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Effect of Resistant Starch on the Glycemic Effect of a Meal

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EFFECT OF RESISTANT STARCH ON THE GLYCEMIC EFFECT OF A MEAL

by

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The purpose of this study was to determine if supplementation of a high glycemic load breakfast with resistant starch attenuates the glycemic response in young healthy adults. Twenty-one subjects, 23.8 ± 3.3 yrs, reported for two days of data collection, having fasted 8-12h. On day 1(control), fasting BG was measured using glucometers, then subjects consumed 2 slices white bread and 250mL apple juice within 15min, the repeated BG measurement at 15, 30, 60, 90, and 120min after baseline. Day 2, the protocol was repeated, except that 10g of resistant starch supplement was added to the apple juice. There was no significant difference in fasting BG between the data collection days. There was no significant difference in the spike in BG (difference between peak and baseline) between the control and resistant starch supplemented meal(52.6±22.7 vs 52.0±20.6mg/dL, respectively) or incremental area under the glucose curve (137.2±78.9 vs 164.0±83.2mmol/L/120min,respectively). BG peaked at 15, 30, or 60 min for all subjects. The resistant starch supplement had no effect on when BG peaked after the meal. The results of this study indicate that 10g supplementation of resistant starch does not affect the spike in BG or overall glycemic response to a high glycemic load meal.

TABLE OF CONTENTS

Table of Contents – Continued

Introduction

Statement of the Problem

Elevated blood glucose (BG) levels are one of the main metabolic risk factors that greatly contribute to the development of health consequences. Elevated blood glucose levels increase the production of advanced glycosylated end products (AGEs) by increasing the glycosylation of body proteins.[1-2] AGEs have shown to play a key role in the acceleration of aging as well as the development and progression of numerous diabetes- and age-related diseases.[3-6] The increasing effect of BG levels from the consumption of sugar is proportional to the buildup of AGEs.[7-8]

As well, inflammation caused by elevated blood glucose levels can later develop into chronic inflammation.[9-10] This results in the ongoing release of lymphocytes entering the tissues and releasing chemicals that activate the inflammatory state. These cells damage healthy tissues through several mechanisms that increase the production of free radicals and the production and release of other pro-inflammatory chemicals. Chronic inflammation plays a foundational role in the development of a number of chronic diseases including but not limited to, cardiovascular disease, diabetes, cancer, and Alzheimer's disease.[11-14]

Carbohydrate consumption results in BG elevation, reaching a peak approximately one hour after ingestion. However, the type of food impacts the speed and amount of rise in BG levels. High glycemic index foods, such as simple and refined carbohydrates cause rapid and higher increases in BG than lower glycemic index foods. Low glycemic index diets decrease inflammation and AGEs.[7]

An approach to decreasing inflammation and AGEs would be to limit food with a high index in the diet. Though ideal, implementation of this is difficult because people may be unwilling or unable to limit their consumption of these types of foods because they are more desirable in taste. Since there may be resistance to limiting the consumption of HGI foods,

alternative ways of reducing the BG spike and overall glycemic response of HGI would be useful.

Regular intakes of soluble fiber supplement and resistant starch have beneficial effects on glucose tolerance such as decreased glycemic response, increased insulin sensitivity, lower serum cholesterol concentrations, reduced blood pressure, and lower risk of coronary heart disease along with other vital health benefits.[15] Added soluble fiber to a meal can slow down the digestion and absorption of food as well as regulate metabolic hormones, therefore improving the postprandial glycemic response and insulin concentrations. This is due to soluble fiber's ability to move undigested through the upper digestive tract and later be metabolized by bacteria in the colon.[15] Possessing the same characteristics, resistant starch also positively contributes to the glycemic effect when ingested and may be more desirable compared to a soluble fiber supplement because it is less viscous, thus producing a less "gummy" texture when added to a beverage.[16]

Purpose of the Study

The purpose of this study is to determine if added fiber supplement will decrease the spike in blood glucose as well as the overall glycemic response to a high refined carbohydrate breakfast in healthy young adults.

Research Questions

1. Does added fiber, in the form of 10g resistant starch, decrease the spike in blood glucose after consumption of a high refined carbohydrate breakfast compared to a high refined carbohydrate breakfast without added fiber supplementation?
2. Does added fiber supplement diminish the glycemic response as measured by the incremental area under the curve (iAUC) after consumption of a high refined

carbohydrate breakfast compared to a high refined carbohydrate breakfast without added fiber supplementation?

Hypotheses

1. The addition of 2 tbsp. of Just Better™ fiber supplement to a high refined carbohydrate breakfast, consisting of 2 slices of white bread and 1 cup of apple juice will diminish the spike in BG compared to the meal without the added fiber supplementation.
2. The addition of 2 tbsp. of Just Better™ fiber supplement will decrease the iAUC compared to the meal without the added fiber supplementation.

Significance of the study

If fiber can diminish the spike in BG and overall glycemic effect of a high refined carbohydrate meal, this may be an effective strategy to mitigate negative health outcomes associated with elevated blood glucose. The production of AGEs could be minimized as well as inflammation, and ultimately reduce risk for such health issues as cardiovascular disease, diabetes, and cognitive decline. In addition, although this thesis is focusing on the effect of resistant starch supplementation on the glycemic response to a high carbohydrate breakfast in healthy subjects, controlling BG is especially important for those with diabetes, and could potentially help control BG in that population, reducing risk for the long term complications of the disease.

Literature Review

Overview of the Glycemic Index and Glycemic Load

The glycemic index is a tool used as an indicator of the magnitude of the glycemic response of a food compared to a standard carbohydrate-containing food, either sucrose or white bread. The test and standard food are consumed in a quantity that provides 50 gm carbohydrate.

The glycemic response to a food is determined by measuring fasting BG, consuming a food, then testing BG in 15-30 min increments for 2 hours.[17] The response is typically a curve, since carbohydrate ingestion increases BG to a peak level, followed by a decline resulting from insulin secretion. Area Under the glucose Curve (AUC) is calculated to reflect the magnitude of the glycemic response. For example, if the AUC for a test food is 50% of the AUC of from white bread, the glycemic index of that the test food is 5.

Many factors affect the glycemic index of food such as the type of carbohydrate (refined or simple vs. whole food sources) and anything in the food that influences the rate of digestion or absorption. Fiber tends to lower the glycemic index because it slows the rate of digestion and absorption of monosaccharides.[17] Although the glycemic index can be a useful tool to predict how a food will impact BG, this method may not truly represent the glycemic response to a food that is not consumed in an amount that provides 50 gm carbohydrate. For example, boiled carrots have an approximate glycemic index of 50, but a standard serving of carrots, ½ cup, only has 5 gm carbohydrate. Therefore, the glycemic load, which accounts for the amount of carbohydrate consumed, is preferred by many.[17]

As defined by Medieros et al., to calculate the glycemic load, the glycemic index of a food is multiplied by the amount of digestible carbohydrate in a serving, which is then divided by 100. For consistency, glycemic index will be used in this paper. Foods can either have a high, medium, or low glycemic index. Health professionals often recommend that those desiring to control BG and/or consume a healthy diet choose lower glycemic index foods, as high glycemic index foods tend to be more highly processed, lower in fiber, higher in sugar, and lower in nutrient density than low glycemic index foods. Examples of foods with a high glycemic index include white rice, cookies, candy, sodas, and fruit juices, while whole grains, such as brown rice

as well as legumes, and whole fruits have lower glycemic indices.[17]

Studies have shown that high-carbohydrate, low-fat diets reduce high-density lipids (HDL-C) and increase triglyceride concentrations.[18] Other investigations have shown a decrease in triglycerides, LDL cholesterol, and HDL cholesterol with a lower glycemic index diet. Additionally, a low glycemic load diet has resulted in lower c-creative protein levels, a marker of inflammation.[19] Furthermore, one study concluded that a low glycemic index and energy-restricted diet containing moderate amounts of carbohydrates can be effective in controlling glucose and insulin metabolism.[20] Thus, evidence exists for health benefits of a lower glycemic index diet.

Overview of Blood Glucose

Blood glucose levels increase when carbohydrate is ingested, but in a healthy individual, BG is maintained in a normal range by the hormones insulin and glucagon. Insulin is an anabolic hormone that promotes the lowering of blood glucose concentrations. An elevation in BG stimulates the secretion of insulin by the beta cells of the pancreas. Insulin binds to receptors on adipose and muscle cells, serving as a signal for GLUT4 glucose transport proteins to translocate to the cell surface, facilitating glucose uptake by the cell. In the fed state, insulin prompts protein kinase cascades promoting glycogen synthesis in both muscle and liver while suppressing gluconeogenesis by the liver. So the general effect of insulin is to lower blood glucose.[21]

When blood glucose levels are low, insulin secretion by the pancreas lowers, and glucagon is secreted. This hormone triggers the liver and muscle to convert glycogen into glucose and release it into the bloodstream, preventing hypoglycemia. In short, insulin and glucagon work together in order to maintain normal blood glucose levels.[21]

Type 2 Diabetes Mellitus

Per the Centers for Disease Control and Prevention, the prevalence of type 2 diabetes is increasing. In the United States, 29.1 million people have diabetes. Of those, 8.1 million are unaware that they have the disease.[21] When an individual has diabetes, the pancreas is not able to produce insulin (type 1 diabetes), or the body makes insulin but can't use it properly to control BG (type 2 diabetes). Both conditions result in hyperglycemia if not treated properly. Complications that can arise from uncontrolled BG include retinopathy, nephropathy, neuropathy, cardiovascular disease, and amputations. Thus, it is imperative that those with diabetes control their BG, and strategies that would mitigate post meal hyperglycemia, such as adding fiber to a meal, may aid in BG control.[22]

Cardiovascular Diseases

According to the American Heart Association, adults with diabetes are 2 to 4 times more likely to die from heart disease than those who don't have diabetes. Those with diabetes have an increased risk for dyslipidemia, a lipid disorder associated with insulin resistance.[22] In terms of treatment, tight glucose control resulting in lower A1C levels has been shown to have a beneficial effect on the risk for cardiovascular disease in those with diabetes.[23]

Advanced Glycation End Products

AGEs have shown to play a key role in the acceleration of ageing as well as the development of chronic diseases such as diabetes and cardiovascular disease by increasing oxidant stress and inflammation. Glucose and body proteins bond to form AGES. The cross-linking effect of AGEs alters protein structures and functions. AGE's are often found in the extracellular matrix where modified matrix proteins impair matrix-matrix as well as matrixcell interactions [24].The body can naturally cope with advanced glycation end-products, but if excess

AGEs accumulate, damage can ensue. Arteries can become stiffened and more prone to developing atherosclerotic plaques, for example.

Evidence suggests that advanced glycation end products are found in retinal vessels of those with diabetes. In 2005-2008, 4.2 million people 40 years or older had diabetic retinopathy, damage to the small blood vessels in the retina that could have resulted in the loss of vision. AGE levels in the retinal vessels correlated with those in serum as well as with severity of retinopathy. Accumulation of AGEs in the peripheral nerves inhibits nerve conduction velocities and results in neuronal blood flow abnormalities. Infusing AGEs in nondiabetic rats resulted in the development of morphological changes in the kidneys and an abnormal increase of protein in the urine, known as proteinuria. The first known relation between AG's and diabetes occurred in 1968 through the discovery of an altered form of hemoglobin in red blood cells in diabetic patients. Investigators found that glycation occurred mainly on N-terminal valine chains and that amadori products were formed by a non-enzymatic reaction which had only been seen up to that point in food chemistry.[25] Those with diabetes create more AGEs, and create them more rapidly than those without diabetes, because of increased BG, AGE precursors, and oxidative stress. The AGEs seen in those with type 2 are carboxymethyllysine (CML), methylglyoxal-derived hydroimidazolone, pentosidine, or glucosepane. There is a correlation between the production of AGEs with diabetic complications. The higher the BG, the more AGEs will form, underscoring the need to prevent hyperglycemia.

Resistant maltodextrin (Fibersol 2, referred as FS2)

As defined by Miyazato et al., resistant maltodextrin (RMD) is created through heat and enzymatic treatments of starch which makes it resistant to digestive enzymes. RMD is a non-viscous fermentable soluble source of dietary fiber that is not digestible and therefore is able to

reach the large intestine. Because this kind of dietary fiber is considered a safe and low-caloric ingredient, many companies have incorporated it into their products. RMD has shown positive effects in the areas of bowel movement, improvement of glucose levels, and attenuation of postprandial blood triglycerides.[26] An experimental study on the effect of resistant maltodextrin on apparent mineral absorption in rats conducted by Miyazato et al., found that both RMD and hydrogenated RMD assisted in the absorption of calcium and magnesium through short chain fatty acid (SCFA) production via cecal fermentation. Iron and zinc were also seen absorbed in the gastrointestinal tract.[26]

Effect of Soluble Fiber Supplement and Resistant Starch

Adding fiber to a meal high in sugar and refined carbohydrates may attenuate the spike in blood glucose and overall glycemic response to the meal. Glucose temporarily binds to soluble fiber, which delays its absorption.[27] The positive effect of soluble fiber and resistant starch on glucose tolerance in healthy people is well documented.[28-32] Kandea et al., found powdered green tea containing digestion resistant maltodextrin (DRM), consumed after a meal, lowered postprandial blood glucose levels at 30 minutes and significantly reduced insulin levels in 30 healthy adults.[28] Another study saw a significant decrease in postprandial blood glucose levels after 46 adults consumed indigestible dextrin when added to a beverage.[29] Yogurt containing DRM effectively reduced serum glucose levels and insulin levels in 21 healthy individuals.[30] Additionally, Wakabayashi et al., found that Fibersol-2 (from corn starch) added to a beverage did quickly blunt the spike in blood glucose.[31] Resistant starch supplementation to a beverage did attenuate the glycemic response after a meal.[31]

Soluble fiber and resistant starch have shown to have a positive effect on glucose tolerance in healthy people. A study conducted by Behall et al., focused in determining the

effects of soluble fiber and resistant starch on glycemic variables.[32] Results found that soluble fiber had a greater effect on postprandial insulin response whereas resistant starch showed an effect on reducing glucose levels. A combination of both soluble fiber and resistant starch contributed to the decrease in glycemic response. There was a decrease in postprandial glucose and insulin response in people with normal glucose tolerance when amylose or resistant starch was consumed in the meals.[32] It was concluded that fiber could improve glucose metabolism in both normal and overweight individuals.[32]

Research Methodology

This intervention study used a cross-over design. This study was approved by the Institutional Review Board at University of the Incarnate Word.

Participants

Twenty-one volunteers were recruited from announcements in nutrition classes taught at the University of the Incarnate Word as well as the snowball effect in the Fall of 2016. Participants were healthy young adults between the ages of 20-33 with no medical conditions or medications that would affect carbohydrate metabolism and had fasting BG values less than 100 mg/dL. Participants did not have allergies or intolerances to white bread, apple juice or corn (the starch source in the resistant starch).

Exclusion criteria included individuals weighing less than 110 lb., who were pregnant or breastfeeding, individuals with medical conditions and/or taking medications that would affect carbohydrate metabolism as ensured by a clinician who interviewed participants as part of the screening process. Those with a body mass index (BMI) greater than 29.9 were also excluded, as were those with a waist circumference above the cutoff that indicates excess abdominal fat (90 cm for females and 102 cm for males, or 35 and 40 in, respectively), as obesity, especially

abdominal obesity, can adversely affect health and glucose tolerance.[33]

In addition, participants agreed to fast for at least 8 hours prior to data collection and refrain from exercise on the days of data collection. All participants signed an informed consent form prior to participation.

Blood Glucose Measurements

Calibrated glucometers were used for BG measurements. Participants were instructed on how to measure BG using a glucometer (FreeStyle Lite). In preparation for the procedure, the measurement included wiping a finger with an alcohol pad, then allowing the finger to dry. A Freestyle Lite lancet was then used to prick the finger for a small drop of blood. A glucose test strip was placed at the finger where the small drop of blood appeared, then placed in the glucometer for determination of blood glucose level. The finger was then wiped again with the new alcohol pad.

Procedure

On day 1 (control day) of data collection, the participants arrived to a specified room on campus at 8 am after fasting for at least 8 hours, having refrained from exercise the day of data collection. They completed a questionnaire detailing demographic and anthropometric information including gender, age, and ethnicity. Height and weight were measured using a manual height scale and digital weight scale, respectively, and body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Waist circumference was measured at the umbilicus to the nearest 0.5 cm using a tape measure. The participants were given coded data sheets to record BG values as well as a numbered glucometer. The participants measured their BG using the glucometer twice. If the 1st and 2nd measurements were not within 10 mg/dL, the participants performed a third measurement. The two closest values within were

averaged for the baseline glucose value. Immediately after determining baseline BG, participants consumed a breakfast of 2 slices of white bread followed by 250 ml apple juice measured in a graduated cylinder, providing a total of 256 kcal, 60 gm carbohydrate, 4 gm protein, 0 gm fat, and < 2 gm fiber within 15 min. They sat quietly and repeated BG measurements at 15, 30, 60, 90 and 120 min post consumption. Investigators recorded BG values on participant data sheets to ensure accuracy. Investigators collected data sheets at the end of the data collection period; participants had recorded their data sheet code in their cell phones so they could be given the correct data sheet on day 2 of data collection.

On day 2 of data collection (7 days later), participants arrived again at 8am on campus and measured their fasting BG as described earlier using the same glucometer as day 1. Immediately after obtaining their baseline BG value, they consumed the breakfast described for day 1 with the addition of 2 tbsp. of Fibersol-2 in the apple juice which added 10 gm of fiber. Participants sat quietly and measured BG according to the protocol followed on day 1.

Data Analysis

Glucose incremental area under the curve (iAUC) was calculated for each participant using the trapezoidal method for both days of data collection. All statistical analyses were completed using IBM SPSS Statistics software.[34] Descriptive statistics were performed on the demographic, anthropometric, BG measurements, and iAUC. Paired t-test was used to determine any differences in baseline, 15, 30, 60, 90, and 120 min BG values, as well as the magnitude of the spike in BG (peak-baseline) and iAUC between the control and treatment days. Chi-Square was used to determine if there was a difference in the time that peak BG was achieved between the control vs added resistant starch day.

Results

Participant Characteristics

Twenty-one young adults comprising of 18 females and 3 males, age 24.1± 3.5 completed the study. Fifteen were Hispanic, five were non-Hispanic Caucasian, and one was African American. See Table 1 for a summary of participant characteristics.

Effect of Added Resistant Starch on BG Spike and iAUC

There was no significant difference in the spike in BG (peak minus baseline) between the control and added resistant starch days (52.6±22.7 vs 52.0±20.6mg/dL, respectively). There was no significant difference in (iAUC) between the two groups. The peaking of BG occurred later on the resistant starch day than on the control day. Forty-three percent of the participants in the control group peaked at 15 minutes postprandial compared to 24% in the treatment group; 43% in the control peaked at 30min compared to 53% in the treatment group; and 14% peaked at 60 min in the control group vs 24 % in the treatment group. Although the peak appeared to be delayed by the resistant starch, there was no significant difference in timing of the peak between the control and treatment day. See Table 2 for a comparison of BG parameters and iAUC, and Figures 1, 2, and 3 for graphic representation.

Table 1. Participant Characteristics

Variable	Mean ± SD *(N=21)*	Range
Age (yrs)	23.8±3.3	20.0-33.0
Height (cm)	162.8±7.6	153.0-183.1
Weight (kg)	70.1±16.0	51.1-112.8
BMI[2]	26.5±5.6	19.4-39.5

[1]BMI = Body Mass Index; weight in kilograms divided by height in meters squared

Table 2. Blood Glucose Indicators on Control and Treatment Days[1]

Variable	Control *(N=21)*	Added Resistant Starch *(N=21)*
Fasting BG[2] (mg/dL)	88.8±9.2	88.1±10.7
15 min postprandial	130.0±21.1	121.5±19.0
30 min postprandial	128.9±27.2	134.0±21.5
60 min postprandial	112.9±29.0	119.0±23.6
90 min postprandial	96.4±23.7	102.9±18.9
120 min postprandial	89.4 ±10.5	89.4±15.1
Peak–Baseline[3] (mg/dL)	52.6 ± 22.7	52.0 ± 20.6
iAUC (mg/dL/120 min)[4]	137.2±78.9	164.0±83.2

[1]There were no significant difference for any variable between the treatment and control days
[2]BG = Blood Glucose
[3]The difference between Peak BG and Baseline BG (spike)
[4]iAUC = Incremental Area Under the Glucose Curve

Figure 1. Comparison of Peak-Baseline BG on Control vs. Treatment Day

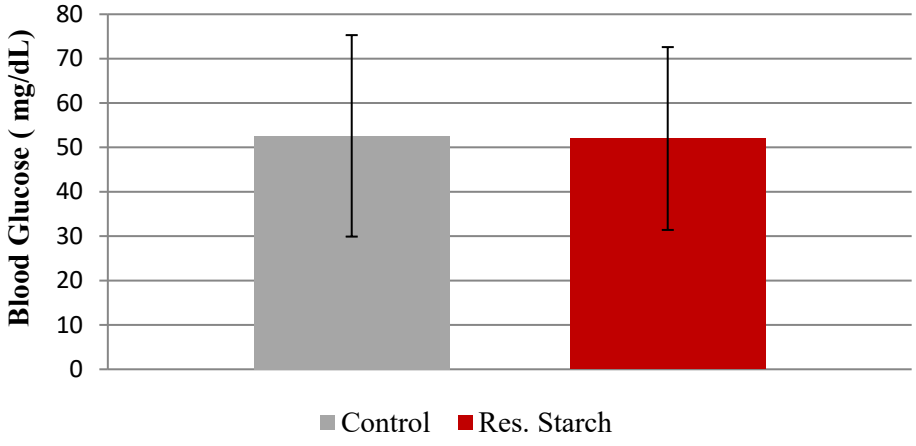


Figure 2. Glycemic Response on Control vs. Treatment Day

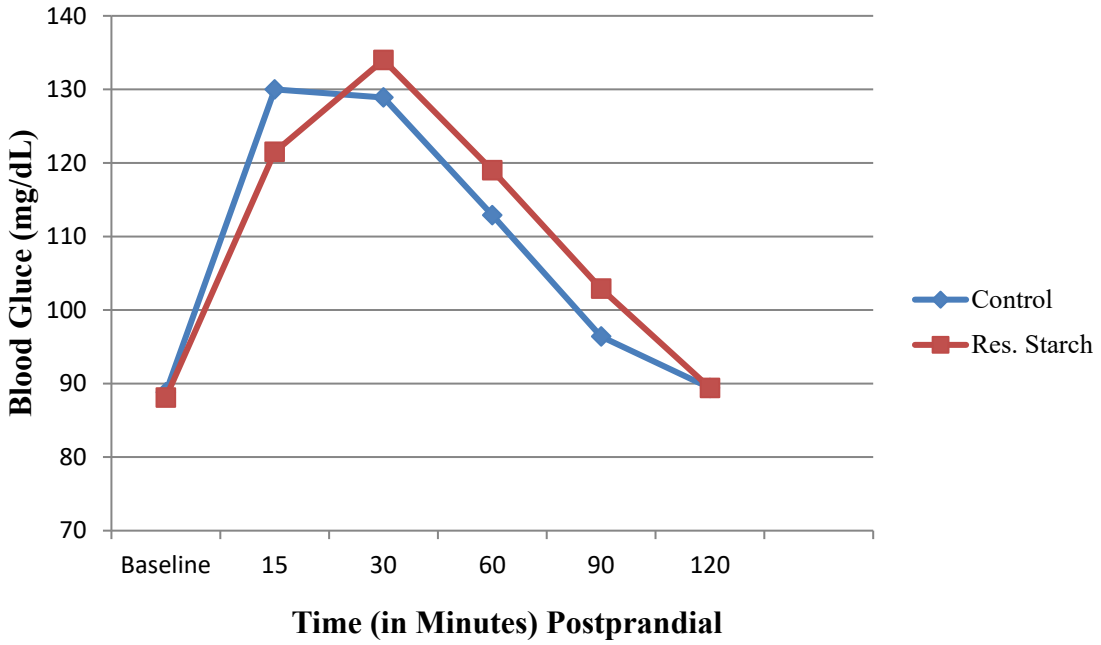
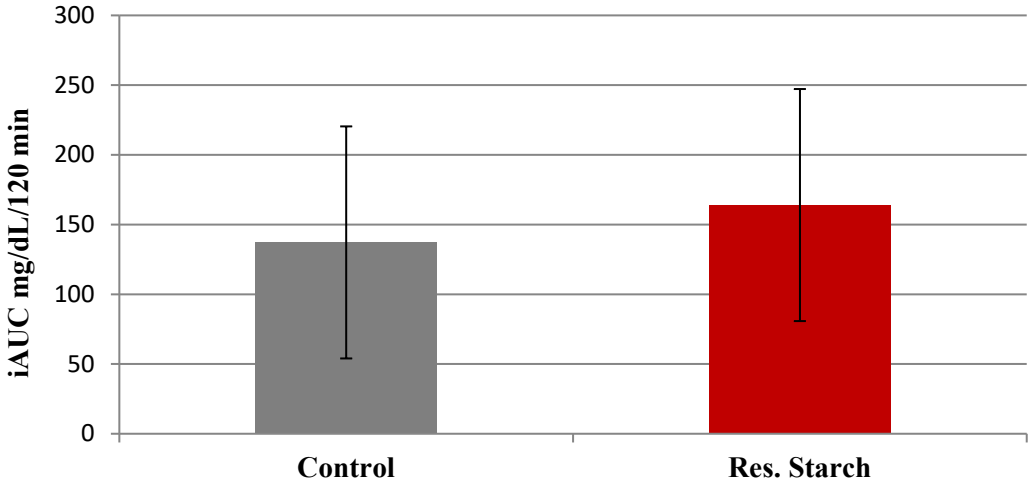


Figure 3. Comparison of iAUC on Control vs. Res. Starch



Discussion and Conclusion

The findings of this study are in disagreement with some studies[28-32] which did indicate a favorable outcome of resistant starch on the glycemic effect of a meal. In the present study, participants ingested a little more resistant starch in their beverage compared to the amounts used in previously published studies.[28-32] Fibersol-2 was used in four studies. In the first experiment, Fibersol-2 significantly lowered postprandial blood glucose levels after 30 minutes in 30 healthy subjects who drank green tea containing 6.6 g of the resistant starch after a meal (302.8 kcal; 103.6 g carb, 7.4 g protein, 2.2 g fat). Additionally, researchers also observed a significant decrease in insulin levels.[28] Wakabayashi et al., found a decreasing affect in blood glucose levels at 30 minutes after 28 participants drank 7 g of Fibersol-2 in their coffee with their meal of a sweet roll and bean jam (553 kcal; 114 g carb, 13 g protein, 5 g fat).[31] In another study, research investigators saw an attenuation of postprandial blood glucose achieved after 30 and 60 minutes of consuming 5.1 g of Fibersol-2. The Fibersol-2 was added to a young barely leaf tea (8.9 kcal; 100 ml) after a meal of three rice balls (565 kcal; 122.3 g carb, 15 g protein, 1.8 g fat). Morita et al., incorporated 7.2 g of Fibersol-2 with100 g of yogurt for each subject (10 men, 11 women) along with a single dose meal that consisted of a pastry with jam filling and tea (633 kcal; 11.9 g protein, 5.5 g fat and 122.4 g carb). The treatment had a positive effect on both serum glucose and insulin levels. Serum glucose levels were significantly lower at 30, 60, and 120 minutes. Insulin levels were significantly lower at 60 minutes. The researchers reported having no subjects with fasting blood glucose >126 mg/dl or > 200mg/dl two hours after consumption of the meal. In comparison to the control group, a decrease in serum glucose levels was observed at 30, 60, and 120 minutes 30.

Lastly, Ito et al., reported an attenuation of BG levels at 30 minutes after RS treatment in

one group. Before receiving the treatment meal, these 22 individuals were identified as having blood glucose levels > 144mg/dl post ingestion of the control meal.[29] The second group, whose BG levels were less than 144mg/dl at 30 minutes post ingestion of the control meal, showed an increase in BG levels at 60 minutes after Fibersol-2 treatment. Researchers mentioned there may have been a delayed effect of the resistant starch in the second group due to the amount of resistant starch being below the threshold needed to reduce the glycemic response of the second group.[29,32,35]

In the present study, it is believed that some of the starch from the resistant starch was absorbed, negating the possible beneficial effect. Also, the apple juice was consumed with the meal which may have influenced the timing of the ingestion of the resistant starch (before vs. during vs. after) and influenced the impact of resistant starch on the glycemic response.[32,35] Additionally, other researchers have experimented with different types and amounts of resistant starch. Studies using varying types and amounts of resistant starch have produced different results. In example, other studies used varying types and amounts of added resistant starch.

Behall et al., examined positive effects in adding soluble fiber and resistant starch on the glycemic response in both 10 overweight and 10 normal-weight females (avg. BMI 30.4, 22.0 kg/m2, respectively). Two days prior and the day of sample collection, the control meal contained 55% carbohydrate, 15% protein, and 30% fat. To assess energy intake, body weight was also factored in in order for subjects to have the same amount of energy throughout all meals during the entire study. Meals consisted of 1g carbohydrate/kg with either glucose alone or the treatment meals with muffins made with three different amounts (0.3, 0.9, or 3.7 g β-glucan/100 g muffin) of beta- glucans and three different quantities of amylose-cornstarch (0.9, 3.4, or 6.5 g/100 g muffin).[32] There were no significant differences in amount of resistant starch and fiber

between the two groups (overweight vs. normal weight). The higher amount of treatment, a greater AUC reduction was seen. The greatest significant decrease in AUC was observed in subjects who consumed their meals with both high amylose cornstarch and high beta-glucan amounts (33 and 59%, respectively). The researchers concluded beta-glucans and resistant starch could be used as effective additives on the glycemic response and can be attained through a healthy diet.[32] Although the study mentioned above showed significant AUCs within subjects consuming the resistant starch with the meal, in the present study, there was no significant difference in overall AUCs or between the groups when subjects ingested the resistant starch treatment after their meal.

In interpreting the results of the present study, limitations must be considered. The study included a low participant number, limiting generalization of results, as was the case in similar studies. The results of this study cannot be generalized beyond the healthy, young adult population. In addition, due to the type of food consumed, the study was not able to blind the intervention (resistant starch) from the control. There was no inspection of the cup participants drank from to ensure that the entire resistant starch supplement was consumed and no residue was left in the cup.

Ideally, venous blood samples assayed in a standardized laboratory would have been used; in this study glucometers were used. There were also variations in time between control and resistant starch treatment days. Most participants completed the control and resistant starch intervention days seven days apart, and all completed both days of data collection within one month. There was also a lack of randomization of timing. All participants performed control first and intervention second, which could introduce a possible ordering bias.

Glucose levels are simultaneously influenced by several factors to include absorption,

clearance, and release from internal organs which need to be carefully controlled in order to produce reliable and valid results. Additional research is required to further establish the health outcomes of various types of resistant starch, as well as the mechanism/s of the effect of resistant starch consumption on glycemic control.

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Appendix
Institutional Review Board Approval Form

9/7/2016

UNIVERSITY OF THE INCARNATE WORD

Cynthia Heiss
4301 Broadway, CPO 311 San Antonio, TX 78209

Dear Cynthia:

Your request to conduct the study titled *Effect of Fiber supplementation on the glycemic effect of a high carbohydrate breakfast* was approved by expedited review on 9/7/2016. Your IRB approval number is 16-09-002. Any written communication with potential subjects or subjects must be approved and include the IRB approval number.

Please keep in mind these additional IRB requirements:

- This approval is for one year from the date of the IRB approval.
- Request for continuing review must be completed for projects extending past one year. Use the **IRB Continuation/Completion form**.
- Changes in protocol procedures must be approved by the IRB prior to implementation except when necessary to eliminate apparent immediate hazards to the subjects. Use the **Protocol Revision and Amendment form**.
- Any unanticipated problems involving risks to subjects or others must be reported immediately.

Approved protocols are filed by their number. Please refer to this number when communicating about this protocol.

Approval may be suspended or terminated if there is evidence of a) noncompliance with federal regulations or university policy or b) any aberration from the current, approved protocol.

Congratulations and best wishes for successful completion of your research. If you need any assistance, please contact the UIW IRB representative for your college/school or the Office of Research Development.

Sincerely,

Ana Wandless-Hagendorf, PhD, CPRA

Ana Wandless-Hagendorf, PhD,
CPRA Research Officer
University of the Incarnate Word IRB

Code

Consent to Participate in a Research Study
Effect of added fiber on the glycemic effect of a high carbohydrate breakfast

You are being asked to participate in a research study conducted by Cindy Heiss, PhD, RD, LD and her graduate student, Sofia Maragoudakis. The purpose of this study is to determine if added fiber will blunt the blood glucose response to a high carbohydrate breakfast. We will also use data collected from this study to determine if the presence of risk factors for type 2 diabetes and activity level are associated with fasting blood glucose and the glycemic response to a high carbohydrate meal.

For this study, you will complete a screening questionnaire determine eligibility for the study. To be eligible to participate in the study, you must be a healthy adult over age 18, with no diseases or disorders that could affect blood glucose levels, who doesn't smoke. You must not be taking any medications which affect blood glucose. You must also be a non-pregnant, over 110lb, with no allergies or intolerances to gluten (which is in the white bread) or peanuts and must be willing to consume 2 pieces of white bread, 1 cup apple juice, and on test days, either 1 Tbsp of a fiber supplement called Just Better™, which is derived from corn, or 2 Tbsp of smooth peanut butter. You must be willing to fast for at least 8 hours, but no longer than 12 hours, prior to data collection. In addition, we will ask you to provide a list of medications you are taking before you begin the study so we can determine that you are not on any medications that affect blood glucose.

You understand that your participation in the study involves the following procedures:

You will be instructed on how to measure blood glucose using the glucometer provided for this study. The measurement includes wiping a finger with an alcohol pad, then allowing the finger to dry. A special lance is then used to prick the finger for a small drop of blood. There will be a "pin- prick" sensation that may result in some slight discomfort. A glucose test strip is placed at the finger where the small drop of blood appears, then placed in the glucometer for determination of blood glucose level. The finger is then wiped again with the alcohol pad. Lancets, glucose test strips, and alcohol pads will be disposed of in a sharps container. Trained technicians and trained personnel will be available to help with the blood glucose measurement if needed.

Each day of data collection will require no more than three hours of your time, and you will report to Bonilla Science Hall, room 316, on the UIW campus between 8 and 9am for each data collection day. Body measurements (height, weight, and waist circumference) will be done in a private area, but other subjects will be in the same room for breakfast and blood glucose measurements. Thus, although all of your data will be confidential, your participation in the study will not be anonymous.

On day 1 of data collection, you will fill out a questionnaire that included demographic information, such as age, gender, ethnicity, and family history of diabetes. Your height, weight, and waist circumference will be measured. You will be given a data collection sheet with a code. Since the investigators will collect your data sheets at the end of each data collection day, you will need to record this code in your cell phone or on a card to keep in your wallet so we can give you the correct data collection form on the second and third days of data collection. You will measure

your blood glucose using a glucometer twice. If the two measures are not within 10mg/dl, you will measure a 3rd time. You will record all measurements on a form provided to you. After obtaining your fasting blood glucose, you will eat two pieces of white bread and drink 1 cup of applejuice.
Then you will sit quietly and measure your blood glucose at 15, 30, 60, 90 and 120 minutes after you finished breakfast. You will use a timer on your smartphone if you have one to time the measurements, or a timer will be provided for you. You will then give your data collection sheet to the investigator.

On day 2 of data collection, you will again measure your fasting blood glucose as described earlier, then consume two pieces of white bread, drink 1 cup of apple juice with 1 Tbsp Just Better™ fiber supplement OR two pieces of white bread with 2 Tbsp peanut butter and 1 cup of apple juice within 15 minutes. Immediately after consumption, you sit quietly for the remainder of the data collection. You will measure your blood glucose at 15, 30, 60, 90 and 120 minutes

On day 3 of data collection, you will again measure your fasting blood glucose as described earlier, then consume the breakfast described in day 2 (added peanut butter or fiber) that you did not consume on day 2. You will measure your blood glucose at 15, 30, 60, 90 and 120 minutes after you finished your breakfast. You will then give your data collection sheet to the investigator.

As compensation for completing this study, you will receive a $50 gift certificate upon completion of day 3 of data collection.

A possible benefit of this study is to see if eating a protein+fat and consuming fiber can blunt the effect of glucose in response to carbohydrate intake. If this is the case, health benefits, including reduced inflammation (which has the potential of reducing the risk of many chronic diseases) and prevention of swings in blood glucose could occur. It is also important to determine if someone with risk factors for type 2 diabetes but does not have the disease has a greater blood glucose response to a meal than those who don't have risk factors so possible interventions can be implemented that would reduce problems associated with elevated blood glucose.

We will input the data from your questionnaires into a spreadsheet for analysis. Your name will not be included in the data spreadsheet, only your study "code number." All data will be confidential, and no copies will be maintained by the study investigator.

Your identity will be protected and any publication that follows this study will only display data of groups, not of individuals.

Participation is voluntary and you have the right to refuse participation without penalty of any kind. You have the right, at the end of the study, to be informed of the findings of this study.

If you have questions, please ask them at any time. If you have additional questions later or you wish to report a problem that may be related to this study, contact:

Cindy Heiss, PhD, RD, LD Phone: 210-829-6354 email: heiss@uiwtx.edu

To contact the University of the Incarnate Word committee that reviews and approves research with human subjects, the Institutional Review Board (IRB), and ask any questions about your rights as a research participant, call: UIW IRB, Office of Research Development (210) 805-3036.

If you completely understand the expectations and rights of participants in this

study, all of your questions have been answered to your satisfaction, and you are willing to participate in this study please sign and date this consent form in the space provided. To sign this consent form, you must be 18-years-old or older by today's date.

_____	_____	_____
Participant Name (printed)	Participant signature	Date Signed

Name_____ Email_

Screening Questionnaire

Effect of fiber or protein+fat supplementation on the glycemic effect of a high carbohydrate breakfast

Please answer the following questions by circling the appropriate answer.

1. Are you over the age of 18? Yes No
2. Are you pregnant or breastfeeding? Yes No
3. Do you weigh at least 110 pounds? Yes No
4. Are you in general good health? Yes No
5. Do you smoke? Yes No
6. Are you on any prescription medications that you know affect your blood sugar?

Yes No

7. Do you have any medical disorder that can affect your blood glucose? Yes No
8. Do you have any allergies or intolerances that would make you unable to consume white bread which contains gluten, Just Better™ fiber supplement (which is derived from corn), or peanut butter?

Yes No

9. Are you willing to consume 2 slices of white bread and applie juice on three occasions?

Yes No

10. Are you willing to fast for at least 8 hours prior to data collection, which will occur on 3 separate days?

    Yes No

Please email this completed form back to Sofia Maragoudakis (maragoud@student.uiwtx.edu) and she will contact you to let you know if you qualify for the study and give you further instructions. You will need to sign an Informed Consent From prior to participation.