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Effects of Soybean Pectin on Blood Glucose and Insulin Responses in Healthy Men

Melissa Ann Jones
University of Arkansas, Fayetteville

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Melissa Jones
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Dr. Sun-Ok Lee
Thesis Director

Dr. Jamie Baum
Committee Member

Dr. Suresh Kumar Thallapuranam
Committee Member

ABSTRACT

The goal of this study was to examine the effects of soy pectin on postprandial plasma glucose and insulin responses in healthy adult males. A second goal was to analyze the subjects' intestinal microbiota to determine if differences in weight or body mass index would be linked to differences in microbiota populations. Pectin is a soluble fiber with demonstrated health benefits such as the ability to lower blood glucose and cholesterol, increase satiety leading to lower caloric intake, improve insulin resistance and lower inflammation. These benefits suggest that pectin may help in prevention and treatment of type II diabetes. Soy pectin is extracted from hulls, a waste product of soy processing in the food industry. Using a randomized-crossover design, thirty male subjects consumed two treatments (control glucose solution and glucose solution with added soy pectin) with a one-week washout period between the treatments. Baseline blood samples were collected 15 minutes prior to consumption and at 0, 15, 30, 45, 60, 75, 90, 120, and 180 minutes. Fecal samples were collected from 16 subjects (5 overweight/obese and 11 normal weight) to identify their gut microbiota profiles. Soy pectin reduced the postprandial plasma glucose and insulin levels. The mean incremental area under the curve (iAUC) responses of plasma glucose and insulin were reduced by ~10.4% and ~19.4%, respectively. With soy pectin, the insulin response was significantly lower at 30 minute ($P<0.05$). None of the time points for plasma glucose were statistically significant. Analysis of results based on subject BMI showed a greater lowering of glucose and insulin response in normal weight subjects than in overweight and obese subjects. Microbiota sequencing demonstrated an increase in the ratio of *Bacteroidetes* to *Firmicutes* in normal weight subjects compared to overweight subjects. Results suggest that soy pectin has potential to assist in improving plasma glucose and insulin concentrations.

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INTRODUCTION

According to the United States Centers for Disease Control and Prevention (CDC), in 2012, 9.3% of the US population of all ages had diabetes and 37% was prediabetic (CDC, 2014). This is a dramatic increase over previous years and has been attributed poor diet and insufficient physical activity (Hu 2011). There are three main types of diabetes: type one, which is a genetic immune related disease, type two, which is caused by environmental factors as well genetic susceptibility and finally, gestational diabetes which affects pregnant women (NIH, 2015). All three types of diabetes carry an increased risk for many serious complications like heart disease, blindness and kidney failure (Brownlee 2001).

Studies show that changes in diet and exercise can help control diabetes and lower the need for medication (Lindstrom et al. 2006; Knowler et al. 2002; Tuomilehto et al. 2001; Franscato et al. 2014). Diets high in fiber have been shown to improve glycemic control (Lindstrom et al. 2006; McIntosh 2001) and reduce the risk and incidence of diabetes (Anderson et al. 2009; de Munter et al. 2007; Meyer et al. 2000). Soluble fiber specifically has been linked to improved insulin resistance (Anderson et al. 2009; Erkkila and Lichtenstein 2006; Marlett et al. 2002). Further, soluble fiber lowers plasma lipids, cholesterol, and glucose concentration (Braaten et al. 1994; Kim et al. 2009; Liatis et al. 2009).

Pectin is a soluble fiber found in the cell wall of plants. Pectin can have varied chemical properties from one plant source to another, such as differing water solubility and gel forming capacity as well as a differing speed or ease fermentation in the human intestine (Caffall and Mohnen 2009). Soy pectin, however, has received little attention, despite the low expense and high shelf stability of soy hulls from which it is extracted (Crandall and McCain 2002).

The goal of this study is to examine the effects of soy pectin on blood glucose and insulin responses in healthy adult males. The hypothesis of the study is that soy pectin will reduce the postprandial glucose and insulin responses as compared with a control. The objectives are (1) to compare how the glucose and insulin responses of healthy males after consumption of glucose solution with added soy pectin differs from the response of the same subject to a control glucose solution and (2) to identify and quantify the composition of gut microbiota between normal weight and overweight/obese subjects.

LITERATURE REVIEW

Diabetes

Incidence and prevalence of diabetes in the United States has been increasing rapidly in the last 30 years. This steady increase has been documented in the National Health and Nutrition Examination Survey (NHANES) reports. The NHANES from 1988-1994 as reported by Harris et al. (1998) showed that 5.1% of US adults $\geq$ 20 years of age were diagnosed with diabetes. Prevalence of undiagnosed diabetes was 2.7%, and the impaired fasting glucose (IFG) was 6.9%. IFG is a fasting blood glucose level between 110 and 126 mg/dL. Taken together, this means 14.7% of US adults had some manner of hyperglycemia (Harris et al. 1998). When Cowie et al. (2009) prepared a report with the data collected from the NHANES from 2005-2006, these numbers had all increased. In this new report, US adults $\geq$ 20 years of age with diagnosed diabetes had risen to 7.7%. The rate of undiagnosed diabetes was 5.2% and that of impaired fasting glucose was 25.9%. The parameters for IFG, however, had been lowered to the range of 100 to < 126 mg/dL (Cowie et al. 2009). The latest data in the US shows another increase in all these statistics.

By 2014, the CDC reported that 9.3% of US adults had been diagnosed with diabetes. This increase in diabetes occurred in both sexes, all age groups and all races. As in the NHANES reports, the incidence of diabetes increased with age and was greatest in persons of African, Hispanic, and Native American ethnicities. While all areas of the US are affected, the impact differs from region to region, with the southeastern parts of the country being particularly affected (CDC 2014).

This increase in not just limited to the US. In 2010, Shaw and colleagues published a large scale epidemiological study of 91 countries (Shaw et al. 2010). This data was used not only to estimate the prevalence of diabetes worldwide in 2010, but also to create trends from the statistics in order to predict prevalence of diabetes across the globe in 2030. The data was grouped together

by geographical location and predicted the greatest increases in T2DM would be in economically developing countries or areas, such as the Middle East, as opposed to developed countries like the US. While there is still an increase predicted, the change is not anticipated to be as large as that in countries that are showing an increase in per capita incomes or gross domestic products. (Shaw et al. 2010).

Again, these increases have been demonstrated in both sexes, across all age groups and in all ethnicities, and are even demonstrated in children under age 14 (Cowie et al. 2009; NCHS, 2013; Fagot-Campagna 2000). This increase has been attributed to cultural changes such as an increasingly calorie rich but nutritionally poor diet, and a global shift toward less physical activity (Hu 2011). Studies have also implicated increased stress and urbanization worldwide as a contributing factor (Zimmet 2001; Shaw et al. 2010).

In healthy subjects, beta cells in the pancreas produce insulin in response to high glucose concentration in the blood stream. Insulin stimulates cells to absorb glucose, reducing the glucose concentration in the blood. Diabetes occurs when either the pancreas does not produce insulin as in T1DM, or the body cannot make appropriate use the insulin it makes, as in T2DM. In T1DM the body destroys the insulin-producing pancreatic beta cells in an auto-immune response. This eliminates the body's ability to produce its own insulin, making it dependent on injections of exogenous insulin to maintain an appropriate concentration of glucose in the blood. In T2DM, cells no longer respond appropriately to insulin, and therefore don't absorb glucose from the bloodstream. In the first stage of T2DM, called insulin resistance, the beta cells simply release more insulin to compensate for this reduced absorption. Eventually, however, the beta cells fail to keep up with the increased need and the body progresses from insulin resistance to prediabetes

(also called impaired glucose tolerance) and finally to overt diabetes (Defronzo and Ferrannini 1991; Fonseca 2009).

Chronic high levels of blood glucose (also called glucose toxicity) create an environment of oxidative stress (Robertson 2006). People with all types of diabetes have an increased risk for blindness, neuropathy, end-stage renal disease, amputations, heart attack, stroke, and early death (Brownlee 2001). Studies have shown that lifestyle changes such as a more balanced diet and increased exercise improve blood glucose control (Lindstrom et al. 2006; Tuomilehto 2001; Pan et al. 1997). While these studies deal primarily with T2DM, there is evidence that exercise has an inverse relationship with complications due to T1DM as well (Caffall and Mohnen 2009; Burani and Longo 2006). The Diabetes Prevention Program found that lifestyle intervention alone was better able to reduce diabetes incidence than Metformin, an anti-diabetic drug (Knowler et al. 2002). Further, in the 10 year follow-up to this study, intensive lifestyle change reduced incidence of diabetes by 34%, but Metformin only reduced incidence 18% compared to placebo (Bray et al. 2009). While lifestyle change can involve both improved nutrition and increased exercise, dietary changes alone, such as increased dietary fiber have shown benefit for the prevention and management of diabetes (McIntosh 2001).

Fiber and Diabetes

Dietary fiber (DF) consists of the portions of plant material (fruit, vegetables, and grains) which are non-digestible to humans (Gropper, 2009). This includes cellulose, hemicelluloses, pectin, lignin, gums, β-glucans, fructans, and resistant starches. DF is further delineated into soluble and insoluble fiber. Insoluble fibers include lignin, cellulose and some hemicelluloses. Soluble fiber includes pectin, gums, β-glucans, and some hemicelluloses. Enzymes in the human

alimentary system are not able to break down the bonds (e.g. the α1-4 galacturonic acid bond of pectin's structure) that link together these carbohydrate chains. The fiber is therefore passed undigested through the stomach and small intestine into the large intestine (Gropper, 2009).

Both soluble and insoluble dietary fibers move from the small intestine to the colon where the fiber is fermented to varying degrees by intestinal microbiota (Gropper, 2009). The most readily fermented fibers are fructans, pectin, gums and resistant starch. Some cellulose and hemicelluloses are also fermented but at a much slower rate. Fermentation produces lactate and short chain fatty-acids (SCFS), the most common being butyrate, propionate and acetate. These products are absorbed into the body and can be metabolized (Gropper, 2009).

Unlike insoluble fiber, soluble fiber has the capacity to form thick viscous solutions when dissolved in water (Gropper, 2009). This gel slows down the progress of ingested material through the digestive tract. As a consequence, soluble fiber increases a feeling of satiety or ' fullness ' for a longer period than insoluble fiber. Further, the gel-like matrix inhibits interaction between food and digestive enzymes (Gropper, 2009).

Diets high in DF have been demonstrated to reduce the risk of diabetes. While the American Diabetes Association (ADA) recommends 25 to 35 grams of fiber each day (ADA, 2012), the average American consumes less than half that (Anderson et al. 2009). In the meta-analysis, Anderson and colleagues (2009) found several health benefits of higher fiber diets, including improved cardiovascular health, better gastrointestinal function and health, lower prevalence of obesity as well as a lower risk and better management of T2DM. This study found similar benefits for fiber in the diets of children. This study did not differentiate between the sources of DF and included data from studies involving fiber from fruits, vegetables, legumes, and whole grains as well as fiber from dietary supplements (Anderson et al. 2009).

De Munter and colleagues (2007) conducted a meta-analysis of several studies including the Nurses Health Surveys one and two. This study correlated the data of 150,000 women in their 20s through 60s. They found a 21% reduction in risk of T2DM for every 2 serving per day increase in whole grain consumption (de Munter et al. 2007). They further reported that the high fiber germ portion of grain showed a similar inverse relationship, but the low fiber germ portion did not (de Munter et al. 2007).

Meyer and colleagues (2000) collected data from 35,988 post-menopausal women in Iowa. The subjects were mailed 16 page questionnaires and asked to report on lifestyle factors including physical activity, cigarette smoking and diet. Those who were free of diabetes at baseline were given 3 follow-up questionnaires over 6 years. In this study, in keeping with de Munter's meta-analysis, intake of DF was shown to have an inverse relationship with incidence of diabetes. Women in the highest quintile of DF were 22% less likely to develop diabetes. As with the meta-analysis, Meyer et al. (2000) found that cereal grains rather than fruits and veggies were responsible for this reduction in risk. The authors mentioned that absorption of carbohydrates from cereal grain is different in milled, whole grain meal compared with intact, un-milled grains (such as those in granola) which are more resistant or inaccessible to digestive enzymes. However, while the study made a point to distinguish between the whole grains and refined grains, it did not separate whole grain meal from intact grains. Further, no similar distinction was made with regard to the processing of fruits and vegetables. In other words, a serving of canned green beans were evaluated as the same as a serving of raw carrots (Meyer et al. 2000).

A study by Schultze and colleagues (2007) used data collected by the European Prospective Investigation into Cancer and Nutrition to do a meta-analysis of incidence of diabetes related to types of fiber. As with the other studies, they found an inverse relationship between cereal fiber

and diabetes, but found no statistical difference between soluble and insoluble fiber. The authors did note that the intake of soluble fiber was much lower than that of insoluble fiber and theorized that the soluble fiber intake was too low to have a statistically evident impact on the risk of diabetes (Schultze et al. 2007).

Chandalia et al. (2000) conducted a randomized control study to show the effect of DF intake of 50 g of DF with 50% from soluble fiber. This study showed improvement over the ADA diet with respect to several indicators of glycemic control in subjects with T2DM. For example, subjects on a the higher fiber diet had lower mean plasma glucose concentrations (8.9% less) in addition to 10% lower daily plasma glucose levels. Similarly, plasma insulin concentrations were 12% lower (Chandalia et al. 2000).

Marlett et al. (2002) in a paper elucidating the position of the American Dietetic Association on dietary fiber, asserted that even modest increases in fruits and vegetables would bring most Americans in line with the recommended daily intake of DF (adding both soluble and insoluble). The authors stated that this would have a dramatic effect on the impact and management of T2DM and other related diseases for North American adults (Marlett et al. 2002).

An increase in soluble fiber specifically has been shown to increase glycemic control, decrease plasma lipids, and decrease hyperinsulinemia (Anderson et al. 2009). Studies have also demonstrated its ability to improve insulin resistance (Erkkila et al. 2006; Marlett et al. 2002). Brennan focused on soluble fiber's ability to change the structure and viscosity of food which contributes to the regulation of postprandial glucose and insulin levels (Brennan 2005). Beta glucan, a soluble fiber present in oats and barley, has received much attention for this trait. In addition to its ability to lower cholesterol when consumed with high carbohydrate meals, beta glucan also increased viscosity of the food matrix in the intestine, reducing absorption of

carbohydrates, resulting in a 50% reduction of the glycemic peak (Wursch and PiSunyer, 1997). Indeed, many studies have linked diets high in beta-glucan to increased regulation of postprandial glucose levels and improved insulin resistance (Braaten et al. 1994; Kim et al. 2009; Liatis et al. 2009). Even moderate amounts (3 g) have displayed a beneficial effect on glycemic control (Kabir et al. 2002). Inulin is another soluble fiber found in rhizomes and roots such as chicory and has also been studied to in order to determine its impact on glycemic control. While some studies have been less than conclusive (Raninen et al. 2011), others have had more positive results (Jackson et al. 1999; Tarini and Wolever, 2010), confirming its effects on postprandial insulin response and consequent reduced risk of T2DM.

Pectin

Pectins are soluble fibers and make up a class of polysaccharides which are found in the primary cell walls of plants. The main back bone of pectin is a chain of α 1-4 linked D-galacturonic acid units. Other sugar units such as rhamnose, arabinose, fructose, glucose, galactose and zylose may be attached as side chains.

The main distinguishing characteristics of pectin are its water solubility and gel-forming capacity. These characteristics differ depending on the moiety attached to the carboxyl group on carbon number 6 of the galacturonic acid unit. Pectins with a large number of units which are methoxylated at this position will have a lower affinity to cross–link, to form gels, and thereby trap water. These pectins require the presence of acid, high temperature and sugar to form gels. Low methoxylated pectins have a negative charge due to the carboxylic acid in this same C6 position and are more likely to interact with other areas of the polymeric chain (cross-linking). These pectins form gels at low temperatures with the addition of ions such as Ca^{2+} and have more

capacity for ion exchange (Cafall and Mohnen, 2009). This variation in structure has an impact on its capacity to exert health benefits (Sanchez et al. 2008).

Pectin can come from many diverse sources such as citrus fruit, apple and grape pomace, as well as legumes, nuts and some vegetables. Most commercial pectins are derived from citrus and apple processing, but researchers are attempting to find novel sources. Studies have tested health benefits of pectin from seaweed (Goni et al. 2002), sugar beet (Peng et al. 2013; Dongowski and Plass 1998), potato (Schwab et al. 2006, Laerke et al 2007), corn cob (Hazan and Madar, 1993), passiflora fruit (de Queiroz et al. 2012, Silva, et al. 2011), and prickly pear (Wolfram et al. 2002). Not all of these attempts were successful (e.g. potato, sugar-beet). Others might not present a practical resource in the US (e.g passiflora).

Pectin and Health Effects

Studies with pectin have demonstrated the soluble fiber's potential for the treatment and prevention of diabetes and blood lipid concentrations. Weinstock and Levine reported that when compared with certain other fibers, guar gum and pectin were more effective at lowering blood glucose and cholesterol levels (Weinstock and Levine, 1988). Jakobstottir and colleagues found that three fiber treatments (pectin, guar gum, and a mix of both) lowered liver fat, cholesterol and triglycerides in rats fed both a low fat diet (LFD, 12% of daily intake) and a high fat diet (HFD, 51% daily intake). Pectin maintained its effect over time as well, lowering liver weight, fat, cholesterol, and triglycerides significantly over six weeks (Jakobstottir et al. 2013). Kim demonstrated that citrus pectin's ability to increase viscosity leads to lower intestinal absorption of glucose (Kim 2005). Sanchez further tied increased viscosity due to high methoxy apple pectin to decreased blood glucose, triglycerides, and cholesterol (Sanchez et al. 2008). Viscosity of pectin

also slows gastric emptying (Dilorenzo et al. 1988), which means subjects feel fuller longer, leading to lower intake of food and therefore total calories subsequent to pectin ingestion (Savastano et al. 2014). This characteristic is thought to be part of the mechanism that makes pectin more effective than other soluble fibers at reducing weight gain (Sanchez et al. 2011; Titgemeyer et al. 1991; Silva et al. 2011). Pectin from edible seaweeds also has been shown to inhibit the digestion of starch, leading to lower absorption of glucose (Goni et al. 2002).

Recent research with pectin has focused its effect on inflammatory markers (Sanchez et al. 2011; Silva et al. 2011). Diabetes has a demonstrated connection to inflammation, leading it to be studied and discussed as an inflammatory disease. Apple pectin as well as pectin from passion flower fruit have been shown to reduce inflammatory markers such as TNF-α, and myeloperoxidase (Sanchez et al. 2011; Silva et al. 2011). Sanchez and colleagues (2011) fed 10 Zucker fatty rats with a high fat diet (HFD) plus 10% apple pectin ad libitum for 15 weeks. The rats displayed a trend (but not significant difference) for lower plasma levels of TNF-α than a control group of rats fed the same HFD with no added pectin. The pectin fed rats had significantly lower levels (~0.75 μmol/mL) of plasma malondialdehyde (MDA), an indicator of oxidative stress associated with T2DM, compared to the HFD control group (~3.0 μmol/mL).The level in the lean/healthy control group was ~0.50 μmol/ml, which was not statistically different from the pectin fed rats (Sanchez et al. 2011). In the study by Silva et al. (2011), pectin derived from passion flower fruit reduced the release of myeloperoxidase (MPO), a biomarker of inflammation from human neutrophils. This reduction occurred in a dose-dependent manner, and rats receiving higher doses (50 and 100 mg/kg) were not significantly different from a group treated with indomethacin, an anti-inflammatory medication. The animals also had swelling induced by injecting carrageenan into the right hind paw. Compared to positive control, animals pretreated with 10 mg/kg of pectin

had reduced TNF-α and inducible nitric oxide (iNOS) in the inflamed tissues. The reduction of MPO, TNF-α, and iNOS demonstrate the anti-inflammatory role of pectin (Silva et al. 2011).

Intestinal Microbiota

The term 'microbiota' refers to the population of microorganisms that live in the human alimentary tract or gut. There are roughly 10 times more cells in the human body belonging to the microbiota than human cells (O'Hara and Shanahan 2006). The largest number populate the distal gut (Gill et al. 2006). These organisms live either in the lumen of the intestine or in the thin layer of epithelial tissue. Human feces contains organisms which have sloughed off the epithelial layer as well as the species which reside in the lumen. Most of these bacteria are considered commensal species which exist in a symbiosis, bringing benefit rather than harm to the human host. The human microbiome has received much attention and there are attempts to create extensive metagenomic gene references of the human gastrointestinal (GI) tract microbiota (Gill et al. 2006, Eckburg et al. 2005, Qin et al. 2012). The main benefit gained from this relationship is the bacterial breakdown of DFs which pass through the stomach undigested by the host. The end products of this break down are short chain fatty acids (SCFA), the most common being propionic, acetic, and butyric acids. Propionic acid has been shown to lower cholesterol levels, and acetic acid is reabsorbed and moves into the liver and muscles to be used as an energy source. Butyric acid is used as an energy source for colonocytes and to induce colonic cell differentiation (Wong et al. 2006).

Peng and colleagues (2013), sought to determine if different DF sources could influence changes in the species type or number of the gut microbiota and therefore, the SCFAs produced as well. The study included 16 groups of mice with 6% DF from either pectin, resistant starch (RS), fructooligosaccharide (FOS), cellulose or a mixture thereof. The mice were fed these diets for 4 weeks and then the entire contents of the intestinal tract were extracted to analyze the total gut

microbiota and SCFA production. The microbiota DNA was purified, 16S rRNA was amplified by PCR, and these rRNA fragments were digested by a restriction enzyme (Hha 1). This procedure produced fragments of the rRNA of different lengths depending on the number and spacing of sites where Hha 1 can cleave the fragment. A larger number of species in the microbiota would therefor produce larger variation in the fragment sizes produced by the enzyme digestion. The largest variety of fragment sizes, and by implication, the largest diversity of microbiota came from mixtures of DFs. Further, the largest production of SCFA came from the mixtures of DFs as well. This study focused on weight loss or maintenance as an indicator of health, and all the test groups gained significantly less body weight than the control diet, possibly helping to demonstrate that gut microbiota can be changed through DFs and these changes can be made to improve the health of the host organism (Peng et al. 2013).

Eckburg et al. (2005) attempted to map the whole intestinal microbiota. This study was conducted on 3 human subjects who were participating in another study. The researchers were able to obtain mucosal tissue from these subjects who were undergoing colonoscopies. The hypothesis was that the microbiota would vary from different anatomical cites within the intestine. Therefore, tissue was obtained from the cecum, ascending, transverse, descending and sigmoid colon as well as the rectum. Stool samples were also collected one month after the colonoscopies. These researchers sequenced the cloned 16S rRNA from each of these tissues separately to assemble a census of the resident species and their preferred sites for colonization. Several archaeal sequences were found, however most of the species sequenced were bacterial phylotypes from the *Firmicutes* and *Bacteroidetes* phyla. The *Firmicutes* sequences made up the largest portion of bacteria with most sequences (95%) belonging to the *Clostridia* class. There were fewer *Bacteroidetes* phylotypes, but these were more varied than the *Firmicutes*. However, the variance in species

between mucosal sites was not as large as anticipated. There were small pockets of bacterial colonies which contained a subset of the overall population of enzymes. However this 'patchiness' was not enough to make the overall makeup of the mucosa less homogenous. In addition, though the population from the stool samples and that of the mucosa was different, it was not enough to be significant. For instance, the percentage of *Bacteroidetes* found in the three subjects' mucosal samples were approximately 30%, 23%, and 10% lower than the percentages found in the subjects' stool samples, but none of these was reported as a statistically significant difference (Eckburg et al. 2005).

Microbiota and Diabetes

Serino and colleagues (2012) demonstrated that the microbiota of a diabetic animal would be different than that of a healthy animal. The study was performed with 100 male C57BL/6 (in-bred and genetically sequenced) mice fed a high-fat diet (72% of daily energy intake) for 3 months. After 3 months, the mice were tested for diabetes and separated into diabetic (HFD-D) and diabetic resistant (HFD-DR) groups. Despite the mice being genetic clones and ingesting the same diet, some developed diabetes and others did not. A group of 10 mice were fed the same high-fat diet plus a supplement of glucooligosaccharide (HFD-GOS). Glucooligosaccharide, a known prebiotic, was given at 10% of daily energy intake to simulate the influence of dietary fiber on the microbiota. The microbiota of the two phenotypes was different. The HFD-D mice had a higher percentage than HFD-DR mice of *Deferribacteres*, S24-7 (a family in the order *Bacterodales*) and *Mucispirillum* with lower counts of *Lachnospiraceae, Coreiobacteriaceae, Oscillibacter, Olsenella* and *helicobacter*. However, the HFD-D mice also had increased *Bacteroides*, a contradiction with the study of Ley et al. (2005). The authors explained this difference by noting

that the diet used in the experiment was specifically intended to induce diabetes without obesity. In fact, the mice did not change body weight over the course of the study period. The mice in the HFD-GOS group developed a distinct microbiota. The mice in this group developed the highest percentage of *Bacteroidetes* with very few *Firmicutes* and almost no *Actinobacteria*. This group also developed *Proteobacteria* at the expense of *Deferrbacteres*. There was a very large increase in the S24-7 family, and in *Parabacteroides*. These distinct microbiota indicated that supplementing the diet with dietary fiber had an impact on the composition of the gut population.

Further, the authors measured biochemical markers of inflammation such as TNF-α, IL-6, PAI-1. The HFD-D mice showed the highest cytokine concentration, fasted lipopolysaccharide (LPS), as well as cellular permeability. Added dietary fiber (HFD-GOS group) lessened liver weight and visceral fat. The HFD-GOS mice had improved gut permeability, plasma cytokines concentrations, and inflammatory markers that were not statistically significant from the HDF-DR mice. Additionally, the HFD-GOS mice had significantly lower LPS and TNF-α levels than the HDF-DR mice. This research would indicate that the gut microbiota was distinct for diabetic versus diabetic resistant mice. Further, it demonstrated the effect of diet, specifically dietary fiber, on gut microbiota and the expression of diabetes (Serino et al. 2012).

In a similar study, Cani et al. (2008) investigated C57bl6/J mice and sequenced their microbiota. A treatment group was fed a high fat diet (HF, 72% of calories from fat) to induce diabetes, and a control group (CT) was given a standard diet. Broad spectrum antibiotics were given to half of the group receiving the high fat diet (HF-Ab) and half of the control group (CT-Ab). After four weeks, markers indicating diabetes were measured for all groups. These markers including plasma endotoxin, intestinal permeability, inflammation (TNF-a, IL-1 and PAI-1 levels), oxidative stress, macrophage infiltration, plasma glucose and insulin, and changes in adipose

tissues (visceral and subcutaneous). Further, a group of *ob/ob* mice (genetically susceptible to obesity) were fed a high fat diet to induce glucose intolerance and half were treated with antibiotics as with the other two groups. Gut microbiota for 8 mice from each group was sequenced. While only three specific bacteria were sequenced (*Lactobacillus, Bifidobacterium*, and *Bacteroides-Prevotella*), the denaturing gradient gel electrophoresis (DGGE) analysis showed the dramatic reduction of bacteria after antibiotic treatment. The mice were also tested for signs of inflammation and oxidative stress as well as gut permeability. As expected, the mice on the control diet showed very little evidence of metabolic distress. The antibiotic-treated control mice showed no significant difference from the non-antibiotic control mice in any parameter. However, the HF group showed much higher levels plasma endotoxin, intestinal permeability, TNF-α, IL-1 and other markers of metabolic distress. The HF group treated with antibiotics, however, measured at levels close to the control groups (Cani et al. 2008). This indicates that microbiota play a role in the treatment or management of metabolic distress and diabetes.

A study by Vijay-Kumar et al. (2010) induced metabolic distress in mice which produced insulin resistance, obesity, hepatic steatosis, hyperglycemia and hyperlipidemia. This stressed state was used to mimic the insulin resistance and inflammation that leads to or is present in T2DM in humans. This study used mice lacking the toll-like receptor 5 (TLR5) gene to bring about the diseased state. The TLR5 gene codes for a receptor protein which is part of the innate immune system and stimulates several inflammatory responses when activated by pathogenic bacteria. At 20 weeks of age, mice lacking this TLR5 gene (T5KO) had increased body weight and fat mass, triglycerides, blood pressure, cholesterol and proinflammatory cytokines in adipose tissue when compared to the wild-type (WT) control mice. TKO5 mice also demonstrated impaired response to glucose challenge and reduced response to exogenous glucose. These mice were not given a

high fat diet, but displayed hyperphagia. Diet restriction eliminated some of the metabolic abnormalities, but did not reduce insulin resistance, indicating calorie restriction was not the only change that was needed. After the T5KO mice were given broad spectrum antibiotics, eliminating 90% of the bacteria in the gut, the metabolic distress was reduced. Fasting blood glucose, food intake, weight gain, were all reduced to levels of the control, WT mice. The gut microbiota from the T5KO mice was then transplanted to the gut of the WT mice. This, in turn caused the WT mice to develop insulin resistance, hyperglycemia, obesity, inflammation, and other markers of T5KO induced metabolic stress (Vijay-Kumar et al. 2010).

These studies demonstrate that diet causes change in intestinal microbiota, and links this change to the subsequent metabolic distress. Antibiotic treatment drastically reduced diversity, essentially razing the intestinal environment and mitigated the severity of diet-induced distress so that the biomarkers of the antibiotic group closely resembled that of the control group. In this way, poor diet appears to bring about T2DM and other metabolic disorders by causing changes in the gut microbiota.

Shin et al. (2014) conducted a similar study with mice on a high fat diet (HFD) or on a normal control diet (NCD). After 8 weeks, half of each group was treated with Metformin, an anti-diabetic drug (HFD-Met, and NCD-Met). After an additional 6 weeks, the HFD-Met mice showed improved glucose tolerance, which was independent of body weight and adiposity. The HFD-Met mice also had a change in the microbiota, with a specific increase in *Akkermansia muciniphila*. Interestingly, the NCD-Met mice had a similar shift of gut microbiota, but it was not as pronounced. The increase in *Akkermansia* correlated with improved epithelial cell health. This suggests that at least part of the anti-diabetic mechanism of Metformin results from a change in the microbiota (Shin et al. 2014).

Microbiota and pectin

Jakobstottir and colleagues (2013) fed a high fat diet (HFD, 51% of daily energy intake) or a low fat diet (LFD, 12% daily energy intake) to rats then given 3 fiber treatments (pectin, guar gum, and a mix of both) or a no fiber control diet. Pectin produced the highest concentration of acetic acid, a fermentation product by gut microbiota, in both HFD and LFD, although between weeks 4 and 6, this was not as pronounced. The guar gum fed mice had a higher level of *Bacteroides* in their ceca than the pectin group. The pectin fed mice showed a greater diversity in their microbiota, as evidenced by an increase in number of different fragment lengths obtained by terminal restriction fragment length polymorphism (T-RFLP), although the authors did not identify specific species which had increased. Pectin also had a greater reduction on the accumulation of fat in the liver. (Jakobstottir et al. 2013). This study showed pectin and its health effects with the interaction of pectin, gut microflora, and the production of SCFA.

MATERIALS AND METHODS

Materials

Soy pectin was obtained from Dr. Phillip Crandall, University of Arkansas (Crandall and McCain 2002). Crandall and McCain of the University of Arkansas have developed a novel process to extract the pectin from soybean hulls, a waste product generated in processing (Crandall and McCain 2002). Glucose solution (50g, orange flavor) was purchased from Azer Scientific (Morgantown, PA, USA). Reagents and chemicals for glucose determination were purchased through Alfa Wasserman Diagnostic Technologies, LLC (West Caldwell, NJ, USA).

Subjects and Study Design

The Institute of Research Board (IRB) at the University of Arkansas approved a human study (Protocol #: 12-10-147) to be conducted at the University of Arkansas Food Science Department. Subjects were recruited from the University of Arkansas and surrounding community (Fayetteville, Arkansas, USA) using flyers posted on public bulletin boards. One hundred and four male subjects were screened to confirm that they were non-smokers, with no diagnosed illness, not taking any medication and did not consume more than two alcohol servings per week. The screening also determined with at least two fasting blood glucose (FBG) readings that the subjects a normal, healthy blood glucose level (<100 mg/dL). Thirty subjects between the ages of 18 and 45 were chosen and signed the consent forms before the study began.

The study was conducted using a randomized crossover design. Subjects randomly underwent two weekend studies: one control weekend and one treatment weekend, with a wash-out period of one week in between. After a 10-hour overnight fasting period, subjects then consumed a control or a treatment solution within two minutes.

Treatment Preparation

Ten grams of soy pectin were dissolved in 50 mL warm water. This was added to the glucose solution (10 fluid ounces) containing 50 grams of glucose. The pectin solution was then stirred vigorously. The pectin solution was then left covered at room temperature overnight in order to further dissolve the pectin. Subjects were given 200 mL tap water to consume with the pectin solution on the experiment day. For the control, subjects simply consumed 10 oz. of prepared glucose solution with 250 mL of tap water in order to keep the amount of fluid equal for both treatments.

Food Frequency Questionnaires

Food Frequency Questionnaires (FFQ) were given to the subjects after the first week's treatment. These were then entered into Nutritionist Pro version 4.3.0 from Axxya Systems (Stattord, TX, USA) to create an overview of the nutrient content of the subjects' reported diet.

Blood Collection and Analysis

A lancet was used to prick the subjects' finger and approximately 0.4 mL of blood was collected into heparinized capillary tubes. This was performed at each of 10 time points. The first blood collection (time point -15, baseline) was taken as the subjects arrived. Subsequent collections took place immediately after drinking the glucose solution (time point 0), and again at 15, 30, 45, 60, 75, 90, 120, and 180 minutes after ingestion.

Blood was drained from the capillary tubes in to centrifuge tubes and spun at 7000 rpm for 10 minutes at 4°C with a Microfuge® 22R Centrifuge (Beckman Coulter, Inc., Brea, CA, USA). Plasma was then separated and stored at -20°C. Plasma insulin concentration was measured using an insulin ELISA kit from Mercodia (Uppsala, Sweden). Plasma glucose concentration was determined using an ACE Alera™ Clinical Analyzer (West Caldwell, NJ, USA). Incremental area under the curve (iAUC) was calculated using trapezoidal rule (Matthews et al. 1990).

Microbiota Analysis

Subjects were given Commode Specimen Collection System (Fisher Scientific, Waltham, MA) and instructions to obtain fecal samples at home. Samples brought into the lab were store at -18 °C. Whole bacterial DNA from fecal samples was purified using QIAamp Fast DNA Stool

Mini Kit (Qiagen, Gaithersburg, MD). DNA concentration and purity of the samples were measured using a NanoDrop ND1000 (Thermo Fisher Scientific) and diluted to 10 ng/uL with DNase-RNase free water. DNA sequencing based on 16S rRNA V3 region was performed using an Illumina MiSeq platform followed previous report developed by Kozich et al. (2013). Raw sequencing data were acquired from an Illumnia BaseSpace website and processed with a bioinformatics tool QIIME pipeline to analyze data included taxonomic level classification, alpha rarefaction and PCoA plots generation (Caporaso et al. 2010).

Statistical Analysis

All statistical analyses were performed using Statistical Analysis System (SAS, Release 9.4, SAS Institute, Inc. Cary, NC, USA). Values are expressed as means $\pm$ standard error of the mean (SEM). Significance of differences among mean values were computed using paired t-test, and were considered significant if $P<0.05$.

RESULTS & DISCUSSION

Subject Profile

After screening, 30 male subjects were selected. The average age was 25.8 $\pm$ 5.49 yrs. Average fasting blood glucose (FBG) was 88.8 $\pm$ 1.3 mg/dL, and the average body mass index (BMI) was 25.1 $\pm$ 0.9 kg/m^2. The average fasting blood glucose is in the healthy range (< 100 mg/dL), but the BMI is considered overweight/obese ($\geq$ 25). Nine subjects were overweight/obese, and 21 were normal weight. Of the 30 subjects, 4 were Asian, 13 Caucasian, 2 African, 6 Latino or Hispanic, and 5 were Asian from India.

Nutrient Intake

Table 1 shows total energy and macronutrient intakes from food frequency questionnaires. Using the Nutritionist Pro software, the mean energy intake per day was calculated at 3011.7 $\pm$ 30.6 calories. This is higher than the 2000-2600 recommended by the U.S. Department of Agriculture for sedentary adult males and even higher than the 2200-2800 recommended for active adult males. Daily protein and carbohydrates expressed as percentages of total calories were both within their recommended ranges of 10-35% and 45-65% respectively. Reported daily dietary fiber was 27.6 $\pm$ 3.0 grams per day. The recommended intake of dietary fiber for males (21-45yrs) is roughly 10 grams higher at 38 grams per day.

The mean intake of total fat was 36.1 $\pm$ 1.5% which is higher than the recommended 20-35%. Additionally, the recommended intake of saturated fat (based on a 2,500 calorie active adult male diet) is 25g, but the reported intake was 42.7 $\pm$ 5.69 grams, which is quite a bit higher. The USDA has begun recommending omega-6 and omega-3 fatty acids rather than monounsaturated and polyunsaturated fatty acids. However, in the present study, the mean reported intake of monounsaturated fat (47.1 $\pm$ 6.33 grams) and polyunsaturated fat (23.9 $\pm$ 2.25 grams) together (~ 71 grams) were much larger than the 55 grams recommended for daily non-saturated fat (based on a 2,500 calorie active adult male diet). Based on the food frequency questionnaire, subjects participating in this study had a diet low in fiber, but high in calories and fat.

Blood Glucose and iAUC

In the present study, soy pectin treatment showed a trend toward lower postprandial blood glucose concentration over three hours (Figure 1). There was no time point at which the difference was statistically significant. This was more apparent when the overweight/obese and normal

weight subjects were separated (Figure 2). There were 21 subjects with normal weight (BMI<25) and 9 subjects who were overweight/obese (BMI≥25). In the normal weight group, mean incremental change was significantly different at 30 min ($P<0.05$), but the overweight/obese group had no significant differences between soy pectin and control.

In table 2, the incremental area under the curve (iAUC) for blood glucose concentration was reduced by 10.4%. When subject data was divided based on BMI, the iAUC for subjects with a normal BMI, blood glucose was significantly reduced by 15.5% ($P<0.05$). However, the iAUC of blood glucose for subjects with an overweight/obese BMI was increased by 2.6%, but this was not statistically significant.

Our results are in line with current research which has shown that pectin has the ability to lower fasting blood glucose levels (Wolfram et al. 2002; de Queiroz et al. 2012). In the study by Wolfram et al. (2002), subjects were non-obese non-diabetic males who were either suffering from hypercholesterolemia (n=12) or hyperlipidemia (n=12). These two groups had blood drawn (time point 1), and maintained a diet self-controlled for consistent macronutrient content for 8 weeks. Blood draws were taken again (time point 2) and subjects were given 250 g/day of prickly pear pectin (*Oputina robusta*) to incorporate into 8 more weeks of standardized meals. Blood draws were taken again (time point 3) at the end of the pectin treatments. Fasting blood glucose at the last two time points (after treatment) showed reduction of 11% (Wolfram et al. 2002). De Queiroz and colleagues (2012) used the pectin from yellow passion fruit peel and saw a reduced fasting blood glucose level. This study involved 43 diabetic adults (15 males and 28 females) who were taking medication to control their diabetes. Subjects were given pectin to take as a diet supplement. Blood was taken before the pectin supplement time, after 30 days and again after 60 days. Fasting blood glucose was lowered by 14.6% after the 30 days of pectin supplementation, by 13.0% after

the second 30 days, and by 25.7% over the combined time periods. In each of these studies, a novel source of pectin was used to lower fasting blood glucose levels for unhealthy subjects over a treatment time of 8 weeks (de Queiroz et al. 2012).

Blood Insulin and iAUC

Soy pectin significantly lowered blood insulin response at 30 min compared to the control (Figure 3) ($P<0.05$). When the data from overweight/obese subjects were separated from the normal weight subjects, this difference is more significant (Figure 4). The normal weight group showed a significant difference at time points 30 and 90 minutes ($P<0.05$). Although there was no significant difference between soy pectin and control in the overweight/obese group, soy pectin tended to lower the blood insulin responses compared to control. There was a larger insulin response for the overweight/obese group (0-85 mU/L) than the normal weight group (0-53 mU/L). All subjects in this study were screened for healthy fasting blood glucose range. However, this increased insulin response coupled with the overweight/obese BMI could indicate a minor increase in insulin resistance.

The mean incremental area under the curve (iAUC) for blood insulin showed that when all subjects were combined (n=30), there was significantly reduced by 19.4% in the soy pectin group compared to the control (Table 3) ($P<0.05$). When we separate the data for the normal weight subjects from that of the overweight/obese subjects, this difference was increased. Normal weight subjects showed a significant difference of 23% ($P<0.05$), while the overweight/obese group had a non-significant difference of 12.2%.

The overweight/obese group (n=9) did not have a statistical significance in either the iAUC or any time point. Increased number of subjects in this group would possibly lead to a significant change.

The study by Wolfram et al. (2002) lowered blood insulin as well as glucose. As with the fasting blood glucose, blood insulin concentration was reduced by 11% after 8 weeks of treatment with prickly pear pectin (Wolfram et al. 2002). The study by de Queiroz et al. (2012) reported that there was no significant difference in blood insulin concentration between the three time points (before pectin treatment, at 30 days and 60 days of treatment) (de Queiroz et al. 2012).

Another human study (Ranganathan et al. 1994) tested pectin's effect on insulin on 6 healthy adult male subjects. After fasting overnight, subjects drank a solution of 50 g glucose + 30 g pectin or a solution of the 50 g glucose alone. Blood was drawn every 30 minutes after consumption. Mean insulin concentration in the blood was significantly lower with added pectin when compared to the control at time points 30, 60, and 90 minutes. The largest difference occurred at 30 minutes, when the blood insulin concentration of the control (~325 pmol/L) was more than twice that of the pectin group (~150 pmol/L) (Ranganathan et al. 1994). Incremental area under the curve was not reported.

In the animal study, Sanchez and colleagues (2008) found that feeding rats a diet including apple pectin lowered blood insulin levels to a level that was not statistically different than a lean genetic control group. In this study, Zucker fatty rats were fed a diet 10% high methoxylated apple pectin (HMAP), while a control group of Zucker fatty rats (FAT) and one of Zucker lean rats (LEAN) were fed diets with no added fiber. After 7 weeks, FAT rats had a fasting insulin concentration > 4 μg/L, while those on the HMAP rats had a concentration < 1 μg/L. Insulin levels of HMAP rats showed no statistical difference from the LEAN rats (< 1μg/L) (Sanchez et al. 2008).

Microbiota composition

Microbiota composition was analyzed with fecal samples from 16 subjects, 5 overweight/obese and 11 normal weight subjects. The data from sequencing the collected DNA was summarized in tables 4 and 5, with only the most populous phyla and genera listed. The lower populated categories (especially in the genus classifications) are quite numerous, but contain very few 'hits' or numbers of organisms and so were reported together as "other."

Some differences occurred between the microbiota of overweight/obese subjects and normal weight subjects. In the phylum level classifications, the overweight/obese group had reduced a *Bacteroidetes* percentage (~6.7%) and an increase in *Firmicutes* (~2.2%) and *Proteobacteria* (~3.8%) (Table 4 and Figure 5). In the genus level classifications, the overweight/obese group had ~8.5% fewer *Bacteroides*, ~2.2% fewer *Clostridium*, ~1.2% and fewer *Phascolartobacterium*. This group also had higher *Prevotella* (~8.6%) and *Rosburia* (~2.1%) than the normal weight group (Table 5 and Figure 6). Only slight differences in gut microbiota populations were seen between these groups. This could result from small subject numbers. However, all subjects were screened for healthy blood glucose levels, therefore the small difference is likely due to the relative health of all the subjects.

In this study, the percentage of the total fecal microbiota in the *Bacteroidetes* phyla was lower in the overweight/obese group (Table 4). The overweight/obese subjects (n=5), had and more *Firmicutes* and *Protebacteria* than the normal weight group. This was expected because a relationship between high *Firmicutes* percentage and obesity has been explored in other research (Ley et al. 2005; Turnbaugh et al. 2006, Tremaroli and Backhed 2012). Ley's study discovered that mice with high *Firmicutes* percentages would gain more weight than mice with a high *Bacteroidetes* percentage even if both were on identical diets. In contrast, a study by Duncan and

others (2008) found differences in the microbiota of overweight and normal weight subjects, but did not find a change in the proportion of these two phyla (Duncan 2008).

The percentage of *Proteobacteria* was larger in the overweight/obese group in this study (Table 4). Only one of the normal weight subjects had a *Proteobacteria* percentage over 2% and 5 had a percentage lower than 1%. However, one of the overweight/obese subjects had 11.84%, one had 7.34%, and one had 5.11%. The other two overweight/obese subjects had *Proteobacteria* percentages at 0.99% and 0.96%, which is similar to the normal weight subjects. Zhu et al. (2013) found a connection between obesity and non-alcoholic liver disease and an increase in *Proteobacteria*. The study was conducted with 63 male and female children and adolescents. These subjects were either healthy (n=16), obese (n=25), or had non-alcoholic steatohepatitis (NASH) (n=22). Gut microbiota sequenced and three distinct groups emerged, differing in the percentage of *Proteobacteria*. The healthy subjects had 0.87% *Proteobacteria*, the obese group had 3.3%, and the NASH group had 6.03%. In the NASH subjects, the increase came mostly in the form of *Escherichia*. The authors pointed out that the products formed by this group of bacteria would include alcohol, which would cause the damage leading to NASH. However, the bacteria that was most increased from the healthy subjects to the obese group was *Campylobacter*. Zhu's study was concerned primarily with NASH, however, so there was no discussion of which specific species was responsible for this change, nor was there a discussion of traits this group of bacteria might confer to the host (Zhu et al. 2013).

In the present study, the overweight group also had a higher percentage of *Prevotella* (Table 5). This genus has been found by De Filippo et al. (2010) to be more prevalent in children from Burkina Faso (BF) than in European (EU) children. Children of BF consumed diets much higher in dietary fiber and lower in animal fat and protein than the EU children. The authors found a

number of bacteria (*Xylanibacter*, *Prevotella*, *Butyrivibrio* and *Treponema*) that occurred only in the BF children. These bacteria are thought to work with intestinal enzymes to ferment xylan and cellulose. These bacteria are therefore instrumental in harvesting more energy from these fibers, gaining added caloric value from food consumed. It was anticipated that subjects in the present study with an increased *Prevotella* percentage might also have a higher typical intake of dietary fiber. However these subjects had an average fiber intake of approximately 21.6 g/day, which was lower that the group average of 27.6 g/day. If the high *Prevotella* population in these subjects is harvesting extra caloric energy from the dietary fiber they consume, as it was in the BF children, this may contribute to these subjects' higher than average weight.

Qin and colleagues conducted a meta-genome project, sequencing the microbiota of 368 Chinese volunteers. Subjects were screened for BMI, fasting blood glucose, blood pressure, fasting serum insulin, fasting serum C-peptide, glycosylated hemoglobin, triglycerides and cholesterol. The study a compiled profile of the gut microbiota for subjects with T2DM and a control profile of non-diabetic subjects.

Several of the bacteria in the present study were mentioned in Qin's study. *Faecalibacterium* and *Rosburia* were enhanced in the control group (Qin et al. 2012). *Faecalibacterium* was also higher in the normal weight group of the present study, however, the *Rosburia* were more abundant in the overweight/obese group (Table 5). The normal weight also had higher percentages of *Clostridium* and *Parabacteroides*, but this was in contrast to the study by Qin, where they were both more populous in the T2DM group.

Other genus level differences in gut microbiota populations in the present study were not significant. The lack of marked difference in microbiota could again be due to a lack of difference

in health status among the subjects, and larger study would surely draw more attention to changes. Research with specific gut microbiota and diets needs to be conducted for a clearer understanding.

CONCLUSION

Pectin from soy hulls has been demonstrated to lower postprandial plasma glucose concentration and subsequent insulin response in adult healthy males. Individual microbiota populations did not explain individual variances in subject glucose and insulin responses, however, these variances were not large enough to have an impact on the combined data. The microbiota of overweight/obese subjects had a higher *Firmicutes*:*Bacteroidetes* ratio than normal weight subjects and higher percentage of *Proteobacteria* and *Prevotella*.

This lowering of plasma glucose and insulin coupled with the low cost and availability of source material indicate that soy pectin may have potential as a value added food ingredient and more study in this area is warranted.

Table 1. Total Energy and Macronutrient Intake from Food Frequency Questionnaire

Calories (kcal)	3011.7 ± 30.6
Protein (%)	17.0 ± 0.9
Carbohydrates (%)	44.4 ± 2.2
Dietary fiber (g)	27.6 ± 3.0
Fat (%)	36.1 ± 1.5
Saturated fatty acid (g)	42.7 ± 5.7
Monounsaturated fatty acid (g)	47.1 ± 6.3
Polyunsaturated fatty acid (g)	23.9 ± 2.3

All values are means ± SEM (n=26)

Table 2. Mean Incremental Area under the Curve (iAUC) for Blood Glucose Responses

Glucose	Control (mg·(~3h)/dL)	Soy Pectin (mg·(~3h)/dL)	Difference
Combined	4896.5 ± 337.11	4387.94 ± 269.16	-10.40%
Normal weight (BMI< 25)	5028.04 ± 447.99	4249.02 ± 326.99	*-15.50%
Overweight/obese (BMI ≥25)	4589.58 ± 430.83	4712.08 ± 482.59	2.60%

All values are means ± SEM; * $P < 0.05$; Normal weight subjects, (n=21); Overweight/obese subjects, (n=9).

Table 3. Mean Incremental Area under the Curve (iAUC) for Blood Insulin Responses

Insulin	Control (μU·(~3h)/L)	Soy Pectin (μU·(~3h)/L)	Difference
Combined	2997.25 ± 410.60	2414.55 + 279.23	*-19.40%
Normal weight BMI< 25	2940.30 + 490.09	2264.57 + 297.43	*-23.0%
Overweight/obese BMI ≥25	3149.03 ± 603.89	2764.51 ± 635.81	-12.20%

All values are means ± SEM, * P < 0.05; Normal weight subjects, (n=21); Overweight/obese Subjects, (n=9)

Table 4. Microbiota Phyla

Phylum	All subjects (%)	Normal weight (BMI<25) (%)	Overweight/obese (BMI ≥ 25) (%)
Bacteroidetes	51.9 ± 3.7	54.2 ± 3.5	47.5 ± 8.7
Firmicutes	41.9 ± 3.1	41.1 ± 3.0	43.3 ± 7.8
Proteobacteria	2.7 ± 0.8	1.4 ± 0.2	5.2 ± 2.1
Verrucomicrobia	1.3 ± 0.6	1.3 ± 0.7	1.2 ± 1.1
Actinobacteria	1.1 ± 0.5	1.1 ± 0.6	1.3 ± 0.6
other	1.1 ± 0.3	0.9 ± 0.1	1.5 ± 1.0

Top 5 phyla out of 22 by percentage for all subjects (n=16); subjects with normal BMI i.e. less than 25 (n=11); and overweight/obese BMI i.e. greater than or equal to 25 (n=5). This table shows top 10 out of 27 classifications. The category 'other' includes all 17 less populous phyla including organisms unclassified at this level.

Table 5. Microbiota Genera

Genus	All subjects (%)	Normal weight BMI<25 (%)	Overweight/obese BMI≥ 25 (%)
Bacteroides	31.0 ± 4.7	34.9 ± 5.4	26.4 ± 9.3
Prevotella	8.1 ± 2.4	4.4 ± 1.6	13.0 ± 7.1
Faecalibacterium	8.0 ± 1.2	8.3 ± 1.6	7.6 ± 1.9
Ruminococcus	5.9 ± 0.8	6.1 ± 1.1	5.3 ± 1.0
Oscillospira	5.9 ± 1.0	6.0 ± 1.3	5.3 ± 1.7
Blautia	3.7 ± 0.6	3.7 ± 0.8	3.5± .05
Clostridium	2.9 ± 0.6	3.7 ± 0.8	1.5 ± 1.0
Roseburia	2.8 ± 0.7	2.1 ± 0.8	4.2 ± 1.4
Phascolarctobacterium	2.4 ± 0.6	2.8 ± 0.7	1.6 ± 0.9
Parabacteroides	2.1 ± 0.3	2.3 ± 0.4	1.9 ± 0.6
other	27.2 ± 2.9	25.7 ± 3.6	29.7 ± 3.5

Top 10 genera by percentage for all subjects (n=16), subjects with BMI less than 25 (n=11), and BMI greater than or equal to 25 (n=5). This table shows top 10 out of 341 classifications. The category of "Other" includes all 331 less populous genus classifications including those unclassified at genus level.

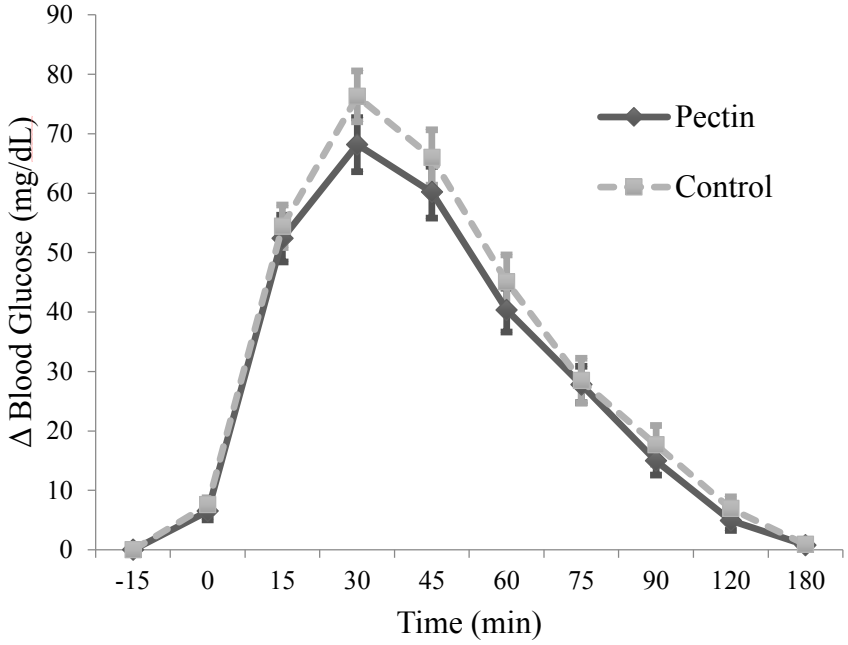

Figure 1. Mean Incremental Changes of Blood Glucose Concentration, in healthy men (n=30), means ± SEM

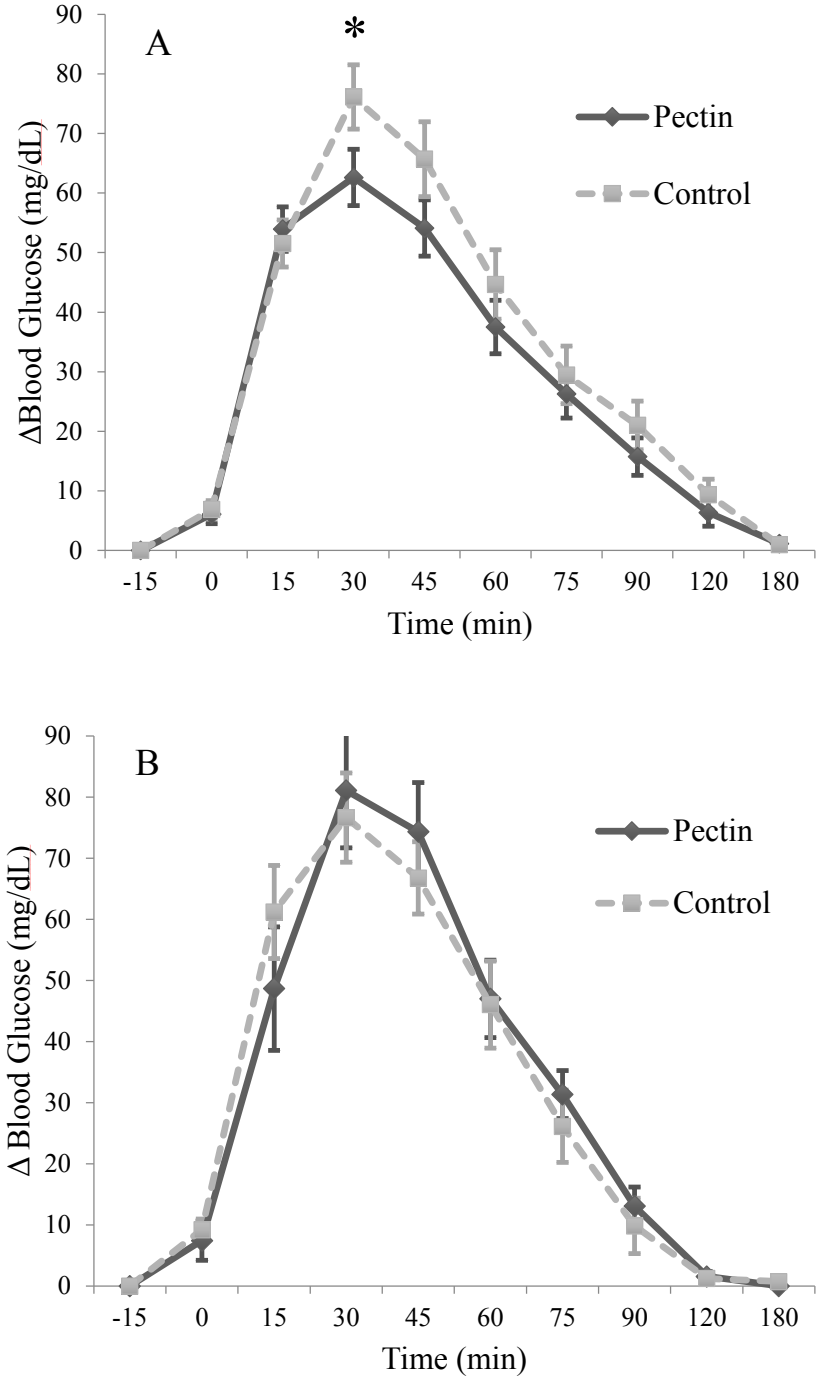

Figure 2. Mean Incremental Changes of Blood Glucose Concentration, A) Normal weight (BMI < 25) (n=21); B) Overweight/obese (BMI ≥ 25) (n=9); means ± SEM, *indicates significant difference ($P < 0.05$)

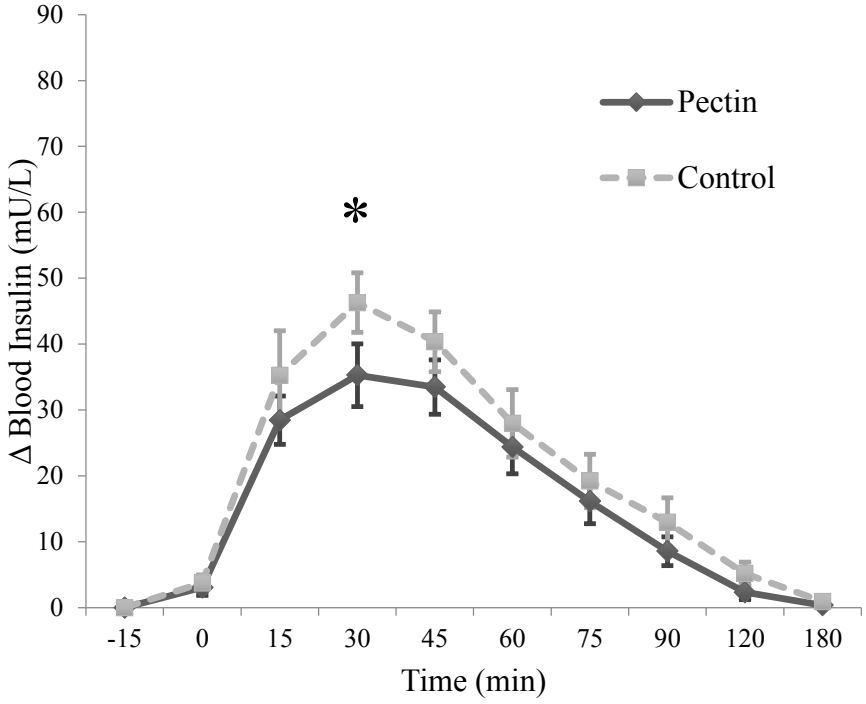

Figure 3. Mean Incremental Changes of Blood Insulin Concentration in healthy men, (n=30) means ± SEM * $P < 0.05$

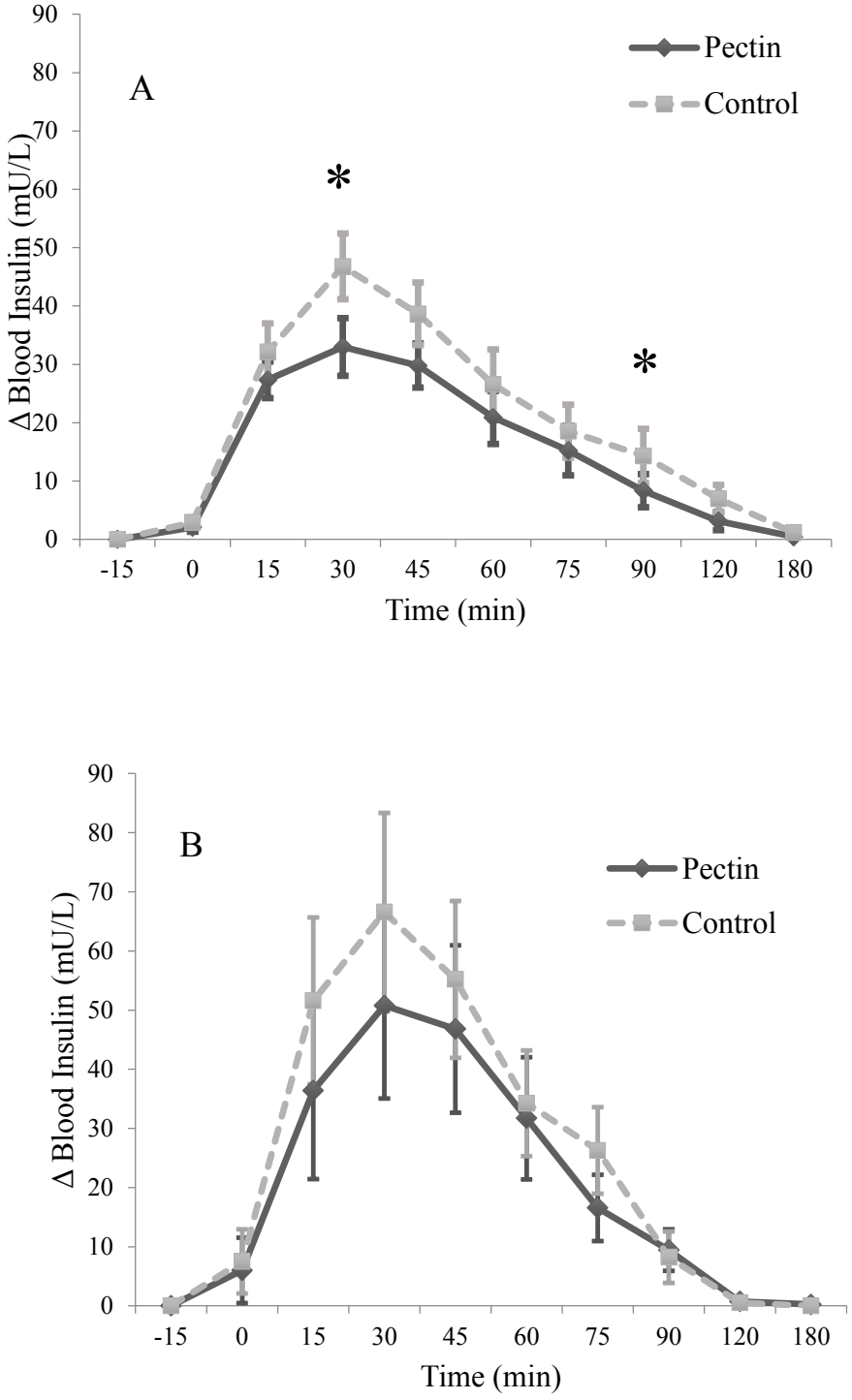

Figure 4. Mean Incremental Changes of Blood Insulin Concentration, A) Normal weight subjects (BMI< 25), (n=21); B) Overweight/obese Subjects (BMI ≥ 25), (n=9); means ± SEM; * $P < 0.05$

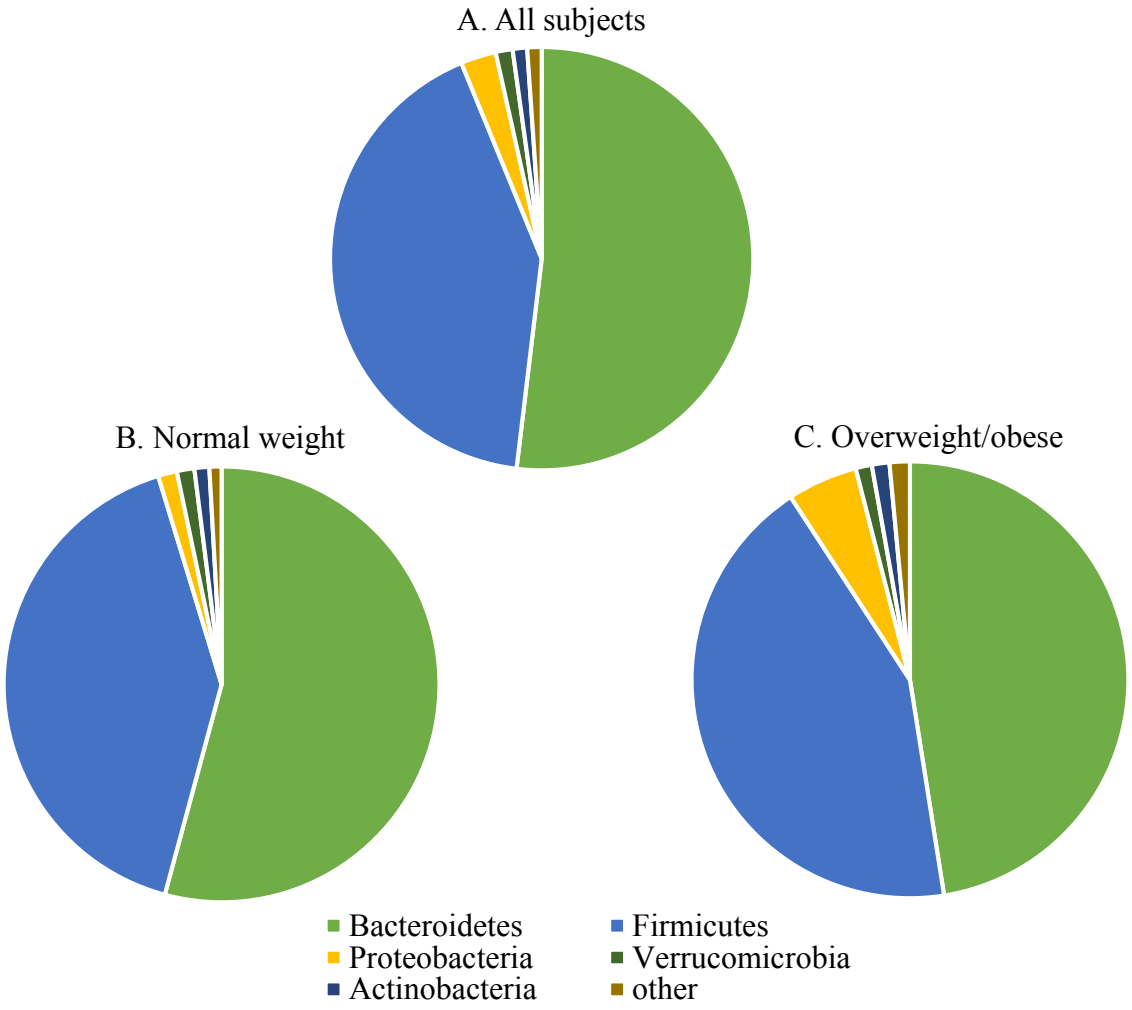

Figure 5. Top 5 Microbiota Phyla, A) All subjects (n=16); B) Normal weight (BMI < 25) (n=11); C) Overweight/obese (BMI ≥ 25) (n=5)

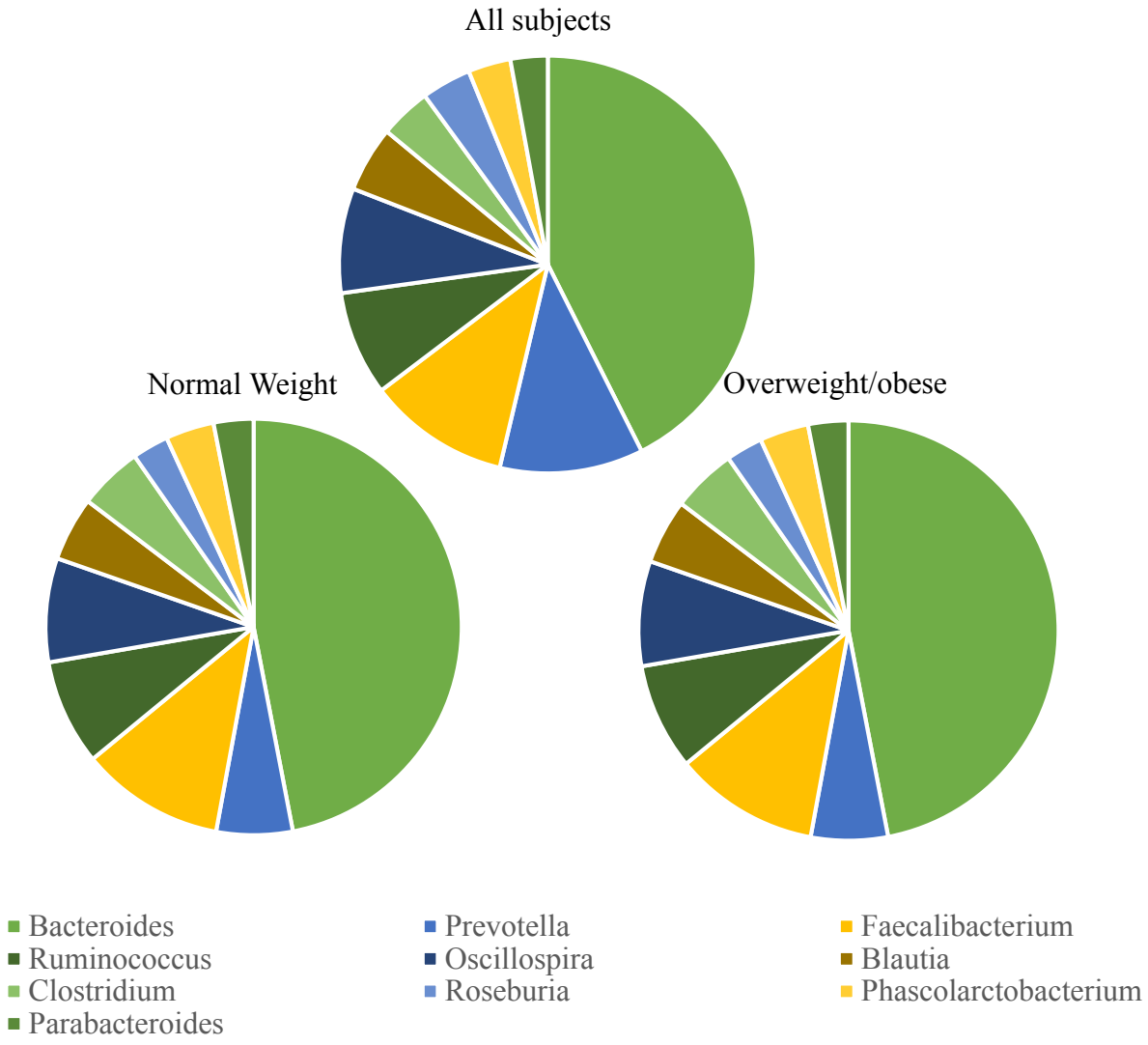

Figure 6. Top 10 Microbiota Genera, A) All subjects (n=16); B) Normal weight (BMI < 25) (n=11); C) Overweight/obese (BMI ≥ 25) (n=5)

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Appendix A:

Food Frequency Questionnaire

Name:_____Subject ID number _____
last, first

This questionnaire asks you about your eating patterns over the past week, which includes the time from exactly one week ago until the last meal you had before you fill out this questionnaire. For each food item listed, respond by indicating your usual intake of that food per day or week. Check "X" on the Day/Week column if you don't eat the food or if you have it once or twice a year. This questionnaire will take about 15 minutes to complete.

Description	Amt	Unit	Quantity	Day/ Week
Breads Cereals and Grain Products				
Whole grain breads (whole wheat, rye, pumpernickel)	1.00	slice		
White breads (burger/hot dog bun-1/2 item, French bread-1 slice)	1.00	serving		
English muffin, bagel, pita bread	0.50	item		
Whole grain crackers: Triscuits, Wheat Thins, etc. (4-6 each)	5.00	item		
Other crackers: Saltines, Ritz, etc. (4-6 each)	5.00	item		
Tortilla, corn, 6 inch diameter (medium)	1.00	item		
Muffins	1.00	item		
Pancakes (2), waffles (1-7 inch diameter)	1.00	serving		
Whole grain hot cereal: rolled oats, rolled wheat	0.50	cup		
Instant or quick hot cereal: cream of wheat, cream of rice	0.50	cup		
Cold cereals: shredded wheat, raisin bran, or bran flakes	0.75	cup		
Cold cereals: Frosted Flakes, Sugar Smacks, etc.	0.75	cup		
Rice, cooked	0.50	cup		
Pasta, cooked	0.50	cup		

Fruits and Juices				
Apple or pear, fresh, medium	1.00	item		
Banana, medium	1.00	item		
Orange (1 item) or grapefruit (1/2 item)	1.00	serving		
Peach (1), nectarine (1/2) or apricots (2)	1.00	serving		
Berries (in season)	0.75	cup		
Cantaloupe, medium (in season)	0.25	cup		
Other melon (watermelon, honeydew, casaba)	1.00	cup		
Pineapple, fresh	0.50	cup		
Dried fruits: raisins (2 Tbsp), dates (2), prunes (2), dried apricots (4)	0.25	cup		

Canned fruit or frozen fruit	0.50	cup		
Orange or grapefruit juice	0.50	cup		
Tomato juice or vegetable juice	0.50	cup		
Other juices: apple, grape, pineapple, or cranberry	0.50	cup		
Fruit drinks: lemonade, punch, Koolaid	0.50	cup		

Fats and Oils				
Vegetable oils: corn, safflower, soy, etc	1.00	Tbsp		
Olive oil	1.00	Tbsp		
Shortening	1.00	Tbsp		
Lard	1.00	Tbsp		
Margarine	1.00	tsp		
Butter	1.00	tsp		
Mayonnaise	1.00	Tbsp		
Regular salad dressings	1.00	Tbsp		
Low-calorie dressings	1.00	Tbsp		
Sour cream	1.00	Tbsp		
Cream cheese	1.00	Tbsp		
Half & Half, table cream	1.00	Tbsp		

Milk, Yogurt and Cheeses				
Skim milk or low fat milk	1.00	cup		
Whole milk	1.00	cup		
Chocolate milk	1.00	cup		
Yogurt	1.00	cup		
Cheese: cheddar, Colby, American, Monterey Jack, etc.	1.00	oz.		
Other cheeses: Swiss, mozzarella, ricotta, string, etc.	1.00	oz.		
Cottage cheese	0.50	cup		

Vegetables				
Salads: lettuce, celery, green peppers, onions	1.00	cup		
Dark green leafy vegetables, raw or cooked	0.50	cup		
Carrots, raw or cooked	0.50	cup		
Tomatoes, fresh, medium	1.00	item		
Starchy vegetables, cooked: corn, peas, mixed vegetables	0.50	cup		
Other vegetables, cooked: green beans, beets, zucchini	0.50	cup		
Cauliflower, broccoli, brussel sprouts, cabbage	0.50	cup		
Winter squash, cooked: acron, butternut, hubbard	0.50	cup		
White potato, baked, broiled, or mashed	1.00	item		
Sweet potatoes or yams, cooked	0.50	cup		

Beverages				

Cola drinks (1 can = 12 fl. oz)	12.00	fl.oz.		
Diet cola drinks (1 can = 12 fl. oz)	12.00	fl.oz.		
Non-cola drinks: 7-Up, Sprite, Slice, etc. (1 can/12 fl. oz)	12.00	fl.oz.		
Diet non-cola drinks (1 can = 12 fl. oz)	12.00	fl.oz.		
Coffee or tea (1 cup = 8 fl. oz)	8.00	fl.oz.		
Decaffeinated coffee or teas: Sanka, herbal tea, etc.	8.00	fl.oz.		
Hot chocolate or cocoa	1.00	cup		
Beer (1 can = 12 fl. oz)	12.00	fl.oz.		
Wine, dry or table (red, white, or blush)	4.00	fl.oz.		
Liquor: vokda, whiskey, gin, rum, etc.	1.50	fl.oz.		

Protein Foods				
Legumes: lentils, pinto beans, navy beans, cooked	1.00	cup		
Nuts and seeds: peanuts, almonds, sunflower seeds, etc.	0.25	cup		
Peanut butter, nut butters	1.00	Tbsp		
Tofu or other meat substitutes	3.00	oz.		
Beef: rib roast, steak, pot roast, veal, etc.	3.00	oz.		
Beef, ground, cooked	3.00	oz.		
Pork: chops, roast, ham	3.00	oz.		
Lamb: chops, roast	3.00	oz.		
Poultry: chicken, turkey, duck	3.00	oz.		
Fish, canned with oil: tuna, sardines	3.00	oz.		
Tuna, water packed	3.00	oz.		
Fish, fresh or frozen, no breading: trout, halibut, sole, etc.	3.00	oz.		
Shellfish: shrimp, scallops, lobster, clams	3.00	oz.		
Eggs, whole, large	1.00	item		
Egg substitutes or egg whites	0.25	cup		
Lunch meats: bologna, salami, etc.	1.00	item		
Frankfurters or sausage link (4 in x 1 1/8 in)	1.00	item		

Desserts and Sweets				
Cookies: chocolate chip, oatmeal, peanut butter, etc.	2.00	item		
Brownies, 2 in.	1.00	item		
Doughnut or sweet roll	1.00	item		
Cake, 1/12 of 9 in.	1.00	slice		
Granola bars (1 item) or granola (1/2 cup)	1.00	item		
Pie, 1/8 of whole pie	1.00	slice		
Gelatin, flavored	0.50	cup		
Pudding or custard	0.50	cup		
Ice Cream	0.50	cup		
Ice Milk	0.50	cup		
Sherbet	0.50	cup		

Candy bar, chocolate bar (1 bar), M&Ms (1 pkg.)	1.00	item		
Hard candy, gum drops, Lifesavers	1.00	item		

Miscellaneous Foods				
Fast food - pizza	1.00	slice		
Fast food - hamburger or cheeseburger	1.00	item		
Fast food - burrito or taco	1.00	item		
Bacon	2.00	slice		
Popcorn, popped	2.00	cup		
Potato chips, corn chips, tortilla chips	1.00	oz.		
Catsup or chili sauce	1.00	Tbsp		
Tomato based sauce (spaghetti sauce)	0.50	cup		
Pickles or pickle relish (1 Tbsp)	1.00	Tbsp		
Olives	5.00	item		
Sauces: soy sauce, steak sauce, barbeque sauce	1.00	Tbsp		
Brown gravy, giblet gravy, or white sauce	0.25	cup		
Soups, vegetable or noodle type	1.00	cup		
Soups, cream	1.00	cup		
Chewing gum	1.00	item		
Sugar, honey, jam, jelly, syrups	1.00	Tbsp		

Can you think of any other food or drink that you had in the past week that was not on this form? If so, what was it? What was the amount? How many times did you have this in the past week?

Food _____

Amount _____ How often? _____ per day, _____ per week

Food _____

Amount _____ How often? _____ per day, _____ per week

Food _____

Amount _____ How often? _____ per day, _____ per week

Food _____

Amount _____ How often? _____ per day, _____ per week

Appendix B:

IRB approval

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

April 21, 2014

MEMORANDUM

TO:	Sun-Ok Lee Melissa Jones Xuan Gu
FROM:	Ro Windwalker IRB Coordinator
RE:	New Protocol Approval
IRB Protocol #:	14-04-654
Protocol Title:	*Fermentation Patterns of Fibers from Soybean and Rice Bran on the Digestive Microbiota*
Review Type:	☐ EXEMPT ☒ EXPEDITED ☐ FULL IRB
Approved Project Period:	Start Date: 04/18/2014 Expiration Date: 04/17/2015

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 120 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.

210 Administration Building • 1 University of Arkansas • Fayetteville, AR 72701
Voice (479) 575-2208 • Fax (479) 575-3846 • Email irb@uark.edu