</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Description</td>
<td>Intervention Details</td>
<td>Outcome Measures</td>
<td>Findings</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
</tbody>
</table>
| Behall & Scholfield 2005 | n=24  
Age: 25-57 y.o.  
Sex: 12 M, 12 F  
HI and non-HI adults of ideal body weight | Four test meals: of muffins or corn chips or made with starch or cornmeal that contained 70% amylose or amylopectin  
RS2 in corn chips (8 g) and muffins (24g) in 60-70 g carbohydrate meal  
(American Maize-Products Company, Hammond, IN) | Postprandial glucose and insulin response  
High amylose chips and muffins resulted in lower average insulin and glucose response  
HI subjects had higher insulin and glucose responses compared to normal individuals |                                                         |
| Kendall et al. 2010    | n=22  
Age: 26 y.o.  
Sex: 9 F, 13 M  
Healthy adults of ideal body weight | 5 tests meals consisting of cereal bar and beverage of varying levels of RS  
RS3 (58%, 0g, 5g, 15g, & 25g) in cereal bar  
(PROMITOR™ Tate and Lyle Ingredients America, Decatur, IL) | Postprandial glucose and insulin responses (fingerpick analysis) | Glucose & insulin AUC were not different  
Acute (<60 mins) insulin and glucose responses were not different |
| Hasjim et al. 2010     | n=20  
Age: 19-38 y.o.  
Sex: M  
Adults of ideal, overweight, and obese body weight | Randomized cross-over design of 2 test meals consisting of RS bread treatment and white bread control  
RS5 (50g, 37%) in bread  
(Iowa State University, Ames, IA) | Postprandial glucose and insulin response | Glucose (55%) and insulin (43%) iAUC reduced compared to control  
Mean glucose and insulin concentration were different by treatment |
<table>
<thead>
<tr>
<th>Study</th>
<th>n=16 (age and gender)</th>
<th>Study Design</th>
<th>Test Meal Properties</th>
<th>Postprandial glucose and insulin response</th>
<th>Summary of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. 2010</td>
<td>n=16 Age: 24 y.o. Sex: 7 F, 9 M Adults of ideal body weight</td>
<td>Cross-over design of 3 test meals of glucose, RS rice and white RS rice</td>
<td>RS2 (8g, 20%) in rice in 40g carbohydrate meal (Agricultural College of Yangzhou University, Jiangsu Province, China).</td>
<td>Postprandial glucose and insulin response</td>
<td>Glucose and insulin AUC reduced by RS</td>
</tr>
<tr>
<td>Van amelsvoort &amp; Weststrate 1992</td>
<td>n=22 Age: 40 y.o Sex: M Adults of overweight and ideal body weight</td>
<td>Cross-over design of test meals (hot mixed lunch) of low-amylose (0%) and high amylose (55%)</td>
<td>RS3 in Rice in 65-122 g carbohydrate per meal (Hylon VII, National Starch and Chemical Co)</td>
<td>Postprandial glucose and insulin response</td>
<td>Glucose lower at 0.5, 1 hours and higher 2, 4, 6 hours in high-amylose meal Insulin higher at 0.5, 1 hours and greater net and absolute AUC in low-amylose meal</td>
</tr>
<tr>
<td>Robertson et al. 2003</td>
<td>n=10 Age: 47 y.o. Sex: 6 F, 4 M Adults of overweight and ideal body weight</td>
<td>Cross-over design of meal tolerance test after 24hr diets of low and high RS</td>
<td>RS2 (100g, 60%) in jelly (Novelose 260, National Starch and Chemical, Manchester, UK)</td>
<td>Postprandial glucose and insulin response</td>
<td>Glucose and insulin response lower and insulin sensitivity improved after 24-hr diet of high RS</td>
</tr>
</tbody>
</table>
Collectively, these studies (Table 2) indicate that doses of 40-60 grams of starch containing 50-70% amylose starch and ≥ 15% RS2 can lower postprandial insulin and glucose response and higher doses of RS3 may be needed to produce similar effects. To improve insulin sensitivity, 100 gram daily doses of total RS over a period of weeks may be needed to produce significant effects. The results also reiterate the idea that normal starch is digested immediately and can cause major spikes in postprandial blood glucose. Slower rates of starch absorption from RS may be a useful tool for reducing postprandial glycemia and insulinemia by releasing glucose over a longer period and thereby providing better control of blood glucose and insulin that would be helpful for individuals with diabetes.

Several studies have shown improved postprandial blood glucose and insulin in DM2 individuals after consumption of RS. In a study comparing insulin and glucose responses in subjects with DM2 after consumption of a resistant starch bar, traditional energy bar, and candy bar; the resistant starch bar had 50% reduction in glucose AUC, and similar trends in
plasma insulin between the RS and traditional bar, but not the candy bar (Reader et al., 2002). Johnston and others (2010) found 40g of 60% RS2 improved insulin sensitivity after 12 weeks of supplementation in 20 volunteers with Type 2 Diabetes. Furthermore, Mitra and colleagues (2007) found 12 weeks of 150g/day of RS3 rice (8-10% RS) lowered fasting blood glucose, total cholesterol, and LDL cholesterol in persons with DM2. These results confirm RS supplementation can be beneficial tool for both normal and diabetic individuals in controlling postprandial glucose and insulin levels.

Fermentation of Resistant Starch

Unhydrolyzed starch is fermented by bacteria in the large intestine resulting in the fermentation products such as carbon dioxide, methane, hydrogen, organic acids, and short-chain fatty acids (SCFA). Fermentation can be measured through hydrogen breath tests and SCFA concentration in feces. Consumption of 17-45 grams of RS2 and RS3 (RS3 at lower doses and RS2 and the higher doses) meals have been shown to increase fecal bulk (Phillips et. Al 1995, Noakes et al., 1996), breath hydrogen (Van Munster, 1994) and fecal SCFA (Phillips,1995; Cummings,1996; Noakes et al. 1996; Jenkins,1998). Increase in fecal bulk is a direct result of increased RS in the large intestine and is correlated (r=0.70) with RS consumption (Phillips; 1995). It is used as a measure for confirmation of indigestibility. For example, 26-30 grams of RS2 from banana and potato flour has been shown to increase stool weight 31 and 34 grams per day respectively, while 17-19 g of RS3 from wheat flour and maize has been shown to increase stool weight by 46 and 49 g per day (Cummings 1996). Confirmation of indigestibility can also be measured through breath hydrogen.
Van Munster et al. (1994) measured breath hydrogen concentration after consumption of 15 g of RS2 three times a day for 7 days in 22 healthy male volunteers, and breath hydrogen began to increase 4 hours and peaked 12 hours after consumption. SCFAs acetate, propionate, and butyrate increased after the RS supplementation in stool samples collected after a 4-week high amylose diet compromising of approximately 33 and 48 grams of RS for 10 overweight women and 13 overweight men respectively (Noakes; 1996). This also resulted in a reduced colonic pH compared to a 4-week low amylose diet. Phillips et al. (1995) had similar results after 11 ideal and overweight, male and female subjects consumed a diet of mixed RS types containing 4.67 mg RS/kJ for an average 38 g of RS per day. They found significant correlations between RS consumption and total fecal output (r=0.85), fecal pH (r=-0.82), and fecal butyrate excretion (r=0.84). Total SCFAs were also increased in Jenkins’ et al. (1998) experiment after subjects consumed separate, 30g RS2 and RS3 breakfast meals for 2 weeks, and butyrate showed the greatest increase of the all SCFA compared to control. In Cummings et al. (1996) acute human feeding study of 3 separate daily meals containing a total of 26-30g RS2, 17-19 grams of RS3, and a control, results showed no significant increases in SCFA. However, there was a trend for increase in total SCFA with no change in butyrate, but increase in acetate and propionate in RS2 and RS3 respectively. Lack of findings may be due to short duration and lower levels of RS consumption in subjects.

These studies indicate 30g or more of RS a day is needed to provide biologically significant increase in short chain fatty acids, and decrease in colonic pH. It also suggests type of SCFA produced may depend on profile of microflora or even type of RS in the large
intestine as many of these studies had mixed results of SFCA production. Metabolism of different SCFA is an interesting topic and important to consider when looking into colonic health benefits.

**Benefits of Fermentation**

Increased SFCA’s from RS consumption provide benefits to the colon through lowering pH and reducing DNA damage (Conlon et al. 2012). Fermentation of resistant carbohydrates in the large intestine provide benefit by reducing ammonia and phenol excretion in feces (Birkett, 1996). Unhydrolyzed starch adds bulk to feces and acts as substrate for fermentation by bacteria allowing SCFA to be produced and pH to be reduced. Lower colonic pH has been shown to have anti-carcinogenic effects in both rats and humans. Rats fed high amylose resistant starch that increased butyrate production showed higher rates of apoptosis in DNA damaged colonocytes (Clark et al. 2012) and butyrate appears to have a mechanistic role for inducement of apoptosis in colorectal cancer cell lines (Shin et al. 2012). Furthermore, Walker et al (1986) studied human populations in South Africa with differing levels of colon cancer incidence and found lower fecal pH in the low disease incidence populations despite similar fiber intake, but RS was not measured in this study and the populations of the low colorectal incidence consumed a greater amount foods high in RS such as maize and bean products. These studies suggest lower pH and possibly specific SCFA butyrate, play a role in protection from colon carcinogenesis.

SFCA's acetate, butyrate, and propionate produced in the colon are absorbed by the intestinal cells to be used for energy or enter the portal vein for distribution in the body.
Additionally, butyrate appears to be the preferred energy source for cultured cells in the colonic mucosa (Shin et al. 2012). Butyrate health benefits tend to relate towards colonocyte health and propionate and acetate have been proposed to provide hypophagic effects by acting as ligands to G-protein coupled receptors mediating lipid metabolism through adipocyte formation by adipokine release (Arora, Sharma, & Frost, 2011). SCFAs from resistant starch consumption play a complex role in colon health as RS has also been shown to reduce DNA damage in rats. Conlon and others (2012) found sprague-dawley rats fed a high amylose RS diet had significantly less (70%) single strand DNA breaks compared to rats fat low RS diet. They also found increased fecal SCFA (high concentration of butyrate), lower phenol and ammonia excretion in feces, and increased colonic microbiota in rats fat high RS diets. Phenols and ammonia are toxic compounds produced as byproducts of protein fermentation in the colon. Phenols produce carcinogenic effects and ammonia is a cytotoxic compound (Visek, 1978). Protein fermentation by-products were reduced in fecal samples collected by Birkett and colleagues (1996) from 5 male and 6 female subjects after a 3 week cross-over design of low-and high RS diet containing RS1 and RS2 from wheat and banana flours distributed in daily diets. They found the high RS diet reduced ammonia and phenol, and increased nitrogen excretion. Reduced excretion of toxic byproducts occurs because the bacteria in the large intestine readily use the unhydrolzed starch for energy decreasing the amount of protein that is fermented and nitrogen is increased from the growth in microbiotic mass (Birkett, 1996). Resistant Starch plays an important role in colonic health, but further research is needed to determine
specific mechanisms behind digestion and metabolism of RS in the colon that lead to these health benefits.

**Resistant Starch influence on Satiety and Weight Control**

Food intake is determined by internal and external factors that produce different satiation effects (hunger, fullness, satisfaction and desire to eat). Satiety and appetite regulation are very complex and are influenced by several factors such as environmental cues, psychological perceptions of hunger, and individual metabolism and physiology that produce neuronal cues leading to ingestion of food (Bornet et al. 2007). Meal factors influence amount of macronutrients and rate of digestion. This in turn affects gut hormones and peptides such as insulin, ghrelin, cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP) that regulate appetite (Table 3).

Table 3: Effect of satiety peptides on appetite

<table>
<thead>
<tr>
<th>Satiety Peptide</th>
<th>Origin</th>
<th>Effect on Appetite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin</td>
<td>Stomach</td>
<td>↑ appetite</td>
</tr>
<tr>
<td>PYY</td>
<td>Large intestine</td>
<td>↓ appetite</td>
</tr>
<tr>
<td>CCK</td>
<td>Small intestine</td>
<td>↓ appetite</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Large intestine</td>
<td>↓ appetite</td>
</tr>
<tr>
<td>Leptin</td>
<td>Adipose tissue</td>
<td>↓ appetite</td>
</tr>
</tbody>
</table>

Resistant starch has been proposed to influence satiety and control weight through several different mechanisms. One mechanism is the steady release of postprandial blood glucose from consumption of resistant starch. This delays the decline in blood glucose to pre-meal levels that would signal hunger and increase appetite. This relates to Jean Mayer’s glucostatic theory (1953) elucidating the effect of glucose levels
on appetite as he found low glucose levels are associated with hunger in both normal and insulin resistant individuals.

A second mechanism of decreased satiety through resistant starch supplementation is the interaction between SCFAs in the colon and satiety hormones. Short-chain fatty acids in large intestine are proposed to increase satiety by their release form the hepatic, portal, and venous blood that reduce production of fatty-acids from visceral adipose tissue (Cummings et al. 1987) and consequently effects physiology of metabolism and neuronal pathways of food intake.

A few studies have shown interaction between fermentation of RS, increase SCFAs, and changes in satiety hormones in both rats and humans. In a series of experiments by Zhou and others (2008), Sprague-Dawley rats fed resistant starch showed increased GLP-1 and PYY expression and secretion compared to control. When seven overweight and obese human subjects consumed 4 g of RS3 (barely and oat mixture) per day for 14 weeks, fasting GLP-1 and PYY were increased as well as satiety 1 hour after a standard meal (Greenway et al. 2007). Decreased satiety through SCFA effects on satiety hormones has basis, but more research is needed to substantiate these claims and identify mechanisms. Controlling body weight by RS consumption is achieved through to basic mechanisms: 1) increased satiation reduces subsequent food intake 2) less metabolizable energy in resistant starch compared to normal starch of same load give less energy intake and less energy intake leads to weight loss or weight control. The energy contribution of digested RS can range from 0 to 100% and up to 70% of RS can be metabolized in the large intestine. This depends on type and product of RS and subsequent processing in the colon (Cummings et al. 1996).
There are a limited number of studies examining the effects of RS on satiety and food intake and even fewer on corresponding satiety peptides. Moreover, evidence for increased satiation and reduced food intake has shown mixed results. Anderson et al. (2010) examined second meal intake of 17 healthy males 2 hours after consumption of a 3 tomato soups containing 50 g of RS. Amount of RS in soup treatment was correlated with reduced food intake at 120 min. The whole-grain soup (27g RS2), high-amylose soup (23g RS2) and regular cornstarch soup (19g RS2) had a 17%, 10%, and 10% decline in food intake respectively. Additionally, whole-grain soup was the only treatment that resulted in lower cumulative energy intake and there was a trend for decline in average appetite area under the curve after RS treatments compared to control. The decline in food intake was thought to be caused by the release of SDS that occurs up to 120 minutes post-prandial and types fiber sources may play a role in differences between results. In a study where postprandial satiety was compared in 5 muffins of different fiber content (9g of RS2, β-glucan, oat, corn bran, polydextrose) in 20 subjects, Willis and others (2009) found RS muffins were the most satiating in satisfaction and fullness up to 3 hours postprandial, and corn bran muffins had similar results for fullness only. Prospective consumption measured on 100mm VAS questionnaire was only lower in corn bran muffin. These results indicate fiber type is an important component in effects of subjective satiety. Subjective satiety is highly variable between subjects and increased satiety does not always lead to reduced intake and vice versa. Bodinham et al. (2010) showed 80g of RS (60%) split between breakfast and lunch meals in 20 young adults lowered energy intake in ad-libitum dinner meal and over the entire 24-hour period as measured by diet diaries, but had no effect on subjective appetite.
ratings. Kendall and others (2010) found opposite results in that subject appetite ratings measured by satiety quotient were lower at 15, 30, and 45 minutes postprandial and found a trend for average satiety to be lower during entire 2-hour post-meal time period with a 25g dose of a RS2 cereal bar compared to control. After 2 hours, subjects were then given an ad-libitum test meal consisting of pizza, and there were no difference in food intake with treatment. To further complicate findings, Raben and colleagues compared 50g raw potato starch (54% RS2) with fully-digestible starch mixed with non-nutritive syrups in 5 male participants. They found the fully digestible starch supplementation increased satiation up to 6 hours post prandial compared to RS supplementation, but RS treatment lowered postprandial glucose and insulin. Differences in satiation of these results may be due to differences between physical forms of treatments. The RS treatment was of liquid form and control was of gel form and research has shown liquids produce less satiating effects postprandial than solid foods (Mourao et al. 2007).

Table 4: Effects of resistant starch on satiety responses and food-intake in humans

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Methodology</th>
<th>Test Meal</th>
<th>Measurements</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raben et al. 1994</td>
<td>n=5</td>
<td>Cross-over design of two test meals preceded by 3 day controlled diet (60% carbohydrate, 28% fat, 12% protein)</td>
<td>50g RS2 (54.1%) in potato starch or 50 g pregelatinized potato starch (100% digestible) mixed into 500 mL diluted artificially sweetened fruit syrup</td>
<td>Satiety every 30 minutes after the meal, using 100-mm VAS Postprandial satiety GIP and GLP-1 satiety peptides</td>
<td>Significant ↑ in fullness and satisfaction 6 hours after consumption in starch meal not RS Starch test meal had spikes in GLP-1 and GIP with no change from RS meal</td>
</tr>
<tr>
<td>Study</td>
<td>n=</td>
<td>Age:</td>
<td>Sex:</td>
<td>Design</td>
<td>Starch Type</td>
</tr>
<tr>
<td>--------------------</td>
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<td>-------------</td>
</tr>
<tr>
<td>Anderson et al. 2010</td>
<td>17</td>
<td>20 y.o.</td>
<td>M</td>
<td>Cross-over design of 5 soups containing 50 g maltodextrin, whole-grain, high-amylose, regular cornstarch, or no added starch at 1-wk intervals.</td>
<td>50g RS2 (dry weight) in tomato soup from whole-grain (Hi-maize), high-amylose (Hi-maize 260) regular cornstarch (MELOJEL)</td>
</tr>
<tr>
<td>Willis et al. 2009</td>
<td>20</td>
<td>29 y.o.</td>
<td>13 F, 7 M</td>
<td>Cross-over design of 5 test meals of muffins containing different fiber types and control</td>
<td>9g RS2 of β-glucan, oat, corn bran, polydextrose</td>
</tr>
<tr>
<td>Bodinham et al. 2010</td>
<td>20</td>
<td>25 y.o.</td>
<td>M</td>
<td>Cross-over design of 2 day test breakfast and lunch meals containing mousse of high and low RS</td>
<td>80g (60%) and 40 % RDS (Hi-Maize® 260 product)</td>
</tr>
</tbody>
</table>

*Note: RS = Resistant Starch, Hi-maize = High-amylose maize, MELOJEL = Regular cornstarch, β-glucan = β-glucan, oat, corn bran, polydextrose.*
To summarize, studies examining resistant starch, satiety, and food intake in humans have provided mixed results (Table 4). The level of subjective satiation does not always predict food intake, but reduction in food intake is the primary goal of increased satiety to improve health. If decreased food intake is the primary goal, regardless of the effects on subjective satiety, then resistant starch content needs to be at least 50% of total starch ingested in doses of 15 g or more per meal to reduce food intake in subsequent meals and 24-hour intake. However, further research is needed to substantiate the effects of RS on increasing satiety and lowering food intake and to determine specific recommendations for RS supplementation to experience these effects.

### Table 4. (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Intervention</th>
<th>Dependent Variables</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kendall et al. 2010</td>
<td>N=22</td>
<td>5 tests meals consisting of cereal bar and beverage of varying levels of RS</td>
<td>Satiety quotient using VAS questionnaire</td>
<td>Greater satiety quotient in 25g RS than all other treatments up to 1 hour postprandial</td>
</tr>
</tbody>
</table>

RS=resistant starch, VAS= visual analog scale,

**Resistant Starch and Adverse Effects**

There appears to be minimal adverse effects associated with resistant starch consumption. In all aforementioned studies, those noting gastrointestinal discomfort all showed minimal effects of RS on negative GI symptoms such as bloating, pain, gas, and diarrhea. In a study of 41 healthy young adults, Storey et al. (2007) examined adverse GI
symptoms from acute and chronic consumption of varying levels of RS3. Subjects consumed 20, 40, 60, 80, 100 or 120 g (total starch) of RS3 containing starch products (> 50% RS of total starch) into daily meals on individual test days. Subjects recorded incidence and magnitude of GI responses and bowel movements 24 hours following each treatment. Results showed no significant differences in the number of subjects experiencing any symptom following consumption of 0–60 g RS3 (20-120g servings of total starch) or in frequency and consistency of bowel movements in 24 h following consumption. When long-term consumption was analyzed, subjects consumed RS3 for a period of 3 weeks, with increasing doses reaching 10 grams above the level noted to induce gastrointestinal symptoms from the short-term study, they found a small yet significant increase in report of GI symptoms. Flatulence, bowel frequency, watery feces increased as RS3 dose increased. From these results, it can be concluded that RS doses needed to produce significant changes in postprandial blood glucose, insulin, and satiety (120g of 50% RS) are not associated with any significant discomforting GI symptoms.

**Summary of findings**

High glycemic foods appear to be detrimental to health as diets with greatest amount of high GI foods are associated with obesity, abdominal obesity, insulin resistance, and Type 2 Diabetes. GI of foods have profound effects on postprandial glucose and insulin metabolism that can be contributed to the development of chronic disease and reduced health. This is why encouragement of low glycemic foods and high fiber intake through resistant starch supplementation is suggested to control glucose and insulin levels and
prevent disease progression. Several studies have confirmed this in normal, overweight, hyperinsulinemic and Type 2 diabetic adults. To attain significant biological effects, approximately 40-60 g doses (total starch dry weight) are needed for reductions in postprandial glucose and insulin and 100 g doses are needed to improve insulin sensitivity. Moreover, because resistant starch lowers peak blood glucose levels, delays blood glucose decline and escapes digestion to be fermented in the large intestine it is thought to decrease appetite, control weight, and improve bowel health. The ability for RS to decrease appetite, improve satiation, reduce intake, and control weight is less clear and more research is need to confirm these benefits. Subjective appetite is very complex and there is not conclusive evidence to determine increased satiation with fiber type and dose. However, current research suggests 80-100 gram doses over a 24-hour period may be needed to reduce food intake (Table 2). Fiber can be associated with uncomfortable gastrointestinal side effects such as bloating, diarrhea, gas, and pain. Although, minimal gastrointestinal side effects are seen with levels needed to produce biological significant effects on postprandial glucose, insulin and satiety. Resistant starch is a relatively new discovery, and its use as a tool for control of glucose and insulin metabolism and satiety and weight management is promising. Research developing resistant starch products for commercial use is an area to consider as this will be able to increase fiber intake on a larger scale and help combat the development and globalization of obesity and Type 2 diabetes.
References


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Shin H, Kim JH, Lee YS, Lee YC. Change in gene expression profiles of secreted frizzled-related proteins (SFRPs) by sodium butyrate in gastric cancers: Induction of


CHAPTER III: RESISTANT STARCH CORN PORRIGES LOWER POSTPRANDIAL BLOOD GLUCOSE WITH NO EFFECT ON SUBJECTIVE SATIETY OR 24 HR INTAKE IN NON-OVERWEIGHT AND OVERWEIGHT ADULTS

Abstract

Three resistant starch corn porridges made from cornmeal of differing amylose content were fed to non-overweight and overweight human subjects. Effects of resistant starch level (0.8 g, 2.0 g, and 7.5 g RS or 3%, 8%, and 30% of total starch respectively) on postprandial blood glucose and satiety responses were compared in non-overweight and overweight human subjects. Responses were measured at baseline and 3 hours after consumption of each resistant starch test meal. It was hypothesized that overweight subjects would have higher blood glucose lower subjective satiety after all treatments. Plasma glucose was hypothesized to be lowest and satiety response would be highest in highest resistant starch treatment. Moreover, it was hypothesized that increased satiety with high resistant starch treatment would result in lower food intake over 24-hour period for all subjects. There were no differences between non-overweight and overweight groups in any response. Resistant starch supplementation improved peak acute post-prandial glucose responses and blood glucose area under the curve with the highest RS dose, but had no effect on subject satiety or 24-hr food intake. It may be that doses of RS greater than 7.5 g per meal or 30% of starch ingested are needed to increase satiety and decrease food-intake in 24 hour period.
Introduction

High glycemic foods appear to be detrimental to health, as several observational studies have shown, diets with the highest glycemic index (GI) foods are associated with obesity, abdominal obesity, insulin resistance, and Type 2 Diabetes (Hu et al. 2011; Halton et al. 2008; Krishnan et al. 2007; Villegas, 2007). The GI of foods has profound effects on postprandial glucose and insulin metabolism that can be contributed to the development of chronic disease and reduced health (Musselman et al. 2011; Pawlak et al. 2004). Moreover, high consumption of rapidly digestible starches cause rapid spikes in blood glucose concentration (Ells, 2005) that can lead to hyperinsulinemia and over time may contribute to the decline of insulin function resulting in chronic hyperglycemia.

For example, Ells et al. (2005) compared postprandial blood glucose, insulin and non-esterifried fatty acids (NEFA) after consumption of a meal containing 75 g rapidly digestible starches (RDS) to 75 g slowly digestible starch with 21g of fat in 10 healthy female volunteers. They found more rapid and higher peaks of plasma glucose and insulin in the first 30-60 minutes after consumption of RDS meal and similar high and rapid peaks in NEFA 4-6 hours postprandial indicating starch type effects on carbohydrate and fat metabolism.

In another study, RS produced comparable results in postprandial glucose, insulin, and NEFA after a meal tolerance test in 10 healthy subjects following 24 h consumption of 100g RS2 consisting of 60% RS distributed in 25g doses compared to 40g RDS delivered in 10g dose in a diet of 42% carbohydrate (Robertson et al., 2003). These results show starch type can acutely affect insulin and glucose homeostasis and that long-term intake of simple carbohydrates may affect carbohydrate and fat metabolism resulting in obesity and DM2.
This is further confirmed in research conducted by Pawlak et al. (2004) examining effects of the dietary glycemic index on adiposity, glucose homoeostasis, and plasma lipids in rats fed high and low GI diets. Diets were of the same macronutrient content, (69% carbohydrate, 20% protein, and 11%) but rats fed a high GI diet were fed starch made of 60% amylose and rats fed a low GI diet were fed starch made of 100% amylopectin. Rats fed the high GI food had similar body weight compared to low GI but almost twice the amount of body fat and less lean body mass. Rats fed high GI diet had greater areas under the curve for blood glucose and plasma insulin after a glucose tolerance, lower plasma adiponectin concentrations (levels are inversely associated with obesity), higher plasma triglyceride concentrations, and severe disruption of pancreatic islet-cell architecture. When Pawlak and others (2004) conducted a similar experiment with high and low fat diets, they found no difference in body fat accumulation between treatments, but less glucose uptake and plasma insulin in rats fed the high-fat diet. These findings suggest both excess fat and carbohydrates play a role in the development of DM2. Although when wild-type Drosophila melanogaster (fruit flies) were fed high-fat, high-sugar, and control diets, larvae fed high-sugar diets developed hyperglycemia, obesity and insulin resistance, while insulin resistance was not seen in the high-fat diet suggesting carbohydrate quality plays a greater role in the decline of insulin function (Musselman et al. 2011).

As insulin function declines, glucose intolerance ensues causing high blood glucose levels that are harmful to the macro and micro vascular functions of the body resulting in DM2 diagnosis. Macrovascular complications can lead to heart disease and stroke. Microvascular damage can cause nephropathy leading to kidney failure, retinopathy
resulting in blindness, and neuropathy eventually leading to amputation (Nelms et al. 2007). This is why encouragement of low glycemic foods and high fiber intake through resistant starch supplementation is suggested to control glucose and insulin levels and prevent disease progression. Resistant starch is any starch that escapes digestion in the small intestine to be metabolized in the colon. Several studies have confirmed improved post-prandial plasma glucose and insulin profiles after RS substitution in normal weight, overweight, hyperinsulinemic, and Type 2 diabetic adults (Behall & Scholfield, 2005; Behall & Hallfrisch, 2002; Robertson et al. 2003; Mitra et al. 2007). Moreover, because resistant starch can lower peak blood glucose levels, delay blood glucose decline and escapes digestion to be fermented in the large intestine it is thought to decrease appetite, control weight, and improve bowel health. There is much evidence supporting resistant starch supplementation and improved bowel health. This is due to increased colonic pH and short-chain fatty acid production as well as decline in carcinogenic by-products, ammonia and phenols, of protein fermentation with consumption of resistant starch (Phillips et al. 1995; Noakes et al. 1996; Cummings et al. 1996). However, evidence is less definitive for improvement in appetite and reduction of energy intake as a means of controlling weight with RS supplementation. There have been a few promising studies showing substitution of resistant starch in daily meals can lead to increased feelings of satiety, reduced intake in subsequent meals, and over 24 h periods (Kendall et al. 2010; Bodinham et al. 2010; Anderson et al. 2010). Resistant starch is a relatively new finding (Jenkins et al. 1981), and more research is needed to make clear recommendations on dosing to provide
improvement in both glucose metabolism and satiety measures that will lead to improved long-term health by reducing prevalence of obesity and type 2 diabetes development.

Several diet and lifestyle factors contribute to the development of obesity and DM2. In an observational study examining diet and lifestyles factors of 84,941 nurses over a 16-year period, incidence of DM2, physical inactivity, cigarette smoking, poor quality of fats and carbohydrates, and excessive calorie intake were significant risk factors for the development of DM2 independent of overweight or obese status (Hu, 2001). Although these lifestyle factors contribute to the development of this negative health condition, obesity is the single most important risk factor for DM2, and high abdominal obesity, (absolute waist circumference >102 cm (40 in) in men and >88 cm (35 in) in women) regardless of weight categorization, is another critical risk factor in DM2 development (Hu, 2011). There has been little research conducted comparing glucose and satiety metabolism between non-overweight individuals and overweight and obese individuals. More research examining differences in post-prandial glucose and satiety between these groups may provide useful information about obesity and DM2 development and resistant starch supplementation.

Better understanding of the physiological effects of carbohydrates on metabolism will help develop recommendations for intake of carbohydrates and resistant starch. These will be important for improving health and combating the globalization of obesity and DM2. Therefore, the purpose of this study was to examine the effects and compare differences of resistant starch corn porridges on post-prandial glucose and satiety response in non-overweight weight and overweight adults.
Materials and Methods

Subjects

Twenty healthy subjects (10 males and 10 females) mean age 25 ± 4 years were recruited for the study. Subjects were classified according to body mass index (<24.9 and >25.0 kg/m²) with a mean BMI of 20.9 ± 1.4 and 28.8 ± 4.7 kg/m² respectively. There were five males and five females in each BMI group. Subjects were healthy, limited alcohol drinkers consuming < 1 alcoholic serving per day and not taking any medications in the past 6 months.

Protocol

Subjects were recruited from the Nutrition and Wellness research center at Iowa State University and visited the center 3 times, separated at least 2 days apart. On test days, subjects arrived in the morning after a 12 hour overnight fast. Upon arrival, baseline blood glucose was measured with a glucometer (ReliOn® Confirm, ARKAY USA, Minneapolis, MN), and a Visual Analog Scale (Greenway et al. 2007) was administered to determine, hunger, satisfaction, fullness, and desire. Blood glucose was measured at base 15, 30, 60, 120 minutes after the test meal and the VAS was administered 30, 60, 120, 180 minutes after consumption of test meal. Subjects were given a food log and gastrointestinal symptoms questionnaire upon completion of each visit and asked to record all food and beverage consumption and gastrointestinal symptoms over the following 24 hours.
Resistant starch

Corn samples used were grown at the ISU agronomy farms in 2009. Three different corn lines were planted: a high amylose, low amylose control, and a hybrid of the two. The inbred high amylose corn line: GUAT209:S13//Oh43ae/H99ae-1-2-1)-B-B-02 was planted in 200 rows. The control inbred corn line: AR011050:S01:1082]-09-02 was planted in 200 rows. The hybrid corn line: GUAT209:S13//Oh43ae/H99ae-1-2-1)-B/3/AR011050:S01:1082)-03)-02-10 was planted in 267 rows. The plants were self-pollinated and hand harvested, dried in a forced-air dryer at room temperature, mechanically shelled, and the seed was bulked. Samples were analyzed for mycotoxins at the Veterinary Diagnostic laboratory at North Dakota State University prior to study.

Test Meals

The three corn lines were consumed by subjects as a corn porridge breakfast. Test meals were consumed by participants in a cross-over randomized block design. Each test meal was taken with 200 ml of water and consumed within 15 minutes.

Porridge formulation and Resistant Starch Analysis

The porridge recipe consisted of 3 ingredients: cornmeal, water, and sucralose. Cornmeal was made from the aforementioned 2009 corn lines. The three varieties were ground in the pilot plant at Iowa State University’s Center for Crops Utilization Research. Samples were ground twice using a Fitz Mill, Comminuting Mill Model #DA606 (The Fitzpatrick Company, Elmhurst, IL). A 3.18 mm (screen #1531-0125) sieve was used for the first grind at 3,003
RPMs. And a 1.02 mm sieve (screen #1532-0040) was used for the second grind at 7,000 RPMs. Samples were stored at 4°C. Cornmeal weight per serving was calculated using 25 g dry weight of total starch because 50 g was too large a portion for a single meal, given the volume to be consumed. Total cornmeal needed for single serving of porridge equated to approximately 29 g. Porridges consisted of 9 times their weight in water as this was the least amount of water needed to have acceptable consistency for the assay and similarities of treatments. A small amount (0.025 g) of sucralose was added for sweetness.

Table 1. Resistant Starch (RS), digestible starch (DS), and total starch (TS) contents of porridge (dry basis)\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>RS (%)</th>
<th>DS (%)</th>
<th>TS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GUAT 2009</td>
<td>18.0 ± 0.1</td>
<td>44.2 ± 0.3</td>
<td>62.2 ± 0.4</td>
</tr>
<tr>
<td>AR/GUAT 2009</td>
<td>5.3 ± 0.0</td>
<td>57.8 ± 0.8</td>
<td>63.1 ± 0.8</td>
</tr>
<tr>
<td>AR 2009</td>
<td>2.0 ± 0.1</td>
<td>61.6 ± 0.3</td>
<td>63.7 ± 0.3</td>
</tr>
</tbody>
</table>

\(^a\)The solid content of the porridge was 9.8%.

Resistant starch content of the three corn varieties were analyzed using Megazyme AOAC Method 2002.02. Because analysis was on a wet basis pancreatic amylase was added according to solid content of porridge. Total starch was calculated by subtracting resistant starch from total starch. Starch content of the three corn varieties used in test meal is shown in Table 1. Based on starch content analysis, GUAT, AR/GUAT, and AR contained approximately 18 g of total starch and 5.2 g, 1.5 g, and 0.5 g of resistant starch. This equates to approximately 30%, 8%, and 3% resistant starch doses for each treatment respectively. Levels of RS used in study were based on amount in each corn line variation and appropriate meal size for consumption by subjects.
Blood analysis

Blood glucose was measured through finger prick analysis using a glucometer (ReliOn® Confirm, ARKAY USA, Minneapolis, MN). ReliOn® glucometer coefficient of variation averages 2.9% between consecutive glucose readings and has a correlation coefficient of 0.98.

Appetite Analysis

Satiety was measured using a 100 mm line Visual Analog Scale (Greenway et al. 2007). Subjects were asked to indicate level of hunger, fullness, satisfaction and desire to eat by marking a position along a continuous line between 0 and 100mm (Appendix A).

Food intake

Subjects were instructed to keep a 24-hour food diary recording amounts of all food eaten after completion of each visit. They were provided with a form to record intake and diets were checked and clarified for portion sizes upon return of the forms. Energy intake was measured using Nutritionist Pro™ Dietary Analysis software (AXXYA SYSTEMS LLC, Stanford, TX).

Gastrointestinal Symptoms

Subjects recorded gastrointestinal symptoms with questionnaire of overall symptoms, and time and onset of specific GI symptoms 24-hours after each treatment. Symptoms were
rated on a scale from 0 to 10 with 0 indicating no discomfort and 10 indicating extreme discomfort (Appendix A).

**Statistical Analysis**

Data was fit to a linear model (PROC GLM, v.9.2, SAS Institute, Cary, NC) where subjects were treated as random block effects to create correlations between subjects and treatments. Mean values and AUC were analyzed for plasma-glucose and satiety. Area under the curve was measured using trapezoidal method. Two-way interactions were evaluated between time with treatment, gender and weight classification. Main effects analyzed for blood glucose, satiety, and food intake. The significance level was set at $P < 0.05$.

**Results**

**Mycotoxin analyses of corn meals**

Corn meals from each of the corn lines showed no detectable contamination for any mycotoxin tested at the Veterinary Diagnostic laboratory at North Dakota State University.

**Glucose Responses**

There were no differences in post-prandial glucose responses when treatments were categorized by gender or weight group (Figures 2,4). The post-prandial blood glucose
response of high RS GUAT porridge at 30 minutes was significantly lower compared to GUAT/AR (p=0.0208) and AR porridge (p=0.0003) responses (Figure 3). Baseline-adjusted blood glucose area under the curve was reduced by 10% (p=0.0185) and respectively compared to AR porridge (Figure 1). There were no differences in post-prandial glucose responses between GUAT/AR and AR porridges.

Figure 1. Baseline adjusted post-prandial blood glucose responses measured as area under the curve 120 min after consumption of AR (3% RS), GUAT/AR (8% RS), GUAT (30% RS) corn porridges. Data represented as mean ± SE, n=20. Significance level p < 0.0185*
Figure 2. Comparison of non-overweight (BMI < 24.9) and overweight (BMI > 25) baseline adjusted post-prandial blood glucose responses measured as area under the curve 120 min after consumption of AR (3% RS), GUAT/AR (8% RS), GUAT (30% RS) corn porridges. Data represented as mean ± SE, n=10.

Figure 3. Post-prandial blood glucose responses 120 minutes after consumption of AR (3% RS), GUAT/AR (8% RS), GUAT (30% RS) corn porridges. Data represented as mean ± SE, n=20. Significance level p<0.05*
Figure 4. Comparison of non-weight (BMI <24.9) and overweight (BMI >25) post-prandial blood glucose responses 120 min after consumption of AR (3% RS), GUAT/AR (8% RS), GUAT (30% RS) corn porridges. Data represented change in mean blood glucose after baseline, n=5. Standard Error was omitted for clarity. Results were not significantly different.

Subjective Satiety responses and food intake

Baseline-adjusted satiety responses (hunger, satisfaction, fullness, and desire) were not different between RS porridges (Figure 5). There were also no trends or differences
between ideal weight and overweight subjects (Appendix B).

![Graph showing satiety responses](image)

*Figure 5. Baseline adjusted area under curve of satiety responses after subjects consumed 3 corn porridges with increasing level of resistant starch. Data represented as means ± SE, n=20.*

**Food Intake**

There was a slight tendency for 24 h food intake to decline with increasing RS dosage. Calorie intake was 1907 ±800, 1831± 543, and 1787± 758 kcals during 24 hours after consuming AR, GUAT/AR, and GUAT porridges respectively, but this was not significant.

**Gastrointestinal Symptoms**

Very few subjects reported adverse gastrointestinal symptoms in the 24 hours following porridge consumption. In the lowest RS dosage (AR), 3 people reported mild gas, bloating or diarrhea and five people reported similar results after GUAT/AR porridge ingestion. The
highest RS dosage (GUAT) had one participant report severe pain of short duration but rated overall discomfort in 24 h period as minimal.

Discussion

Glucose Responses

We hypothesized that mean post-prandial glucose response would decline as RS dose was increased in the porridge test meal. This was confirmed with our results as peak glucose responses and blood-glucose AUC were reduced in the 30% RS GUAT corn porridge compared to 8% RS GUAT/AR and 3% RS AR corn porridges (Figures 1, 3). The glucose response tended to decrease with increasing RS dose; however, this was not significant.

These results suggest doses between 30% RS of total starch or approximately 5g doses are needed to produce significant biological changes in post-prandial glycemia. Previous findings of resistant starch dose and postprandial glycemia show effects begin to occur around 15% RS of total starch or approximately 5 g dosages (Behall & Howe, 1995; Behall et al. 1989; Behall et al. 2002). For example, a two-day controlled diet, followed by a tolerance test of either glucose solution or breads containing 2%, 8%, 12% and 15% of RS2 (American Maize-Products Company, Hammond, IN) confirms this effect (Behall et al. 2002).

Moreover, Li et al. (2010) found similar declines in both postprandial glucose and insulin responses after subjects consumed rice providing 20% RS, equal to 8 g of RS in 40 g carbohydrate load. In our study, the resistant starch load was approximately 30% RS, equal to 5 g of RS in a 25 g (dry-weight) carbohydrate load. However, Jenkins et al. (1998) and
Kendall et al. (2010) observed no change in acute postprandial blood glucose and insulin responses after consumption of approximately 30 g and 25 g RS doses respectively. This may be due to flaws in design rather than the effect of high resistant starch doses. Jenkin’s test meals provided equal amounts of available carbohydrate and Kendall did not specify total or available carbohydrate loads in test meals. Equal amounts of carbohydrate loads should produce similar effects regardless the amount of RS because there is an equal amount of starch contributing to the rise is plasma glucose. Moreover, the main mechanism for using resistant starch as a tool for controlling postprandial glycemia is that a percentage of the total starch in a carbohydrate load is resisting digestion; therefore, less available starch is contributing to the rise in plasma-glucose compared to an equal starch load of fully digestible starch. Perhaps standardization of RS dosage in treatments is needed to help make better sense of results and develop concise recommendations for RS supplementation. Based on previous literature and our current findings, 5 g doses of RS or 15% RS of total starch in a standard 25-50 g carbohydrate load is needed to improve postprandial blood glucose.

**Satiety Responses**

Our study did not show any differences in satiety measures between RS treatments (Figure 5, Appendix B). Previous research examining the satiety effects of RS have provided mixed results (Raben et al. 1994; Anderson et al. 2010; Bodinham et al. 2010; Kendall et al 2010). We hypothesized that the 30% RS GUAT porridge would be the most satiating by reducing hunger and desire to eat and increasing satisfaction and fullness compared to the 8% and
3% treatments. Satiety and appetite regulation are very complex and are influenced by several factors such as environmental cues, psychological perceptions of hunger, and individual metabolism and physiology that produce neuronal cues leading to ingestion of food (Bornet et al. 2007). Resistant starch has been proposed to influence satiety and control weight through several different mechanisms. One mechanism, and the basis for our hypothesis, is the steady release of postprandial blood glucose from consumption of resistant starch due to delay of gastric emptying into the small intestine. This delay causes a decline in blood glucose to pre-meal leaves that would signal hunger and increase appetite. This relates to Jean Mayer’s glucostatic theory (1953) elucidating the effect of glucose levels on appetite as he found low glucose levels are associated with hunger in both normal and insulin resistant individuals. A second mechanism of increased satiety through resistant starch supplementation is the interaction between SCFAs in the colon and satiety hormones. Short-chain fatty acids in the large intestine are proposed to increase satiety by their release form the hepatic, portal, and venous blood that reduce production of fatty-acids from visceral adipose tissue providing an energy source in post-absorptive state (Cummings et al. 1987). Consequently, this affects physiology of metabolism and neuronal pathways of food intake. A few studies have shown interaction between fermentation of RS, increase SCFAs, and changes in satiety hormones GLP-1 and PYY, associated with increased satiety and reduced food intake, in both rats and humans. In a series of experiments by Zhou and others (2008), Sprague-Dawley rats fed resistant starch showed increased GLP-1 and PYY expression and secretion compared to control. Furthermore, when seven overweight and obese human subjects consumed 4 g of RS3 (barley and oat mixture) per
day for 14 weeks, fasting GLP-1 and PYY were increased, and subjective satiety was increased 1 hour after a standard meal (Greenway et al. 2007). The decrease in subjective satiety through the effects of increased SCFA post-prandially on satiety hormones has shown a few positive results, but more research is needed to substantiate these claims and identify mechanisms. We also hypothesized that this increase in subjective satiety would lead to a reduction in total energy intake over 24 hour period following consumption of RS. Controlling body weight by RS consumption is achieved through two basic mechanisms: 1) increased satiation reduces subsequent food intake 2) less available energy in resistant starch compared to digestible starch of same load give less energy intake and less energy intake leads to weight loss or weight control. As with previous literature looking at subjective satiety and resistant starch substitution, evidence for increased satiation and reduced food intake has shown mixed results. Anderson et al. (2010) examined second meal intake of 17 healthy males 2 hours after consumption of a 3 tomato soups containing 50 g of RS. The amount of RS in soup treatment was correlated with reduced food intake at 120 min. The whole-grain soup (27g RS2), high-amylose soup (23g RS2) and regular cornstarch soup (19g RS2) had a 17%, 10%, and 10% decline in food intake respectively. Additionally, whole-grain soup was the only treatment that resulted in lower cumulative energy intake and there was a trend for decline in average appetite area under the curve after RS treatments compared to control. The decline in food intake was thought to be caused by the release of SDS that occurs up to 120 minutes post-prandial, which delays decline in blood glucose levels to baseline that would initiate feelings of hunger. Additionally, the types of fiber sources (i.e. soluble vs. insoluble) may play a role in differences between
results. In a study where postprandial satiety was compared in 5 muffins of different fiber content (9g of RS2, β-glucan, oat, corn bran, polydextrose) in 20 subjects, Willis and others (2009) found RS muffins were the most satiating in satisfaction and fullness up to 3 hours postprandial, and corn bran muffins had similar results for fullness only. Prospective consumption measured on 100mm VAS questionnaire was only lower in corn bran muffin. These results indicate fiber type is an important component in effects of subjective satiety. Subjective satiety is highly variable between subjects and increased satiety does not always lead to reduced intake and vice versa. Bodinham et al. (2010) showed 80 g of RS (60%) split between breakfast and lunch meals in 20 young adults lowered energy intake in ad-libitum dinner meal and over the entire 24-hour period as measured by diet diaries, but had no effect on subjective appetite ratings. Kendall and others (2010) found opposite results in that subject appetite ratings measured by satiety quotient were lower at 15, 30, and 45 minutes postprandial and found a trend for average satiety to be lower during entire 2-hour post-meal time period with a 25g dose of a RS2 cereal bar compared to control. The level of subjective satiation does not always predict food intake, but a reduction in food intake is the primary goal of increased satiety to improve health. If decreased food intake is the primary goal, regardless of the effects on subjective satiety, then resistant starch content needs to be at least 50% of total starch ingested in doses of 15 g or more per meal to reduce food intake in subsequent meals and 24-hour intake. However, further research is needed to substantiate the effects of RS on increasing satiety and lowering food intake and to determine specific recommendations for RS supplementation to experience these effects.
Differences between weight groups

Our study did not show differences in blood glucose (Figure 2) or satiety between weight groups (Appendix B). This may be due to the fact that the majority of the overweight group had a body mass index between 25.0 – 29.9 kg/m$^2$. Two subjects in the overweight group were considered obese by BMI (30.3 and 41.2 kg/m$^2$). The 2 obese subjects had an approximate 20% higher baseline-adjusted glucose AUC compared to normal and overweight subjects (according to BMI). The 2 obese subjects also had an approximate 50% higher glucose at 60 min compared to normal and overweight subjects (data not shown).

Limitations

Some of the limitations to our study included frequency of blood glucose measurements and small sample size. In a review of GI methodology by Brouns and others (2005), it is suggested to measure blood glucose every 15 min for the first 60 min, than every half hour for at least 3 hours. Sampling less than recommended frequency leads to increase variation between means. A sample size of 10 subjects appears to be adequate for measuring differences in glycemic response, but 20 to 40 subjects are recommended for greater power and precision in detecting differences. We had 10 subjects in each weight group and 20 for each treatment although baseline characteristics (i.e. body mass index) were not the same for the 20 participants which could have influenced results. Moreover, satiety measurement and 24 h food dairies could have been more precise. For example, a secondary meal intake 2-3 h after test meal could have been given to measure satiety
through reduction in food intake, and a scale could have been given to subjects to weigh food intake over the 24-h period following each visit to insure portion size.

**General Conclusions**

Our results indicate that a 7.5 g dose of resistant starch or 30% RS in total starch can reduce postprandial blood glucose. However, this dose was insufficient to increase subjective satiety 3 h postprandial and/or reduce energy intake 24 h following consumption. Literature suggests 5 g doses or 15% RS in total starch is needed to improve postprandial blood glucose, and 15 g doses or 50% RS in total starch is needed to reduce energy intake in subsequent meals and daily intake.

**Future research**

This study provides evidence for use of RS as a tool for lowering postprandial glycemia in normal weight and overweight adults; however, more research needs to be conducted in obese and DM2 individuals as they may benefit most from resistant starch effects. Moreover, long-term studies on satiation and food intake from RS substitution are needed to make better recommendations on supplementation. Finally, developing products with high doses of resistant starch for large scale production and commercial use such as breads, cakes, muffins, and rice are needed to help combat globalization of obesity and DM2.
APPENDIX A: INFORMED CONSENT DOCUMENT AND DATA COLLECTION FORMS

INFORMED CONSENT DOCUMENT

Title of Study: Development of novel digestion-resistant starches from corn to combat human disease

Investigators: Suzanne Hendrich, PhD
Danielle Alexander, Graduate Research Assistant

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

This study is designed to compare the effects of a food product containing a new starch compared with a control starch on blood glucose. It is thought that the new starches, which are considered to be slowly digestible by humans, will cause lower blood glucose than the standard starch. This effect of digestion resistant starch may help people to control and prevent diabetes and obesity. You are being invited to participate in this study because you are an ISU student or local resident who is between the ages of 18-79 years and may be available to participate in this project. You may be eligible for this study if you are healthy, a non-smoker, not allergic to corn, not taking any medications and not drinking more than 3 drinks per day.

DESCRIPTION OF PROCEDURES

The purpose of the study is to observe blood glucose concentrations and monitor possible adverse effects from eating for breakfast a corn porridge product after an overnight (10 hr) fast. Some of these porridges are thought to be digestion-resistant and will be compared together. The porridges will contain corn meal, water, salt and sucralose (a noncaloric sweetener "Splenda").

If you agree to participate in this study, your participation will involve,

- after an overnight fast of at least 10 hours, coming to the Nutrition & Wellness Research Center and answering brief questionnaires on health and eating,
- tasting the porridge and rating its palatability,
- and if eligible providing blood samples just before (time 0) after (15, 30, 60, and 120 minutes) eating a starch-containing food product for breakfast on three days, which are spaced at least one day between each test day. Each blood sample will be ~0.25 mL (6 drops). A sterile automated lancet will be used to obtain blood from the finger or thumb to facilitate blood sampling.
- You will also rate your satiety or fullness on brief questionnaires at 0, 30, 60, 120 and 180 minutes after eating the porridges.
- You will be asked to fast for 10 h before breakfast on each of the days on which you eat a test meal.
- On each of the 3 days, your participation will involve about 4 hours of your time.
- You will be asked to record food intake 24 h after eating the food product on a food log and note any gastrointestinal distress symptoms (distention, pain, gas, diarrhea) on a questionnaire.

RISKS

While participating in this study you may experience the following risks. You may experience minimal discomfort or irritation at the site of blood sampling. There is a slight risk you may experience infection at the site of blood sampling. Blood will be taken under strict aseptic techniques to minimize infection risk. Ingestion of the resistant starch could result in mild to moderate signs of discomfort, which we will ask you to note. Abdominal discomfort such as bloating, gastrointestinal pain, and diarrhea might occur. These symptoms are not likely to persist for more than a day.
BENEFITS
If you decide to participate in this study there will be no direct benefit to you. It is hoped that the information gained in this study will benefit society by showing whether a novel starch can lower blood glucose response after a meal, which might result in healthier food products to become available eventually.

COSTS AND COMPENSATION
You will be compensated for participating in this study and there will be no cost to participate in the study. You will receive $75 for every visit in which you ingest the required amount of food product, provide all 5 blood samples, complete all 5 satiety questionnaires and answer the adverse events questionnaire. A total of $225 will be given for participating in eating all 3 food product, and completing all blood sampling intervals and questionnaires. You will need to provide your social security number (SSN) and address in order to be paid. This information allows the University to fulfill government reporting requirements and confidentiality measures are in place to keep this information secure. You may elect to forego receipt of payment(s) and continue in the research study if you do not wish to provide your social security number and address. If you are not a US citizen, taxes will be subtracted from your compensation. Information regarding documentation required for participant compensation may be obtained from the Controller’s Department; 294-2555 or http://www.controller.iastate.edu.

PARTICIPANT RIGHTS
Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled. If you are not able to comply with any of the required procedures (questionnaires, consuming the test products, providing 5 blood samples per product) for any reason, you may be dropped from the study.

RESEARCH INJURY
Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thelen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance.

CONFIDENTIALITY
Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, federal government regulatory agencies including the Food and Drug Administration and the Institutional Review Board (a committee that reviews and approves human subject research studies) may inspect and/or copy your records for quality assurance and data analysis. These records may contain private information.

To ensure confidentiality to the extent permitted by law, the following measures will be taken. Subjects will be assigned a unique code number, and all samples and questionnaires identified by code number only. The identifiers to the codes will be kept in a locked desk in the investigators’ locked offices. Only the key study personnel (professors and graduate research assistants) will have access to the data. The data identifiers will be retained for approximately 2 years, until the study is published. If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS
You are encouraged to ask questions at any time during this study. For further information about the study contact Suzanne Hendrich, (515) 294-4272, shendric@iastate.edu, 224 MacKay or Danielle Alexander, alexander.danielle.k@gmail.com. If you have any questions about the rights of research subjects or research-related injury, please contact the Office for Responsible Research, IRB@iastate.edu, 1138 Pearson Hall, (515) 294-4566; or the Director, Office of Responsible Research, 1138 Pearson Hall, (515) 294-3115

**************************************************************************************************

PARTICIPANT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the written informed consent prior to your participation in the study.

Participant's Name (printed) ____________________________________________

Participant's Contact information:

Email __________________________________________________________________

Phone number __________________________________________________________________

__________________________ ____________________________
(Participant's Signature) (Date)

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

__________________________
(Signature of Person Obtaining Informed Consent)
Subject ID number ______________

Health information questionnaire: Research study (May-July 2011)—Development of novel digestion-resistant starches from corn to combat human disease, Suzanne Hendrich, PhD, Principal Investigator, Danielle Alexander, study coordinator.

Age: _____ (must be 18 years of age or older) Date of birth: __________________________
                      mo/day/year

Ethnicity (circle as many as appropriate):

African or African American  Asian or Asian American  American Indian  White

Latino/Hispanic  Native Hawaiian or Other Pacific Islander

Body weight____  Height____

For which of the following days are you available to eat a breakfast containing corn porridge and complete testing (lasting from ~ 7 am to 10 am)? (circle all available)

M  Tu  W  Th  Fr  Sa  Su

How much alcohol do you drink? none  1-3 drinks/day  4 or more drinks/day

Do you smoke or use tobacco products? yes  no

Do you currently have any disease or illness? specify:

Are you taking any drugs? specify:

Have you taken antibiotics in the past 6 months? yes  no

Are you allergic to corn? yes  no
### Blood Glucose Recording Sheet

<table>
<thead>
<tr>
<th>Time point</th>
<th>Blood Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>
### Visual Analog Scale

**Time Point** ______________  **Subject ID** ______________  **Date** ______________

Make a mark crossing the scale (line) beneath each question to indicate how you feel (the extremes are described at either end of each line).

#### How hungry do you feel?

<table>
<thead>
<tr>
<th>I am not hungry at all</th>
<th>I have never been more hungry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### How satisfied do you feel?

<table>
<thead>
<tr>
<th>I am completely empty</th>
<th>I cannot eat another bite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### How full do you feel?

<table>
<thead>
<tr>
<th>Not at all full</th>
<th>Totally full</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### How much do you think you can eat?

<table>
<thead>
<tr>
<th>Nothing at all</th>
<th>A lot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Record all foods eaten over the next 24 h, in as much detail as possible, using amounts from package labels or estimation of portion in cups, ounces, milliliters or grams.

<table>
<thead>
<tr>
<th>Time</th>
<th>Food item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Subject ID number ___________________  Date ___________________

**Gastrointestinal symptoms questionnaire:** Research study (2011)—Development of novel digestion-resistant starches from corn to combat human disease, Suzanne Hendrich, PhD, Principal Investigator, Danielle Alexander, study coordinator.

Record any events of gastrointestinal distress (such as discomfort, feeling bloated, gas, pain, burping, flatulence, diarrhea or loose stools) that you experience over the next 24 h.

<table>
<thead>
<tr>
<th>Time of onset H: min am/pm</th>
<th>Time of cessation H: min am/pm</th>
<th>Symptom</th>
<th>Level of discomfort (1 = lowest, 10 = most severe possible); number of gas passages (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Overall, circle the rating of your level of gastrointestinal symptoms over the 24 h after drinking the test beverage:

gastrointestinal bloating (estimated intensity 0 = None; 1 to 5 = Mild; 6 to 7 = Moderate; 8 to 9 = Severe, 10 = Worst possible)

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>

gastrointestinal pain (estimated intensity 0 = No pain; 1 to 5 = Mild pain; 6 to 7 = Moderate pain; 8 to 9 = Severe pain, 10 = Worst pain possible)

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>

diarrhea (estimated severity 0 = None; 1 to 5 = Mild; 6 to 7 = Moderate; 8 to 9 = Severe, 10 = Worst possible)

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>
APPENDIX B: FIGURES OF SATIETY RESPONSES

Figure 6. Comparison of normal weight (BMI ≤ 24.9) and overweight (BMI ≥ 25) post-prandial fullness measured as area under the curve after subjects marked satiety rating on 100mm line visual analog scale. Data are represented as means ± SD, n=10.
Figure 7. Comparison of normal weight (BMI ≤ 24.9) and overweight (BMI ≥ 25) post-prandial satisfaction measured as area under the curve after subjects marked satiety rating on 100mm line visual analog scale. Data are represented as means ± SD, n=10.

Figure 8. Comparison of normal weight (BMI ≤ 24.9) and overweight (BMI ≥ 25) post-prandial desire measured as area under the curve after subjects marked satiety rating on 100mm line visual analog. Data are represented as means ± SD, n=10.
Figure 9. Comparison of normal weight (BMI ≤ 24.9) and overweight (BMI ≥ 25.0) post-prandial hunger measured as area under the curve after subjects marked satiety rating on 100mm line visual analog scale. Data are represented as means.
### APPENDIX C: GASTROINTESTINAL QUESTIONNAIRE RESPONSES

Table 6: Subjects reporting gastrointestinal discomfort 24 hours after consumption of corn porridge

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Treatment</th>
<th>Onset Hour:Min</th>
<th>Cessation Hour:Min</th>
<th>Symptom</th>
<th>Level</th>
<th>GI bloating</th>
<th>GI pain</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>AR</td>
<td>6:00 PM</td>
<td>6:00 PM</td>
<td>Diarrhea</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>AR</td>
<td>9:30 AM</td>
<td>10:00 AM</td>
<td>Bloating</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>AR</td>
<td>10:30 AM</td>
<td>12:30 AM</td>
<td>Gas</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>AR</td>
<td>7:15 AM</td>
<td>11:00 AM</td>
<td>Bloating</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>AR</td>
<td>10:30 PM</td>
<td>6:45 AM</td>
<td>Gas</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>AR</td>
<td>6:45 AM</td>
<td>6:50 AM</td>
<td>Loose stool</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>AR</td>
<td>10:30 AM</td>
<td>11:00 PM</td>
<td>Gas</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>AR/GUAT</td>
<td>11:30 AM</td>
<td>12:00 PM</td>
<td>Gas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>AR/GUAT</td>
<td></td>
<td></td>
<td>Loose stools</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>AR/GUAT</td>
<td></td>
<td></td>
<td>Pain</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>AR/GUAT</td>
<td>8:10 PM</td>
<td>8:25 PM</td>
<td>Diarrhea</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>AR/GUAT</td>
<td></td>
<td></td>
<td>Bloating, pain, Diarrhea</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>AR/GUAT</td>
<td>6:55 AM</td>
<td>7:01 AM</td>
<td>Loose stools</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>AR/GUAT</td>
<td>8:00 AM</td>
<td>6:00 AM</td>
<td>Bloating</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>AR/GUAT</td>
<td>10:50 AM</td>
<td>10:50 AM</td>
<td>Bloating</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>AR/GUAT</td>
<td>10:15 PM</td>
<td>10:15 PM</td>
<td>Pain</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>GUAT</td>
<td>9:15 PM</td>
<td>9:30 PM</td>
<td>Bloating</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>GUAT</td>
<td>9:15 PM</td>
<td>9:30 PM</td>
<td>Gas</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>GUAT</td>
<td>8:15 PM</td>
<td>9:30 PM</td>
<td>Pain</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>GUAT</td>
<td>8:15 PM</td>
<td>9:30 PM</td>
<td>Bloating</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: blank spaces indicate specific time was not recorded or general symptoms were described for 24 h period
REFERENCES


ACKNOWLEDGEMENTS

I am happy to thank all individuals who have helped me along the way in completing this thesis. I would first like to express my sincere gratitude to my advisor, Dr. Suzanne Hendrich, because without her continual support, motivation, encouragement, and patience this thesis would not have been possible. I would also like to thank Dr. Terri Boylston for supplying sucralose for the porridges and Dr. Jaylin Jane for letting me use her lab to analyze my samples and Yongfeng Ai for having the patience to help me understand resistant starch analysis and answer my questions along the way. Another thanks to Dr. Hui Wang for fitting me into his busy schedule to prepare my corn samples at the pilot plant and Steve Fox for his direction. I greatly appreciate Jeanne Stewarts help with organizing data collection at the Nutrition and Wellness Research Center and my helpers Sylvia King, Tyler Roskam, and Dan Chen. I also appreciate Esther Haugabrook’s random advice along the way that helped to keep me in a positive mindset. Lastly, I would like to thank my committee members, Dr. Christina Campbell and Dr. Doug King, for their time commitment, flexibility, and feedback to help me improve my knowledge and skills.