2007

Resistant starch does not reduce body fat in rats fed a high fat diet

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RESISTANT STARCH DOES NOT REDUCE BODY FAT IN RATS FED A HIGH FAT DIET

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Human Ecology

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August 2007

DEDICATION

This thesis is dedicated to my grandfather, Nanda Kishore Tripathy; my father, Ramesh Chandra Tripathy; and my father-in-law, Rama Chandra Das. It is because of their blessings that I am here today.

ACKNOWLEDGEMENTS

First and foremost, I offer my obeisance to the Goddess Maa Mangala for having bestowed her grace on me to complete this work.

My deep sense of reverence and gratitude to my committee chair, Dr. Michael J Keenan, for his sustained interest, meticulous guidance, constructive criticism, critical supervision, wise counsel, affectionate help, moral support and valuable suggestions shown during the period of my investigation, that made it possible to complete my master's degree successfully.

I am extremely thankful to my other committee members, Dr. Roy J Martin and Dr. Richard Tulley. Dr Martin is an outstanding motivator, listener, advisor, and educator. Dr. Tulley has been a positive motivator throughout my tenure at LSU. All of my professors have been role models to me and I thank them for my development as a graduate student.

I owe much gratitude to all of my lab members. I would like to thank especially, Kathy, Shayna, Anne, and Jun, without them this project could not have been done.

Thanks are due to my mother, grandmother, mother-in-law, Babulee, Chagala and Chagulu for their inspiration, everlasting affection and encouragement. I am thankful to my husband Siba, who is my true motivator and who makes me officially, the proudest and happiest wife. I love you very much.

In addition, I would like to especially thank Dr Maren Hegsted, Emily Whelan and Linda Silverman for their invaluable support during my first semester of study.

This research was supported by the LSU AgCenter's Biotechnology Education for Students and Teachers (BEST) and Hatch project 93678.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study examined the effects of resistant starch in a high fat diet (28% of energy) on body weight, abdominal fat, cecal weight and pH, serum gut peptide YY (PYY) and gene expression for PYY and proglucagon in male Sprague Dawley rats. Three groups of rats (8wk) were fed one of the following diets for ten wks: energy control (EC; 3.7 kcal/g), resistant starch (RS; 3.7 kcal/g) and fiber control (FC; 2.9 kcal/g). Results were classified as significant when $p<0.05$. The consumption of RS resulted in significant increases in cecal weight (full and empty), serum PYY, gene expression for PYY and proglucagon in cecum and significant decrease in pH in cecal contents compared to the other groups. However, the disemboweled body weight and abdominal fat for RS were not lower compared to the EC. In our previous studies, we observed that RS in a low fat diet (18% of energy), produced similar gut signaling, as in the current study, but reduced disemboweled body weight and abdominal fat compared to EC. The reason for the unexpected results needs further study. Since high fat diets have been shown to alter brain response to other satiety signals (leptin), a high fat diet might affect neuronal responses to PYY and GLP-1 signaling from the gut caused by dietary RS.

CHAPTER 1

INTRODUCTION

Obesity and overweight status continue to be leading public health concerns in the United States over several decades and its prevalence has been rapidly increasing in the United States, as well as other countries, over the past two decades (1,2). According to 2003-2004 data, 16% of US children and adolescents were at risk for overweight, 17.1% of US children and adolescents were overweight, 66.3% adults were overweight or obese and 32.2% adults were obese (2). Body mass index, expressed as weight/height^2 (BMI; kg/m^2), is commonly used to classify overweight status (BMI 25.0-29.9) and obesity (BMI greater than or equal to 30.0) among adults (age 20 years and over) (3). Obesity has been associated with a relative increase in diabetes, cardiovascular disease, various cancers, respiratory disorders during sleep, gallbladder disease, and osteoarthritis (4). Adverse health effects of obesity justify the need to discover effective treatments, among them possible dietary treatments.

It has been suggested that sufficient fiber in the diet will tend to prevent excessive food intake and fat accumulation by decreasing the caloric density of the diet, by slowing the rate of food ingestion, by increasing the effort involved in eating, by promoting intestinal satiety, and by interfering slightly with the efficiency of energy absorption (5). Resistant starches contain varying amounts of starch that resist amylase digestion in the small intestine and this resistant starch is fermented to short chain fatty acids (SCFA) by the microflora in the large intestine (6,7). When resistant starches are added to the diet, it is similar to adding fiber. Dietary resistant starches are reported to dilute caloric density, improve whole body insulin sensitivity, increase satiety, and increase lipid oxidation (8). Resistant starch in the diet may assist in the prevention and management of obesity via its potential effects on delaying the delivery of glucose as fuel

with subsequent fat utilization and appetite control benefits (9). Our research group has demonstrated reduced body fat in rats fed a low fat diet with resistant starch compared to rats fed an energy control diet (10).The results point to increased energy expenditure because energy intakes were not different.

In order to design effective dietary treatments, it is essential to understand the physiology of appetite control and the pathophysiology of obesity. According to the first law of thermodynamics, energy equilibrium means the energy input in the form of food equals the energy expenditure through exercise, basal metabolism, thermogenesis and fat biosynthesis. The control of body weight concerns the control of adipose tissue and is concerned with the key role that is played by the hypothalamus. Ventromedial hypothalamus, a part of hypothalamus, plays a primary role in feeding behavior. In addition, the paraventricular nuclei (PVN) and arcuate nuclei (ARC) in the periventricular zone of the hypothalamus are the sites where multiple hormones, released from the gut and adipose tissue, converge to regulate food intake and energy expenditure. There are two distinct types of neurons in ARC that are important in control of food intake: (1) pro-opiomelanocortin neurons, which are activated by anorexigenic hormones, and (2) neurons, which are activated by orexigenic peptides. ARC plays a major physiological role in hunger and satiety sensation via orexigenic hormones (ghrelin and orexins) and anorexigenic hormones (cholecystokinin, peptide YY, glucagon-like peptide-1, oxyntomodulin, leptin and others). Thus, the signaling from the gut and adipose tissue before and after a meal reaches to specific receptors in the afferent (mostly vagal) nerves and the hypothalamic neurons, involved in adiposity signaling and the regulation of food intake, and maintains the energy balance (11).

The gut-derived hormone, peptide YY (PYY), plays a role in modulating energy balance and adiposity through actions that regulate food intake, the efficiency of energy uptake, and

tissue metabolism of nutrients. PYY administration induced obese mice to have negative energy balance. This resulted from reduced food intake with a relative maintenance of mass-specific energy expenditure. The respiratory quotient (RQ) was lower indicating increased mobilization and oxidation of fat stores to help meet energy requirements which led to fat loss (12).

Oligofructose, another type of fermentable fiber- a non-starch polysaccharide, increases the mRNA expression of the proglucagon gene in the cecum and colon and blood levels of glucagon-like peptide 1 (GLP-1). Increases in GLP-1 are also involved in satiety and associated with reduced fat mass development and body weight (13).

Current data from our lab demonstrated that dietary resistant starch increases the mRNA expression of PYY and proglucagon in the cecum of rats, and plasma PYY and GLP-1 (10,14). In addition SCFA were increased. Therefore, fermentable resistant starch may decrease the body fat via increases in SCFA in the large intestine, which appear to stimulate mRNA expression of PYY and proglucagon genes by large intestinal cells and increases in PYY and GLP-1 secretion by these cells. All of our lab group's previous work has been done with low fat diets based on the fat level (18% of energy) of the AIN-93 diet (10,14).

Objectives

The objective of the study was to examine the effects of resistant starch (RS) in a high fat diet (28% of energy) on body fat, body weight and gut signaling in rats. The specific objectives addressed by the study were the following:

- To measure the body weights, abdominal, perirenal and epididymal fat pads, and the total body fat composition.
- To determine the pH of the cecal contents and cecal weights.

- To measure the plasma PYY level, and gene expression for PYY and proglucagon in the cecum.

Hypotheses

The hypothesis of the study was that the RS in a high fat diet (28% of energy) will also increase gut signaling to reduce body fat as occurred in our earlier studies on resistant starch in a low fat diet (18% of energy). Specific hypotheses addressed by the study were the following:

- The inclusion of RS in a high fat diet (28% of energy) will increase the gene expression of PYY and proglucagon, and plasma PYY levels in rats.
- The inclusion of RS in a high fat diet (28% of energy) will decrease the body weight, abdominal fat, perirenal fat, epididymal fat and total percent body fat in rats.
- The inclusion of RS in a high fat diet (28% of energy) will decrease the pH of the cecal contents and increase the cecal weights in rats.

Assumptions

- It is assumed that rats from the current study will be similar to rats used in the low fat studies and that all conditions for the studies will be similar.
- It is assumed that rats will be weighed accurately and recorded correctly throughout the study.
- It is assumed all assays will accurately assess *in vivo* conditions.

Limitations

- ARC neuropeptides were not assessed although the hypothalamus plays a role in energy balance.
- An effect of resistant starch in low fat diets on gut signaling was not assessed in this study though done in our earlier studies.

- Effects of different types of fats on body weight and body fat were not examined.
- This was an animal study and results may not be similar for human subjects.

CHAPTER 2

REVIEW OF LITERATURE

Dietary Fiber

The term "dietary fiber" coined by Hipsley, in 1953 is one of the most talked about nutrients for health promotion and disease prevention at this present time (15). In 1976, Trowell and co-workers defined dietary fiber as the digestion-resistant polysaccharides (mostly plant storage saccharides), such as gums, modified celluloses, mucilages, oligosaccharides, and pectins. This definition also includes cellulose, hemicellulose, lignin, gums, modified celluloses, mucilages, oligosaccharides, pectins, and associated minor substances, such as waxes, cutin, and suberin (15). The Association of Official Analytical Chemists (AOAC) in 1995 defined dietary fiber as the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion. It includes polysaccharides, cellulose, and lignin and associated plant substances (15). In this context, the Panel on Macronutrients (DRI) recently defined dietary fiber as the nondigestible carbohydrate and lignin that are intrinsic and intact in plants. Functional fibers are the isolated, nondigestible carbohydrates that have proven beneficial physiological effects in humans. Total fiber is the sum of dietary fiber and functional fiber (16). Many researchers also classify dietary fiber as insoluble dietary fibers which are known to promote bowel regularity; and soluble fibers which are known to reduce blood cholesterol and blood glucose (17). Dietary fibers are also classified as non-fermentable fibers and fermentable fibers by many research groups (18). The non-fermentable fibers increase the fecal bulk, decrease the transit time, help in carcinogen binding and bile salt binding and increase the viscosity (18). The fermentable fibers also pass the small intestine without being digested and then are fermented in the large intestine and produce short chain fatty acids (SCFAs) and decrease the pH (18). These SCFAs produced

in the large intestine after fermentation help in lowering blood cholesterol, restoring normal intestinal flora, inhibiting growth of harmful bacteria and reducing liver toxins, food intolerances and food borne allergies (19). Resistant starch, oligosaccharides, oligofructose, fructan, inulin, and β-glucan are a few examples of fermentable fibers, whereas cellulose, hemicellulose and methylcellulose are a few examples of non-fermentable fibers (18). Despite not having an exact definition and classification of dietary fiber, experts agree on one important thing - dietary fiber is an important weapon in the fight against heart disease, colon cancer, diabetes, and obesity.

Resistant Starch

The term "resistant starch" was coined in 1982 by Englyst and his coworkers to describe a small fraction of starch that was resistant to hydrolysis by exhaustive amylase and pullulanase treatment in vitro, and his group also confirmed that this same type of starch also resisted enzymatic hydrolysis *in vivo* using healthy ileostomy subjects (20,21). Resistant starch (RS) is subdivided into 4 fractions, RS1, RS2, RS3 and RS4. RS1 is physically inaccessible to digestion and is entrapped in a non-digestible matrix, found mainly in whole grains and legumes. RS2 is mainly the ungelatinized starch granules found in raw potato starch, green banana and high amylose cornstarch. RS3 is the retrograded starch found mainly in cooked and cooled potatoes, bread, cornflakes and food products with repeated moist heat treatment. RS4 is chemically modified starch due to cross linking with chemical reagents, found in breads and cakes (6,20). Resistant starches are the non-digestible portion of the various types of foods and like fiber resist amylase digestion in the small intestine and are fermented by the microflora in the large intestine to short chain fatty acids (SCFA) which leads to a decrease in pH (6,7). The amount of resistant starch can vary depending on the degree of processing and time spent in small intestine prior to entry into large intestine.

The major SCFAs produced include butyrate, acetate and propionate, and it is believed that these SCFA in particular mediate many potential physiological effects such as improved glycemic and insulinaemic responses, improved bowel health, improved blood lipid profile, increased satiety and reduced energy intake, increased micronutrient absorption and thermogenesis (6,7). All these factors may be helpful in preventing diseases such as colorectal cancer, cardiovascular disease, osteoporosis and obesity and assist in the management of diabetes, impaired glucose tolerance, inflammatory bowel diseases, diverticulosis and constipation (6,7). Butyrate is the key short chain fatty acid which increases the secretion of gut peptides such as peptide YY (PYY) and glucagon like peptide 1 (GLP-1) through the ileal brake which in turn regulates food intake and increases satiety (22).

PYY and GLP- 1 are mainly synthesized in the intestinal endocrine cells in response to the ingested nutrients. Glucose and fatty acids are the two major nutrients that influence PYY and GLP-1 secretion (14). Thus, our lab designed a study using male Sprague Dawley rats as a model to examine the gene expression patterns for PYY and proglucagon in the duodenum, jejunum, cecum and colon. Rats were randomly divided into 2 groups: control group fed a standard AIN-93G powdered diet and the resistant starch group. The diet of resistant starch fed rats was the same as the diet of the control group except that resistant starch was used to replace the regular starch. Rats were fed for 4 weeks. The results showed that resistant starch fed rats had higher gene expression for PYY and proglucagon in the cecum and colon compared to control rats. Interestingly, it was also found that the proglucagon and PYY gene expressions were unchanged in the jejunum and ileum by RS, but were increased in the cecum and colon, where the fermentation of RS occurred. When cells of the surface layer of the cecum and colon were incubated with butyrate *in vitro*, the PYY and proglucagon gene expressions were also increased

in a dose dependent manner. Thus, it appears that the distal part of the gut senses the butyrate, a short chain fatty acid and up regulates the PYY and proglucagon gene expression (14). Our lab also recently reported at the Experimental Biology 2007 conference that dietary RS promotes a consistent elevation in plasma GLP-1 and PYY levels compared to the non-fermentable fiber diet (EC) in rats. RS-fed rats also had decreased body fat compared to the EC fed rats. Thus, this data strongly support our hypothesis that RS fermentation stimulates GLP-1 and PYY expression in the gut (23).

Retrograded amylose, an example of RS3, resists digestion and passes through the small intestine and enters the large bowel where it is fermented. Retrograded amylose cornstarch was prepared by gelatinization of high amylose cornstarch, followed by storage at 1ºC for 48 hours for a rat study. Twenty four male Wistar rats were randomly divided into 2 groups. One group was fed a diet with retrograded amylose cornstarch and the other a control diet without resistant starch for the study of 14 days. The control diet was prepared by using standard AIN-93 ingredients. The intra-cecal pH was measured by inserting a microelectrode through a small incision. The cecum was subsequently removed and the wet weights of both the collected cecal contents and rinsed tissue were recorded. Cecal size and contents were found to be greater in the resistant starch-fed rats, and cecal pH was significantly lower in resistant starch fed rats compared to the control group. This study confirmed that a substantial proportion of retrograded amylose from cornstarch escapes hydrolysis in the small intestine, and acts as a substrate for bacterial fermentation in the cecum (24).

RS, a prebiotic, is a potential substrate for fermentation by the microbiota, and is capable of stimulating proliferation and activity of endogenous desirable bacteria. It is believed that RS shows its beneficial physiological effects by production of SCFA through fermentation (25).

Ninety six male Sprague-Dawley rats that were 5 weeks old were obtained and randomly divided into 8 experimental groups. Rats were fed experimental diets for 4 weeks. Rats had free access to water and were weighed weekly throughout the study. The experimental diets were based on the AIN-76 standard for purified diets for rats and mice and contained either low-RS or moderate-RS (10% Hi-maize 958). *Lactobacillus acidophilus*, *Bifidobactrium lactis* and a combination of the 2 cultures were added at a concentration of 1% each by weight to semi purified diets containing low-RS or moderate-RS at the expense of sucrose. The 8 experimental diets were low RS, low RS+ *lactobacillus acidophilus,* low RS+ *Bifidobacterium lactis,* low RS+*lactobacillus acidophilus*+*Bifidobacterium lactis,* moderate RS, moderate RS+ *lactobacillus acidophilus,* moderate RS+ *Bifidobacterium lactis* and moderate RS+*lactobacillus acidophilus*+*Bifidobacterium lactis.* The feces and ceca were collected for analysis of SCFA and pH. The fecal pH of moderate RS groups with different bacteria cultures were not significantly different than low RS groups with different bacteria cultures, but moderate RS without any added culture had significantly lowered (p<0.05) fecal pH than low RS without added culture. The fecal SCFA contents were significantly higher (p<0.05) in all moderate RS groups compared to all low RS groups. All moderate RS groups had significantly higher (p<0.05) cecal SCFA content and lower pH (p<0.05) than all four low RS groups. Thus, at least 10% RS can influence the fermentation variables such as pH and SCFA concentrations both in the cecum and feces, and can act as a proper substrate for fermentation and also provide a right condition for bacterial action. (25).

Thirty Wistar rats that were 8 weeks old were used to study the effects of different forms of resistant potato starch (RS) on the SCFA production. The experimental diets were fed for 5 months. The rats had an average initial body weight of 210g, and were adapted for 7 days to a

balanced basal diet containing 60% waxy maize starch devoid of any RS. Then rats were randomly divided into 3 groups each with 10 rats, and were fed diets containing their respective diets. Group 1 rats were fed a rapidly digestible waxy maize starch (basal diet: 0% RS), group 2 rats were fed a mixture of 83.3% waxy maize starch (0% RS) and 16.7% native granular potato starch (60% RS), group 3 rats were fed a mixture of 33.3% waxy maize starch (0%RS) and 66.7% modified retrograded potato starch (15% RS). The final RS content of 10% of the diets was calculated on the basis of raw potato starch and retrograded potato starch for the group 2 and group 3, respectively. At the end of the study, the feces and ceca were collected to examine the effects of 10% raw potato starch and 10% retrograded potato starch on SCFA production. It was found that group 3 rats had a significantly higher ($p<0.05$) concentration of cecal SCFA such as acetate, propionate and butyrate compared to both group 1 and 2 rats. Whereas, in the case of the fecal SCFA, only propionate was found higher ($p<0.05$) in group 3 rats compared to group 1 and 2 rats. These results suggest that fermentation of retrograded potato starch produces higher SCFAs: butyrate, propionate and acetate compared to the raw potato starch (26).

Non-starch polysaccharides, a type of carbohydrate with similar effects as RS, have slower digestion and absorption of carbohydrates in the small intestine and undergo fermentation in the colon. Fructooligosaccharides, oligofructose and xylooligosaccharides are a few examples of the indigestible and fermentable non-starch oligosaccharides. To evaluate the *in vivo* effects of the oligosaccharides on cecal and fecal short chain fatty acids, pH and total large bowel wet weight, fifty male Sprague Dawley rats were acclimatized with a purified powdered diet before starting the experiment. Rats were individually housed in a stainless steel cage and had free access to water. Fifty rats were randomly divided into 5 groups and fed 5 types of experimental diets for 14 days. The five experimental diets were: control diet, control diet +5%

microcrystalline cellulose (5% CC), control diet +5% CC +6% fructooligosaccharides, control diet +5% CC + 6% oligofructose, and control diet +5% CC +6% xylooligosaccharides. It was found that total cecal SCFA pools were higher and pH was lower in rats fed the oligosaccharide containing diets compared with rats fed with control or cellulose diets. Cecal total weight was found higher in all oligosaccharide fed groups compared to the control and cellulose groups, whereas colonic total wet weight was significantly higher for only rats consuming xylooligosaccharides compared with other treatments. Thus, dietary fermentable, indigestible oligosaccharides produce SCFAs through fermentation that lead to lower pH in cecum and higher cecal weight (27).

These data demonstrated that resistant starch and other oligosaccharides that are fermented produce similar effects. Fermentation of oligosaccharides is also associated with an increase in the levels of certain gastrointestinal peptides (24). Resistant starch and oligosaccharides have additional gastrointestinal effects such as transfer of energetic molecules from the small to the large intestine, increased bulking effects and increased fecal excretion of energy (28).

Resistant Starch and Body Fat

To assess the effects of dietary resistant starch on abdominal fat and gene expressions of PYY and GLP-1, our lab conducted two studies. In the first study, thirty, 7-month- old female retired breeder rats were assigned to 3 diets treatment groups: control group (5% fiber, 3.6 kcal/g), fermentable high amylose-resistant cornstarch (RS; 39.9% fiber, 33% RS, 3.2 kcal/g) group and non fermentable methylcellulose (MC; 37.5% fiber, 2.5 kcal/g) group. RS and MC groups were similar in their fiber levels. In the second study, thirty, 8-week-old male rats were randomly assigned to two diet treatment groups: resistant starch (RS; 3.3 kcal/g, 36.3% fiber,

29.7% RS) group and non fermentable cellulose energy control group (EC; 3.3 kcal/g, 15.7% fiber). The study was in three phases: 1, 3 and 5 weeks. The data was combined statistically within a factorial arrangement of treatments with diet and time as independent variables. In the first study, RS fed rats had higher serum levels of PYY and higher gene expressions for PYY and proglucagon in cecum and large intestine compared to the two control groups of rats. RS fed rats had lower pH of the cecal contents and higher cecum weights compared to the control and MC groups. RS rats had lower abdominal fat and disemboweled body weight compared to the control group but there was no difference in disemboweled body weight and abdominal fat between RS and MC group. At the end of study 2, RS fed rats had higher plasma levels of PYY and higher gene expressions for PYY and proglucagon in cecum compared to the EC fed rats. RS fed rats had lower disemboweled body weight, abdominal fat, pH of the cecal contents and higher cecum weights, weights of cecal contents, butyrate, propionate and acetate production in cecal contents compared to the EC group. All these results suggest that RS as a result of fermentation produces SCFA in the large intestine, which then leads to increases in gene expression of PYY and GLP-1. Study 2 demonstrated that when dietary energy is similar, effects of fermentation on reducing body fat and disemboweled body weight are observed (10).

High-fat diets promote obesity by the mechanism of hyperphagia because more energy is consumed as a result of higher energy density of the diet. Current recommendations for the management of obesity include an increase in dietary fibers to dilute the energy density of the diet and control energy intake (13). Our lab group has demonstrated effects of dilution of dietary energy with non-fermentable fiber in a high fat diet (10,29). The effects of fermentation with a high fat diet need to be addressed.

Oligofructose (OFS), a fermentable fiber decreases the food intake and fat mass by increasing GLP-1 and possibly improving satiety. To examine the effect of OFS in a high fat diet on food intake modulation and proglucagon derived peptide and ghrelin levels, 12 male Wistar rats were randomly divided into two groups: high fat OFS diet with a metabolizable energy (MEV) of 4.1kcal/g and high fat control diet with MEV of 4.4kcal/g. At the end of the study, blood, cecum and colon samples were collected. The results showed that high fat OFS rats had lower body weight and energy intake ($p<0.05$) than high fat control rats. Also high fat OFS rats had significantly higher mRNA ($p<0.05$) expression for proglucagon in cecum and proximal colon. GLP-1 levels were found higher ($p<0.05$) and dipeptidyl peptidase IV (DPPIV) levels, an enzyme that degrades active GLP-1, were found lower ($p<0.05$) in portal circulation in high fat OFS rats. There was no change in plasma ghrelin concentration between the high fat OFS and high fat control group. Thus, the addition of OFS in the diet appears to protect against the promotion of energy intake, body weight gain, fat mass development induced by a high-fat diet by increasing the proglucagon mRNA in the cecum and the colon and portal concentration of GLP-1. However, the dietary energy density of the high fat control may also have a role in producing reported effects (13).

To examine the relationship between the RS content in a meal and post absorptive fat oxidation, a study was conducted with 12 human subjects (5 males and 7 females). The subjects received four meals differing only in resistant starch (RS) content in random order, approximately four weeks apart. Meals contained 0%, 2.7%, 5.4%, or 10.7% RS as a percentage of total carbohydrate. Subjects were between 28 to 45 years old and had a BMI ranging from 20 to 28. It was observed that addition of 5.4% RS to the diet significantly decreased the respiratory quotient compared to the meals that contained 0%, 2.7% and 10.7% RS, indicating an increase in

fat oxidation. The consumption of 10.7% RS was not associated with increased fat oxidation. Thus, they suggest at higher RS consumption, only a portion of RS is fermented and the remainder passes through the colon as an insoluble fiber (8).

Administration of PYY and GLP-1 Hormones

PYY and GLP-1 are gastrointestinal-derived hormones that are released post-prandially in proportion to the amount of calories ingested. PYY reduces food intake and body weight and suppresses appetite when intravenously infused into rodents and humans. Circulating PYY concentrations increase after food ingestion and have been low in obese humans. Peripheral infusion of PYY [3-36] reduces the food intake, hunger sensation and body weight gain (12). Peripheral administration of GLP-1 also significantly reduces gastric emptying and food intake in both rodents and humans (30). These gastrointestinal hormones influence central nervous system feeding circuits via vagal–brainstem–hypothalamic pathways. To investigate the role of the vagal–brainstem–hypothalamic pathway in the mediation of the inhibitory effects of peripheral PYY [3–36] and GLP-1 on food intake, a study was conducted using male Wistar rats as the animal model. Rats had undergone either bilateral vagotomy or brain-stem hypothalamic pathway transectioning. The bilateral sub-diaphragmatic total truncal vagotomy was done by freeing the ventral and dorsal vagal trunks from the esophagus below the diaphragm, and then tying and cauterising to minimise nerve regeneration. Rats, while undergoing brainstem–hypothalamic pathway transectioning were placed in a stereotaxic frame with the head fixed 2.4 mm below the interaural line. A wire knife (Model 120, David Kopf Instruments) was lowered in the brain in a coronal plane, bilaterally 0.5 mm from the midline, 1 mm caudal to the lambdoidal suture and 8 mm ventral to the dura, and then rotated to extend the blade 1.5 mm laterally from the midline. The knife was then raised to 3 mm ventral to the dura before being retracted and

removed from the brain. Then food intake was measured after the administration of PYY [3-36] and GLP-1. The results showed that the effects of peripheral administration of both PYY [3–36] and GLP-1on food intake and activation of hypothalamic arcuate feeding neurons were abolished in rats that had undergone either bilateral sub-diaphragmatic total truncal vagotomy or brainstem–hypothalamic pathway transectioning. These findings suggest that the vagal–brainstem–hypothalamic pathway plays a role in the effects of circulating PYY3–36 and GLP-1 on food intake and may play a role in reducing body weight. The findings of the study also support the role of PYY and GLP-1 in food intake regulation (31).

The gut hormone fragment PYY [3–36] with the adipocyte hormone leptin reduces food intake by modulating appetite circuits in the hypothalamus. However, in obesity there is a marked resistance to the action of leptin, which greatly limits its therapeutic effectiveness. A study was conducted to investigate whether obese subjects are resistant to the anorectic effects of PYY. Twelve healthy obese and 12 healthy lean subjects were recruited. The inclusion criteria for the study were BMI of 27 to 40 for the obese group and 17 to 23 for the lean group. The control and experimental groups received a 90-minute infusion of saline or PYY (total dose, 2 nmol per square meter of body-surface area), respectively. Two hours after the termination of the infusion, the subjects were offered a buffet lunch with food in such excess that all appetites could be satisfied. The amounts of food and water were quantified preprandially and postprandially, and the caloric intake was calculated. Six hours after the infusion, plasma levels of PYY, ghrelin, insulin and leptin were measured. Results showed caloric intake during a buffet lunch offered, two hours after the infusion of PYY was decreased by 30 percent in the obese subjects ($p<0.001$) and 31 percent in the lean subjects ($p<0.001$). PYY infusion resulted in a significant decrease in the cumulative 24-hour caloric intake in both obese and lean subjects.

Plasma levels of leptin and insulin were found significantly higher in the obese group and unaffected by PYY infusion. PYY infusion reduced the plasma levels of the appetite-stimulatory hormone ghrelin. Endogenous fasting and postprandial levels of PYY were found significantly lower in obese subjects ($p<0.001$) compared to the lean subjects. Furthermore, the fasting PYY levels were correlated negatively with the BMI ($r=-0.84$, $p<0.001$). These findings suggest that obese subjects are not resistant to the anorectic effects of PYY. Thus, low endogenous PYY levels in the obese subjects, suggest that PYY deficiency may contribute to the pathogenesis of obesity (32).

The effect of the pharmaceutical administration of PYY [3-36] on body weight and adiposity in diet induced obese mice was determined. Obesity was induced in rats by high-fat feeding (58% of energy from fat, 16% from protein, 26% from carbohydrate) at 4 wk of age. Mice were fed this diet throughout the study. The experimental group received continuous PYY [3-36] administration whereas the control group received the vehicle dimethyl sulfide. The parameters, researchers measured, were the respiratory quotient (RQ), food intake, body weight and adiposity. Food intake ($p<0.05$) and body weight ($p<0.001$) were significantly reduced compared to the control group. The RQ of the light cycle was reduced significantly ($p<0.001$) throughout the study whereas the dark cycle RQ was only transiently decreased ($p<0.001$) compared to the control group. Epididymal fat pad weights in PYY [3-36]–treated mice were 50% lower than in controls ($p < 0.01$). A negative energy balance after PYY [3-36] administration in diet-induced obese mice resulted from reduced food intake. Thus, fat loss and reduced RQ after the PYY [3-36] administration highlight the potential role of PYY[3-36] in driving increased mobilization of fat stores to help meet energy requirements (12).

Another research group also examined the endogenous postprandial PYY response and the effect of exogenous PYY [3–36] doses on satiety and food intake in human subjects. The human study included 20 obese and 20 normal-weight subjects. The obese volunteers had lower (p <0.001) fasting plasma PYY levels compared to the healthy subjects. Although a rise in plasma PYY levels in both obese and normal subjects was seen after having the meal, obese subjects had lower postprandial plasma PYY levels (p <0.05) than control subjects. The PYY [3-36] infusion to humans showed a negative correlation between food intake and infused PYY [3-36] concentration. However there was no correlation between the hunger sensation and PYY concentration. In another study researchers studied the effects of a high-fat (HF) and low-fat (LF) diet on plasma PYY in mice. The infusion of PYY [3-36] in mice significantly reduced the food intake in HF mice compared to the HF mice injected with saline. The HF mice without PYY [3-36] infusion had both fasting and postprandial plasma PYY levels lower compared to the LF mice. Thus, the PYY release from the intestinal tract may be inhibited in the obese, leaving obese subjects with a functional deficiency in PYY-induced satiety (33).

GLP-1, another hormone, along with PYY and other possible hormones participates in the regulation of energy intake. To examine the effect of intravenously infused GLP-1 on subjective appetite sensations after an energy-fixed breakfast and on spontaneous energy intake at an ad libitum lunch, 20 young, healthy, normal weight men were recruited. It was a placebo-controlled, randomized, double blinded study. Subjects were tested for 2 different occasions for the study, separated by at least 3 weeks but not more than 7 weeks. The breakfast consisted of yogurt, bread, butter, cheese, jam, kiwi fruit, orange juice, and water. Total available energy content of the meal was calculated to be 20% of each subject's individual energy requirements in a day. The lunch was a homogeneous mixed hotpot consisting of pasta, minced meat, green

pepper, carrots, squash, onions, corn, and cream. For both meals, the distribution of energy was 50% energy from carbohydrates, 37% energy from fat, and 13% energy from protein. Fifty pmol/kg body weight per hr of either saline or GLP-1 was infused simultaneously with initiation of the test meals. GLP-1 infusion enhanced satiety and fullness during breakfast when compared with the saline infused group (treatment effect: $p < 0.03$). Furthermore, spontaneous energy intake at the ad libitum lunch was reduced by 12% by GLP-1 infusion compared with saline infusion ($p < 0.002$). In conclusion, the results show that GLP-1 enhanced satiety and reduced energy intake and thus may play a physiological regulatory role in controlling appetite and energy intake (30).

Exenatide and Body Weight Regulation

Exenatide is a compound that exhibits activity similar to the naturally occurring gut hormone GLP-1. GLP-1 is released from cells in the gut in response to food ingestion and binds to pancreatic beta-cell receptors to stimulate the release of insulin. Exenatide administration mirrors many of the effects of GLP-1, which include glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, slowing of gastric emptying, reduced appetite and enhanced beta-cell function. In addition to positive therapeutic effects on fasting and postprandial glucose levels, exenatide treatment is also associated with progressive reductions in body weight. Exenatide is reported to enable patients with type 2 diabetes to achieve glycemic control and promotes weight loss in obese subjects (34). Exenatide treatment in the amount of 5pg per week for two years to type 2 diabetic subjects in a study also led to a decrease in glycosylated hemoglobin and body weight, suggesting its importance in glycemic control and body weight regulation (35). In another study, eight two week periods of adjunctive exenatide treatment in obese patients with type 2 diabetes also resulted in sustained reduction in

glycosylated hemoglobin and progressive reduction in weight, as well as improvement in some cardiovascular risk factors (36).

Gastric Bypass Surgery and Gut Secreted Hormones

Gastric bypass surgery promotes effective and sustained weight loss. This surgery accelerates the delivery of nutrients to the hindgut, which results in decreased gastric emptying and gastrointestinal transit, dubbed the "ileal brake." GLP-1 and PYY have been suggested as mediators of the "ileal brake." Both GLP-1 and PYY release are augmented after gastric bypass surgery and have been shown independently to decrease food intake in humans. Hunger was decreased and satiety increased with increases in plasma GLP-1 and PYY. Thus, increased postprandial plasma GLP-1 and PYY after gastric bypass surgery may decrease food intake. GLP-1 increases insulin and decreases glucagon secretion and thus helps maintain normal blood glucose responses (37).

To determine whether gastric bypass surgery alters PYY levels or response to glucose, a study was designed by including 6 obese patients that had undergone gastric bypass surgery, 5 lean controls and 12 obese controls. Serum PYY levels after a 75-gram oral glucose tolerance test was measured. After substantial body weight loss induced by gastric bypass, the PYY response to an oral glucose tolerance test was found significantly higher in obese subjects that had gastric bypass surgery than the controls and obese subjects not having gastric bypass surgery (p <0.01). Thus gastric bypass results in a more robust PYY response to caloric intake. These findings provide further evidence for a role of gut-derived hormones in mediating appetite changes after gastric bypass, and also support further efforts to determine whether PYY[3–36] replacement could represent an effective therapy for obesity (38).

All studies discussed above whether on RS or PYY/GLP-1 administration exogenously or exenatide treatment or gastric bypass surgery, agree on one point that PYY and GLP-1 hormones play roles in food intake and body weight regulation. Among all of these treatments, our resistant starch supplementation is a natural way for secretion of these anti-obesity hormones: PYY and GLP-1. Theoretically it could also be possible that PYY produced from the gut conveys the message to the hypothalamus to suppress the hunger by down regulating the agouti related protein (AgRP) and neuropeptide Y (NPY) and by up regulating propiomelanocortin (POMC) hormone in the ARC of the hypothalamus (39). Our lab's gene array results presented at Experimental Biology 2007 conference also showed that not only PYY and GLP-1, but a cluster of other genes, including genes for other secreted peptide hormones, neurotransmitters, immune factors and genes involved in prevention of cancer were also differentially expressed in RS fed rats compared to the rats fed an energy control diet containing cellulose, a non fermentable fiber. These results support that fermentation is the key factor for the increased signaling in RS fed rats. Thus, the up- and down-regulated genes for secreted peptides, neurotransmitters and immune factors in RS rats may play roles in improved local health of the GI tract and the health of the entire organism with possible benefits of reduced risk for obesity, diabetes, heart disease and cancer (40). It was also mentioned earlier that RS fed rats had consistently elevated plasma levels of PYY and GLP-1 compared to the rats fed non fermentable fiber (23). Thus, feeding RS is a natural way for endogenous secretion of these anti-obesity hormones: PYY and GLP-1, and could be a novel approach for the treatment of obesity.

CHAPTER 3

RESISTANT STARCH DOES NOT REDUCE BODY FAT IN RATS FED A HIGH FAT DIET

Introduction

Obesity and overweight status continue to be leading public health concerns in the United States over several decades and its prevalence has been rapidly increasing in the United States, as well as other countries, over the past two decades (1). According to 2003-2004 data, 17.1% of US children and adolescents were overweight and 66.3% of adults were overweight or obese (2). Obesity has been associated with a relative increase in diabetes, cardiovascular disease, various cancers, respiratory disorders during sleep, gallbladder disease, and osteoarthritis (4). Adverse health effects of obesity justify the need to discover effective treatments, among them possible dietary treatments.

Resistant starches (RS) contain varying amounts of starch that resist amylase digestion in the small intestine and this resistant starch is fermented to short chain fatty acids (SCFA) by the microflora in the large intestine (6,7). Thus, adding resistant starches to the diet is the same as adding fermentable fiber. Effects of dietary resistant starch include: reduction of metabolizable energy density, a bulking effect and fermentation (10). In a previous study, our research group controlled for reduction of energy density with the use of cellulose in an energy control diet. The rats fed a low fat diet with resistant starch had reduced body fat compared to rats fed an energy control diet (10). The results point to increased energy expenditure as a result of the bulking effect and/or fermentation because energy intakes were not different.

Feeding resistant starch was associated with increased SCFA in cecal contents, increased mRNA expression of PYY and proglucagon in the cecum of rats, and increased plasma PYY (10) and plasma GLP-1 (14). Therefore, fermentable resistant starch may decrease the body fat via

increases in SCFA in the large intestine, which appear to stimulate mRNA expression of PYY and proglucagon genes by large intestinal cells and increases in PYY and GLP-1 secretion by these cells.

There is some debate about the benefits of higher levels of dietary fat and effectiveness of weight loss (41,42). High fat diets with resistant starch replacing regular starch may be effective in weight loss. Thus, this current study was conducted to examine the effects of resistant starch (RS) in a higher fat diet (28% of energy) on gut signaling and body fat.

Materials and Methods

Design. The study used a randomized experimental design with three groups, and was undertaken to examine the effects of resistant starch in a high fat diet (28% of energy) on body weight, abdominal fat, cecal weight, cecal content pH, plasma PYY and gene expression for PYY and proglucagon in male Sprague Dawley rats. The three experimental groups included an EC (energy control) group, a RS (resistant starch) group, and a FC (fiber control) group. The study required 2 control groups because the independent variables, energy and fiber, were confounded by each other. Rats were fed the diets for 10 weeks.

Animals. Forty-two, 8 week old male Sprague Dawley rats, from the same colony and matched for age and weight, were purchased from Harlan Co. (Indianapolis, IN), for use in the study. The study protocol was approved by the Louisiana State University Institutional Animal Care and Use Committee. The rats were housed individually in wire cages that each contained a food cup and a water bottle. The rats were acclimated for one week to recover from travel and to habituate to a control powdered diet. Each group of rats was fed its respective treatment diet for 10 weeks. The 42 rats were randomly assigned to three groups (n=14), and the initial body weight of the rats ranged from 281g to 285g. After 10 weeks of the study, the rats were killed.

Food intake, food spillage and body weight were recorded three times a week (Monday, Wednesday and Friday) for the entire length of the study.

Diet Preparation. The three diets included AIN93-G recommendations for macronutrients, micronutrients and protein (Table1). Specific treatment differences of the three diet groups were: EC (Starch source was 100% amylopectin, 11% cellulose fiber by weight, diet energy value was 3.7 kcal/g), RS (Starch source was 60% amylose and 40% amylopectin, with 30% resistant starch and 31% fiber, diet energy value was 3.7 kcal/g), and FC (Starch source was 100% amylopectin, 31% cellulose fiber, diet energy value was 2.9 kcal/g). Lard and soybean oil (2:1) were used as the fat source.

Sample Collection. The GI tract was removed and weighed, and the disemboweled body weight was determined from the mass of the GI tract subtracted from the final body weight of the rats. The full ceca were weighed, the cecal contents were removed, the the ceca were rinsed with .9% saline, and the empty ceca were again weighed. Cecal contents were frozen for later pH measurement. Epithelial cells were scraped from the cecum and frozen using liquid nitrogen for later extraction of total RNA and gene expression for PYY and proglucagon. Abdominal fat (retroperitoneal), perirenal fat and epididymal fat were dissected and weighed. Blood plasma was collected for analysis of PYY. After weighing, all materials, except blood and cecal scrapings, were added back for proximate analysis of body fat.

Sample Analysis. Thawed cecal contents were homogenized in distilled water (0.5 g wet sample to 5 ml water), and the pH was measured using a combination electrode. Plasma PYY was measured using a commercial kit (Phoenix, Belmont, CA). Whole body fat was determined by proximate analysis. An RNeasy mini kit was used for the total RNA extraction from the cecum cells for each rat. Gene expressions for PYY and proglucagon were quantified by Real

Time Reverse Transcriptase- Polymerase Chain Reaction (real time RT-PCR). Cyclophilin mRNA levels from each sample were used as an internal control to normalize the mRNA levels. We have determined that cyclophilin, a common housekeeping gene, does not change with our treatments. The sequences of TaqMan probes and primers for cyclophilin (GenBank no. M15933) and PYY (M17523) are available on request. The probe and primers for proglucagon (NM_012707, assay ID Rn00562293_ml) were purchased from Applied Biosystems (Foster City, CA). Results for PYY and proglucagon were expressed as a ratio to cyclophilin.

Statistical Analysis. The data were analyzed using the Statistical Analysis Systems (SAS) statistical software package version 9.1. Data were statistically analyzed by using multiple univariate ANOVAs followed by F protected least significance difference. The independent variable was diet; it had 3 levels: EC, RS and FC. All data were presented as least square means (lsmeans) ± pooled SE. Both F-test in ANOVA and least square differences were considered significant if $p<0.05$.

Results

There were no differences in disemboweled body weight, abdominal fat, epididymal fat, perirenal fat and total fat between EC and RS rats. RS rats had a higher disemboweled body weight ($p<0.007$), abdominal fat ($p<0.0006$), epididymal fat ($p<0.0012$), perirenal fat ($p<0.003$) and total fat ($p<0.0003$) compared to the FC rats; and EC rats also had a higher disemboweled body weight ($p<0.001$), abdominal fat ($p<0.0069$), epididymal fat ($p<0.0317$) and total fat ($p<0.0088$) compared to the FC rats. RS rats had significantly lower pH ($p<0.0001$) and higher cecal weights ($p<0.0001$) compared to EC and FC rats, and there were no differences in pH and cecal weight between EC and FC rats (Table 2). EC, FC and RS groups were not significantly different in their percent body fat (Fig 1).

Plasma PYY was greatest for RS rats ($p<0.0001$) compared to EC and FC rats, and there was no difference in plasma PYY between EC and FC rats (Fig 2A). RS rats had increased gene expressions for PYY ($p<0.0001$) and proglucagon ($p<0.0001$) compared to EC and FC rats. EC and FC rats were not significantly different in PYY and proglucagon gene expressions (Fig 2B and 2C).

Discussion

RS, one type of non-digestible, fermentable fiber, has three major effects when included in the diet: dilution of dietary metabolizable energy, a bulking effect similar to non-fermentable fiber, and fermentation to short-chain fatty acids and increase in the expressions of PYY and proglucagon (a gene for GLP-1) in the gut (7,8,10,14,20). PYY and GLP-1 are the gastrointestinal-derived hormones released post-prandially in proportion to the amount of calories ingested (31). PYY reduces food intake and body weight in rodents and suppresses appetite and food intake when intravenously infused into humans. GLP-1 peripheral administration reduces gastric emptying, food intake and body weight in both rodents and humans (31).

The primary objective of our study was to examine the effects of RS in a high fat diet (28% of energy) on gut signaling and body fat. Our results showed that rats fed with RS in a high fat diet had increased gene expression for PYY and proglucagon in cecal cells and plasma PYY compared to rats fed EC and FC diets. The relative proportion of gene expression for PYY and proglucagon, and plasma PYY levels in the EC group compared to the RS group were 19.9%, 19.2% and 1.28%, respectively. These proportions were similar to those in our low fat diet (18% of energy) study (10). Thus, the signaling from the gut is similar with feeding RS in both a low and high fat diet.

Table 1. Diet Table

Nutrient	EC (3.7kcal/g)		RS (3.7kcal/g)		FC (2.9kcal/g)	
	%(g/100g)	%(kcal/g)	%(g/100g)	%(kcal/g)	%(g/100g)	%(kcal/g)
Protein	20	20	20	20	20	20
Tot. carbs	57	53	68	53	39	47
Fat	12	28	12	28	9	28
RS	0	0	30	22	0	0
Cellulose	11	0	31	0	31	0
Sugar	10	11	10	11	10	14

EC, Energy control; RS, Resistant starch; FC, Fiber control; Tot carbs, total carbohydrate

Table 2. Measurements from the End of the Study

LS means measurements	EC	RS	FC	SE
Disemboweled BW (g)	396.5^{a}	388.3^{a}	356.8^{b}	7.61
Empty cecum (g)	0.682^{a}	2.19^{b}	0.722^{a}	0.07
pH, cecal contents	8.27^{a}	6.23^{b}	8.21^{a}	0.072
Abdominal fat (g)	2.81^{a}	3.05^{a}	2.14^{b}	0.159
Epididymal fat (g)	4.55^{a}	4.92^{a}	3.98^{b}	0.176
Perirenal fat (g)	1.21^{ab}	1.36^{a}	1.00^{b}	0.075
Total fat (g)	8.56^{a}	9.34^{a}	7.12^{b}	0.357

EC, energy control; FC, fiber control; RS, resistant starch; SE, standard error; BW, body weight. Measures for treatments across rows with different superscripts are different ($p<0.05$).

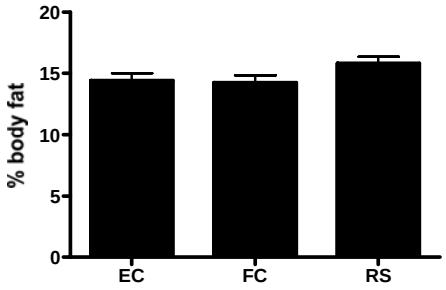

Fig 1. Percent Body Fat for Rats. Data presented as lsmeans ± pooled SE. There was no significant difference among the groups.

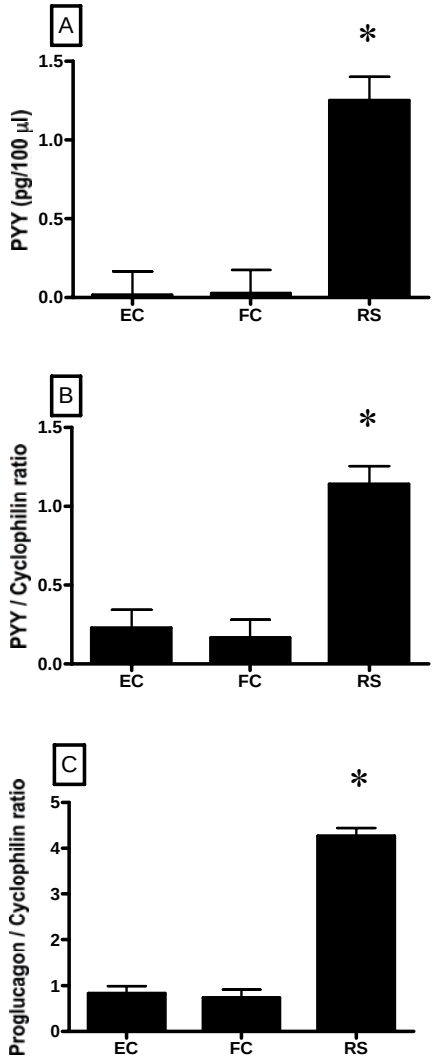

Fig 2. Plasma PYY for Rats (A), PYY Gene Expression in Cecum (B), and Proglucagon Gene Expression in Cecum (C). *: RS is significantly different than EC and FC at $p<0.05$. Data presented as lsmeans ± pooled SE.

RS in a high fat diet resulted in a decrease in pH of cecal contents and an increase in cecal weights compared to the other treatment groups. These results are also consistent with other studies on different types of resistant starches (7,24-27,43) and with our group's previous study with RS in a low fat (18% of energy) diet (10). Lower pH and increased cecal weights are associated with increased fermentation by RS, and we believe fermentation in the large intestine leads to the increased secretion of gut hormones and reduced body fat (10).

RS did not reduce disemboweled body weight, abdominal fat, perirenal fat, epididymal fat and total percent body fat for rats on the high-fat diet in this study compared to our previous study with a low fat diet (10). These results are contradictory to our previous study results.

In our previous study an increased expression of PYY and proglucagon genes in cecum and increased secretion of gut hormones was associated with a reduction in body weight and body fat in rats fed RS in a low fat diet (10). We have also observed a constant elevation of both plasma GLP-1 and PYY in RS fed rats compared to EC fed rats (J. Zhou, M.J. Keenan, M. Hegsted, R. Tulley, S.C Danna, A.M. Raggio, K.L. McCutcheon, L. Shen, S. Tripathy, R.J. Martin, unpublished data). The results of our current study with resistance to an endogenous physiological dose of PYY and GLP-1 with feeding a high fat diet would be similar to the effect on leptin, another satiety signal (42,44,45). Thus, the high fat appears to be blunting the response to PYY and GLP-1 signaling caused by dietary RS. It is also possible that high fat may be increasing ghrelin levels or affecting other signals such as immune factors, neurotransmitters and secreted peptides. Many studies on pharmaceutical administration of PYY [3-36] in mice fed a high fat diet also reported an increased fat oxidation (12,33) and satiety and administration of GLP-1 in humans fed a high fat diet reported a deceased energy intake and increased satiety (30). Although our results are contradictory to their results, in their study the doses of PYY and GLP-1

infused were much higher. In the current study, our blood level for PYY in RS fed rat was 1.25pg/100µl, whereas, in infusion studies of PYY, blood levels ranged from 16.2pg/100µl to 19.8pg/100µl in high fat diet fed mice (33) and 4ng/100µl in pig (46). Thus, effectiveness of infused PYY with high fat feeding appears to be due to non-physiological blood levels.

A report by Cani et al. (2005) demonstrated lower body weight and less body fat with oligofructose fermentable fiber in a high fat diet compared to a control high fat diet (13). Though their results also appear to be contradictory to our results, in their study dietary energy density was not controlled (4.1 vs. 4.4 kcal/g). This may possibly account for the differences in the study by Cani et al. and our study. In the current study and in two of our previous studies, our research group demonstrated the importance of dietary energy density with a high fat diet (10,29). Lowering the energy density of the high fat diet with non-fermentable cellulose for the FC group was very effective in lowering body weight and body fat.

In summary, the study demonstrated that resistant starch in a high fat diet did not reduce body fat and body weight. This was contrary to our previous study with a low fat diet. However, signaling produced by the gut was similar for both the current study and previous low fat study. The possible explanation for this result is that a high fat diet alters the brain response to the satiety signals from the gut. This would be similar to the effect on leptin, another satiety signal. Thus, the high fat is blunting the response to PYY and GLP-1 signaling caused by dietary RS. This result would support the advocacy of RS diets lower in fat content**.** Future studies should be done by adding RS to high fat diets with different types of fat and measuring possible blunting of signaling in the brain.

CHAPTER 4

CONCLUSION

Obesity and overweight are serious health concerns where a person accumulates an abnormally high proportion of body fat. This condition can increase the risk of diabetes, dyslipidemia, hypertension and osteoarthritis. According to 2003-2004 data, 17.1% US children and adolescents were overweight and 66.3% of adults were overweight or obese (2). Obesity results from excessive calorie intake and inadequate expenditure of energy. Theories to explain this condition include hypothalamic dysfunction of hunger and satiety centers, abnormal absorption of nutrients, and impaired action of gut and growth hormones and of hormonal regulators: insulin and leptin. The increased incidence of obesity and its associated health risks justify the need to discover effective treatments, among them possible dietary treatments.

It has been suggested that sufficient fiber in the diet will tend to prevent excessive food intake and fat accumulation by decreasing the caloric density of the diet, by slowing rate of food ingestion, by increasing the effort involved in eating, by promoting intestinal satiety, and by interfering slightly with the efficiency of energy absorption. However, fiber is a complex entity that includes fermentable and non-fermentable compounds. Resistant starches are non-digestible fibers that resist, to varying degrees, amylase digestion in the small intestine and are fermented to short chain fatty acids which lead to decrease in pH by the microflora in the large intestine. The major SCFA produced include butyrate, acetate and propionate. It is believed that these SCFAs, in particular, mediate many potential physiological effects such as improved glycemic and insulinaemic responses, improved bowel health, improved blood lipid profile, increased satiety and reduced energy intake, increased micronutrient absorption and thermogenesis. All these factors may be helpful in preventing diseases such as colorectal cancer, cardiovascular

disease, osteoporosis and obesity and assist in the management of diabetes, impaired glucose tolerance, inflammatory bowel diseases, diverticulosis and constipation.

Based on unpublished data from our lab, we believe butyrate is the key SCFA produced by fermentation of RS and appears to increase the secretion of gut peptides: peptide YY (PYY) and glucagon like peptide 1 (GLP-1). These hormones, in turn regulate energy expenditure. Previous data from our lab reported that RS in a low fat diet (18% of energy) increases the mRNA expression of PYY and proglucagon genes in cecum and also increases plasma PYY and GLP-1 and reduces the body fat (10,14,23). Thus, dietary treatment with RS, a novel approach, may be helpful in reducing body fat and obesity.

Many people consume high fat diets and high fat diets have been promoted for weight loss (47). Therefore, in this study, we tested the effects of resistant starch on gut signaling, body fat and body weight in male Sprague Dawley rats fed a high fat diet (28% of energy). In our study we had three experimental groups: an EC (energy control) group, an RS (resistant starch) group, and an FC (fiber control) group. Each had 14 rats. The study required 2 control groups because two of the independent variables, energy and fiber, were confounded by each other. Rats were fed the diets for 10 weeks. The three diets included AIN93-G recommendations for macro- and micronutrients as well as protein (48).

We found that RS increased the cecum weight and reduced the pH of the cecal contents. This is evidence for increased fermentation in the large intestine. RS also increased the plasma PYY levels and cecal cell gene expressions for PYY and proglucagon compared to the EC and FC groups. This gut signaling was similar to our lab's earlier study on RS in a low fat diet (18% of energy). However, RS did not reduce disemboweled body weight, abdominal fat, perirenal fat, epididymal fat, total percent body fat for rats fed a high-fat diet in this study compared to the

controls. The possible explanation for this result is that a high fat diet alters the brain response to the signals from the gut. This would be similar to the effect on leptin, another satiety signal (42,44,45). Thus, the high fat is blunting the response to PYY and GLP-1 signaling caused by dietary RS.

A report by Cani et al. (2005), however, demonstrated lower body weight and less body fat with oligofructose fermentable fiber in a high fat diet compared to a control high fat diet (13). Their results appear to be contradictory to our results, but in their study, energy density was not controlled (4.1 vs. 4.4 kcal/g). This may account for the differences in the study by Cani et al. and our study. We have found in several past studies and in the current study that dilution of the energy density of a high fat diet with a non-fermentable cellulose was very effective in lowering body weight and body fat (10,29).

Many studies with pharmaceutical administration of PYY [3-36] in mice fed a high fat diet have reported an increased fat oxidation and satiety (12,33). Administration of GLP-1 in humans fed a high fat diet also reported a deceased energy intake and increased satiety (30). These results are also contradictory to our results, but in their study, the doses of PYY and GLP-1 infused were much higher than blood levels in our studies.

The data from the current study demonstrated that resistant starch in a high fat diet was not associated with reduced body fat and body weight. These results were contrary to our previous study with a low fat diet. There was similar gut signaling in both our low and high fat diet studies. The possible explanation for the result with the high fat diet is that a high fat diet alters the brain response to the energy balance signals from the gut. This would be similar to the effect on leptin, another energy balance signal. Thus, the high fat is blunting the response to PYY and GLP-1 signaling caused by dietary RS. This result would support the advocacy of RS

diets lower in fat content. It is possible that different types of fat may have different effects. Therefore, future studies should be done by adding RS to diets with different types of fat to examine the effects on both brain and gut signaling. Our results also demand further research on assessment of gut peptides in obese rats after feeding RS to validate our theory that PYY and GLP-1 play a part as anti-obesity hormones.

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APPENDIX A

STUDY PROTOCOL

LOUISIANA STATE UNIVERSITY

AND AGRICULTURAL AND MECHANICAL COLLEGE
Institutional Animal Care and Use Committee
Division of Laboratory Animal Medicine

Animal Welfare Assurance # A3612-01
License # 72-3
Multiple Assurance # M1128

February 14, 2006

Dr. Michael Keenan
School of Human Ecology

Dear Dr. Keenan:

Protocol #06-017 entitled, "Determination of Differences in Molecular Signaling between Fermentable and Non-fermentable Carbohydrates in Rats" lists you as the principal investigator.

This is to inform you that your protocol was approved at the regularly scheduled IACUC meeting on February 9, 2006. This approval is valid for 3 years, and authorizes the use of 42 rats.

Thank you.

Sincerely,

Kathy L. O'Reilly, Ph.D.
Chair

dbd

School of Veterinary Medicine • Skip Bertman Drive • Baton Rouge • Louisiana • 225/578-9643 • FAX 225/578-9649

APPENDIX B

INGREDIENTS FOR THE THREE TYPES OF DIETS FOR TOTAL SIX KILOGRAMS

Ingredients	Amounts		
	EC	RS	FC
1. Macro mix			
Amioca cornstarch	2.500kg	-	1.489kg
High maize cornstarch	-	3.184kg	-
Sucrose	0.600kg	0.600kg	0.600kg
Cellulose	0.634kg	-	1.860kg
Casein	1.200kg	1.200kg	1.200kg
2. Micro mix			
Mineral mix (AIN-93G)	0.210kg	0.210kg	0.210kg
Vitamin mix	0.060kg	0.060kg	0.060kg
Choline chloride	0.008kg	0.008kg	0.008kg
L-Cystine	0.018kg	0.018kg	0.018kg
3. Oil mix			
Soybean	0.240kg	0.240kg	0.240kg
Crisco or Lard	0.480kg	0.480kg	0.480kg
BHT	0.084g	0.084g	0.084g
Food coloring	¼ tsp	¼ tsp	¼ tsp

APPENDIX C

DIET PREPARATION

To prepare the AIN-93G diet, the macronutrients were weighed and combined in a large mixing bowl. The ingredients were mixed for 10 minutes at low speed. The micronutrients were sieved sequentially into a bowl, ground up, and added to the macro mix. Both the macro mix and micro mix were mixed together for 10 minutes until a uniform distribution of color of micromix throughout the mix was obtained. An oil mix was prepared, added to the mixture, and mixed for 30 minutes. The diet was stored frozen (-20^0C) in Ziploc bags. Each was labeled with the diet type, date, batch number, and initials of the person who prepared the diets. A small sample from each diet batch was placed in a tiny plastic bag and labeled for future diet analysis if necessary.

APPENDIX D

PROCEDURE FOR RECORDING FOOD INTAKE AND BODY WEIGHT

The weight of the food cup plus the food was recorded before and after each feeding. To determine the amount of food consumed the differences were calculated. To weigh the rats, a bucket was placed on a balance, the balance was brought to zero, and the rats were individually placed in the bucket and weighed.

APPENDIX E

METHOD FOR EXTRACTION OF mRNA FROM CECUM CELLS (QAIGEN KIT)

An RNeasy mini kit was used for the total RNA extraction from the cecum cells for each rat. A small piece of each sample was cut and placed in a tube. Buffer RLT was added to the sample piece which was homogenized by using a Rotor-stator homogenizer (Model PCU-11, Kinematica). The samples were centrifuged, a supernatant for each sample was collected in a test tube, and ethanol was added. A sample of 700µl from the test tube was transferred to RNeasy mini column and centrifuged. The liquid collected at bottom of test tube was discarded and then 700µl buffer RW1 was added to the RNeasy mini column, the samples were centrifuged, and the liquid collected was again discarded. In the following step, 500µl buffer RPE was added to the RNeasy mini column, the samples were centrifuged, and the liquid was discarded. The final step was repeated once. At last RNase free water of 50µl was added to each sample. The samples were again centrifuged; the total RNA was collected in a test tube and stored at -80^0C.

APPENDIX F

DETAILS ABOUT STATISTICAL MODEL

The independent variable was diet; it had 3 levels: EC, RS and FC. The 10 dependent variables were the following: disemboweled body weight of the rats, weight of the empty cecum, pH of cecal content, abdominal fat, perirenal fat, epididymal fat, total percent body fat, plasma PYY level, gene expression for PYY, gene expression for proglucagon. ANOVAs were used to analyze overall effects of diets on each dependent variable. The least significance difference followed by ANOVA was used to know the individual diet effect on each dependent variable.

APPENDIX G

DATA USED FOR FIGURE 1, 2A, 2B AND 2C

Group	%Body fat	Plasma PYY (pg/100µl)	PYY: Cyclophilin	Proglucagon: Cyclophilin
EC	14.427 ± 0.6	0.016 ± 0.149	0.227 ± 0.115	0.821 ± 0.174
FC	14.234 ± 0.6	0.025 ± 0.149	0.166 ± 0.115	0.735 ± 0.174
RS	15.8 ± 0.6	1.25 ± 0.149	1.14 ± 0.115	4.27 ± 0.174

All data are presented as lsmean ± pooled standard error

VITA

Sasmita Tripathy was born in Angul, Orissa, India, on June 17, 1978, to parents Ramesh Chandra Tripathy and Pramila Tripathy. Sasmita received her bachelor's degree in home science from Orissa University of Agriculture and Technology, India, in April of 2000. Sasmita was also the recipient of the University Gold Medal for her bachelor's degree. Sasmita received her master's degree in home science a specialization of foods and nutrition from Acharya N.G. Ranga Agricultural University, India, in April of 2002. She had many job experiences such as Field Investigator for six months, Instructor for one year, Dietitian for six months and Junior Scientist for six months before coming to Louisiana to begin her graduate studies at LSU. She received a graduate supplement award from the Graduate School, LSU and also was the recipient of the Neva Nolen Scholarship Award from the School of Human Ecology, LSU. During her tenure as a graduate student, Sasmita worked as a Graduate Teaching Assistant. Sasmita joined Dr Michael Keenan's lab to do her research and presented her part of the research as a poster at the Experimental Biology 2007 Conference. Sasmita plans to graduate from her master's program in August of 2007. Currently, Sasmita is a graduate student in LSU. Sasmita hopes to continue her doctoral program in the near future. After completing her doctoral program, Sasmita will continue to do research in nutrition and contribute to the everlasting resources for the scientific community.