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The effects of non-viscous, fermentable fibers on appetite and food intake in healthy adults

by

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LIST OF ABBREVIATIONS

α-MSH	α-Melanocyte Stimulating Hormone
AACC	American Association of Cereal Chemists
AgRP	Agouti-related Peptide
AMPK	Adenosine Monophosphate-activated Protein Kinase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APO-AIV	Apolipoprotein A-IV
ARC	Arcuate Nucleus
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BMI	Body Mass Index
BRFSS	Behavioral Risk Factor Surveillance System
CART	Cocaine and Amphetamine Regulated Transcript
CCK	Cholecystokinin
CoA	Coenzyme A
CODEX	Codex Alimentarius Commission
CV	Coefficient of Variance
DXA	Dual-energy X-ray Absorptiometry
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FFAR	Free Fatty Acid Receptor
fMRI	functional Magnetic Resonance Imaging

GHS-R1	Growth Hormone Secretagogue Receptor 1 (Ghrelin Receptor)
GIP	Glucose-dependent Insulinotropic Peptide
GLP-1	Glucagon-like Peptide 1
GLP-2	Glucagon-like Peptide 2
GLPR1	Glucagon-like Peptide Receptor 1
GRP	Gastrin-releasing peptide
IOM	Institute of Medicine
IRB	Institutional Review Board
LHA	Lateral Hypothalamus Area
MC4R	Melanocortin 4 Receptor
MCTs	Monocarboxylate Transporters
MSG	Monosodium Glutamate
NHANES	National Health and Nutrition Examination Survey
NIH	National Institute of Health
NPY	Neuropeptide Y
NSP	Non Starch Polysaccharides
NTS	Solitary Nucleus
NWRC	Nutrition Wellness Research Center
PET	Positron Emission Tomography
POMC	Proopiomelanocortin
PVN	Paraventricular Nucleus
PYY	Peptide YY
RIA	Radioimmunoassay

RS	Resistant Starch
SCFA	Short Chain Fatty Acid
SFD	Soluble Fiber Dextrin
SMCTs	Sodium-mediated Monocarboxylate Transporters
SOCS-3	Suppressor of cytokine signaling-3
TEE	Total Estimated Energy Requirement
TCA	Tricarboxylic Acid Cycle
VAS	Visual Analogue Scale
VMN	Ventromedial Nucleus

ABSTRACT

The increase in the number of overweight and obese individuals is a public health concern due to correlations of obesity with increased incidence of chronic diseases such as type 2 diabetes and cardiovascular disease. Population data have shown correlations between increased fiber consumption and lower body weight and body mass index (BMI). However, the exact nature of this relationship is not known.

There is considerable variation between different types of fiber and their potential impacts on the physiological regulatory systems of appetite and body weight. The goal of the research presented in this dissertation is to explore the effects of non-viscous, fermentable fiber on appetite and food intake in healthy adults. Collective results from the studies will be used to evaluate short-term appetite and food intake changes as a potential mechanism linking fiber consumption and lower body weight.

We hypothesized that increased resistant starch (RS4)/resistant dextrin consumption will promote satiation and decrease *ad libitum* food intake at the next meal. We further hypothesized that increased RS4/resistant dextrin consumption will increase postprandial satiety as measured by subjective appetite ratings and that this effect will be modulated through changes in plasma biomarkers of appetite. As addition of fiber to a mixed meal can alter the glycemic response of that meal, we are also interested in effect of RS4/resistant dextrin on postprandial glucose and insulin responses.

To test these hypotheses, a series of experiments were conducted. In the first study, all-purpose flour was replaced with Fibersym® resistant starch flour in a breakfast meal

(control: 2g fiber, treatment: 24g fiber). Twenty-seven healthy adults (age: 23±2 years, BMI: 23.0±3.1 kg/m^2) participated in the study. Although there were no statistically significant treatment effects observed for satiety or blood measures (p > 0.05), caloric intake over the entire test day was lower for the resistant starch treatment group after data was normalized (p=0.05).

To investigate potential timing effects of fiber consumption, we conducted a study using one of three isocaloric beverages providing 0, 10 or 20g fiber from soluble fiber dextrin (SFD) with the lunch meal. Forty-one healthy adults (age: 24±4 years, BMI: 23.4±2.5 kg/m^2) participated in the study. Glucose-dependent Insulinotropic Peptide (GIP) was lower for the 20g fiber from SFD treatment as compared to control (p = 0.0001). However, no other treatment differences in blood measures were observed (p > 0.05). Additionally, there were no treatment effects on subjective appetite or food intake during the first 150 minutes post consumption of treatment beverages (p > 0.05). After participants left the lab, the 20g fiber from SFD treatment group was shown to have lower mean hunger (p = 0.005) and desire to eat (p = 0.0001) and higher fullness (p = 0.002) as compared to control. There was no treatment effect on food intake based on diet diaries or total day consumption (p > 0.05).

In a final study, two sources of SFD (corn and tapioca) and two doses (10 and 20g) of fiber were tested along with a control group. Half of the treatment dose was provided in beverage form at the breakfast meal and half was provided 2 hours later in the form of a snack bar. Participants remained in the lab for 10 hours after breakfast and breath hydrogen measures were taken as an indication of colonic fermentation. Forty-three healthy adults (age: 24±4 years, BMI: 23.6±3.5 kg/m^2) participated in the study. Breath hydrogen showed a

statistically significant dose response of SFD. However, there were no other treatment differences observed for blood measures, appetite or food intake over the test day.

In conclusion, results from these studies demonstrate that under laboratory conditions, increased resistant starch/resistant dextrin consumption did not affect *ad libitum* food intake or subjective appetite ratings. Although in free-living conditions, appetite and food intake changes were observed, they were modest in magnitude and inconsistent between studies. Furthermore, while we report evidence of resistant dextrin fermentation in healthy young adults, there is no robust effect of biomarkers of satiety or glycemic response. These results are important as they show that a single dose of 10-20g non-viscous, fermentable fiber is not sufficient to impact next meal energy intake. Additionally the overall findings do not support short-term changes in appetite as an underlying mechanism to link potential effects of fiber on body weight.

CHAPTER 1

GENERAL INTRODUCTION

Introduction

Research interest in appetite and the physiological regulation of body weight is largely focused on reducing the obesity epidemic. Obesity is a state of excess body fat that has negative impacts on an individual's overall health. Body mass index (BMI) is a ratio of an individual's weight in relation to his or her height and is typically used to classify obesity. Research has shown that BMI is strongly correlated with the gold-standard methods for direct measurement of body fat such hydro-densitometry or dual-energy x-ray absorptiometry (DXA) [1]. Additionally, it is a simple and inexpensive way for clinicians to identify individuals who might be at greater risk of health problems due to their weight [2]. Data from the 2013 Behavioral Risk Factor Surveillance System (BRFSS) survey reported nearly two thirds of the adult US population are overweight ($25.0 \leq BMI \leq 29.9$ kg/m^2) or obese ($BMI \geq 30.0$ kg/m^2). These self-reported data reflect population-sampling data from the National Health and Nutrition Examination Survey (NHANES) [3, 4]. Gender analysis showed that 47% of men and 22% of women were overweight, while the prevalence of obesity was 33% and 36% for men and women respectively [5, 6].

As a person's body fat increases, their risk of developing obesity-related illness such as type 2 diabetes mellitus, cardiovascular disease and certain types of cancer also rises [7-10]. Recent meta-analysis data estimate that for every unit increase in BMI (1 kg/m^2) adults age 30-59 the risk of developing type 2 diabetes increases by 20%. The same is true for the development

of hypertensive disease [11]. Obesity also increases medical costs by as much as 42% as compared to normal weight individuals [12]. For these reasons, decreasing the prevalence of obesity is a major public health concern in the United States as well as other developed countries.

A number of epidemiological studies link increased fiber consumption with and lower body weight [13] lower body fat [14] and lower BMI [15, 16]. Although numerous mechanisms by which fiber could impact satiety and body weight have been suggested [17], establishing a causal relationship is difficult due to the complex nature of fiber. There are numerous types of fiber each with unique physical properties and potential impacts on various aspects of human health. Generally speaking, fiber is the portion of plant material that is resistant to enzymatic digestion and can be separated into three broad categories: (1) complex carbohydrates, such as cellulose and hemicellose, that contribute to the structural integrity of plant cells, so called non-starch polysaccharides (NSPs), (2) non-carbohydrate substances, such as lignin and waxes, that form complexes with NSPs in cell walls, and (3) non-structural carbohydrates that are resistant to digestion [18, 19].

Although increased fiber consumption may be a useful strategy to manage body weight and decrease the number of overweight and obese individuals, dietary fiber intake is below recommended levels in many countries. In the U.S. the average intake for men and women is approximately half of recommended amounts. [20, 21]. Furthermore, interventions focused on increasing the consumption of high fiber foods such as fruits and vegetables have had limited success in increasing fiber intake [22, 23]. Therefore, fiber fortification of commonly eaten foods may be a more effective strategy for appetite and weight management interventions. Unlike

naturally occurring dietary fiber which are primarily NSPs and non-carbohydrate substances, fiber fortification generally utilizes non-structural carbohydrate fibers. Examples include resistant starches and resistant dextrins because their bland taste and stability across a wide range of pH and temperatures makes them easy to incorporate into a wide variety of commercial foods [24, 25]. For that reason, resistant starch and resistant dextrin will be the focus of this dissertation.

Resistant starches (RS) can be classified according to physical structure and nutritional characteristics. RS1 is physically surrounded by indigestible material making the starch inaccessible to digestion. RS1 is found in whole grains, seeds and legumes. RS2 is granular type raw starch found in high amylose corn and unripe bananas whose resistance is conferred by tight packing of starch granules. RS3 is retrograde starch that becomes resistant through the cooking and cooling process. During heating, amylose leaches from starch granules into solution forming densely packed, double helices upon cooling which help stabilize hydrogen bonds and makes the starch resistant to digestion. Starches of the RS4 type have been chemically modified, for example through treatment with distarchphophaste ester which creates phosphate cross linkages between starch molecules, rendering them resistant to digestion [25, 26]. A fifth type of resistant starch has been proposed (RS5) in which starch accessibility to enzymes is limited by the formation of starch-lipid complexes. However, because these lipid complexes are created through chemical modification of the native starch, these complexes may be considered a subset of the RS4 type.

Resistant dextrins are low-molecular-weight polysaccharides produced through modification of individual starch molecules. For example, Soluble fiber dextrin (SFD) is

produced from heat and acid treatment of corn, wheat or tapioca starch to induced branching and increase the number of α-1,6-glycosidic bonds as compared to the original starch [27-29]. The resulting product, SFD, is resistant to enzymatic digestion and represents yet another potential fiber variation. With the creation of numerous chemical modified starches each with their own unique physical properties, the potential physiological impacts of these ingredients vary greatly. In 2009 the Codex Alimentarius Commission (CODEX) proposed that any synthetic or chemical modified carbohydrate polymer must be shown to have a physiological benefit to humans before it can be defined as fiber [30]. As a result, there is a need to test these novel starches for their potential effects on human appetite.

There are a number of physiological mechanisms through which fiber is thought to impact appetite and food intake in humans. These mechanisms are highly variable depending on the food matrix of the fiber and its chemical properties. For example NSP from naturally occurring produce are though do decrease energy intake in part through bulking and increased mastication to slow eating rate [31]. Viscous fibers such guar gum are thought to increase satiety through and decreased gastric emptying and delayed nutrient absorption [32-33]. Finally, of greatest relevance to the fiber types discussed in this dissertation, non-viscous, fermentable fibers are thought to impact appetite through energy capture and downstream signaling of short-chain fatty acids (SCFAs) [34-35]. Although a recent systematic review evaluating the impact of fiber on appetite and food intake in humans included data from over 40 studies, [36] none of these studies investigated RS4 or resistant dextrins. Therefore, the purpose of my dissertation work is to test the impact of RS4 and resistant dextrin on appetite and food intake in humans with particular interest on the physiological changes that may mediate any observed effects.

Dissertation Organization

The dissertation begins with an overall introduction, followed by a review of the physiological regulation of appetite and food intake as well as the influence of fiber on these systems. The next three chapters consist of manuscripts summarizing projects conducted during my PhD work. The first study, "Effect of resistant wheat starch on subjective appetite and food intake in healthy adults" investigates a commercially available RS4 (Fibersym®RW). The following two manuscripts, "The effect of soluble fiber dextrin on postprandial appetite and subsequent food intake in healthy adults" and "The effect of soluble fiber dextrin on subjective and physiological markers of appetite" explore the effects of a novel, propriety source of SFD. These manuscripts include inputs from co-authors, who have contributed to experimental design, data collection and analysis, as well as manuscript preparation. Following the presentation of these manuscripts, a general conclusion chapter is presented to summarize the overall findings, followed by recommendations for future research.

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CHAPTER 2

LITERATURE REVIEW

Physiological Regulation of Body Weight and Food Intake

Theories of body weight regulation

Despite an overall population shift towards higher body weight, individuals are fairly weight stable throughout much of their adult lives with an average annual weight gain of 1-2 pounds [1]. Using the National Institute of Health (NIH) body weight planner, a two pound weight gain averaged over an entire year, assuming no change in physical activity, requires only a 30 kcal/day intake over energy requirements [2]. However, humans do not typically gain weight in an even pattern, usually experiencing long periods of weight stability interspersed with short period of rapid weight change (i.e. the holiday season, freshman fifteen, etc.) [3, 4]. Although most people experience weight gain over decades, these same people still experience long periods of weight stability. The ability to maintain body weight over an extended period of time suggests a robust mechanism that consistently matches energy intake and expenditure.

One of the earliest body weight regulation theories is the Lipostatic Model of Body Fat Regulation or simply the set point theory. Proposed by Gordon Kennedy in 1953, the set point theory postulates the existence of a signal that communicates a person's current state of body fatness to the brain where it is compared to a predetermined "ideal" body fat amount for that individual [5, 6]. A signal reading higher than the set point would drive intake down and increase expenditure to restore the set point. Alternatively, a signal lower than the set point

would increase intake and drive expenditure down to promote weight gain. Despite the discovery of leptin, an adiposity signal, and the ability of the theory to provide an explanation for the common failure of dieting to promote long-term weight loss, there are many limitations to this concept of body weight regulation [7, 8]. Firstly, changes in fat mass only account for small changes in body weight and the set point theory does not account for regulation of lean body mass [9]. Secondly, this theory cannot explain the rapid increase in the incidence of obesity as there is no evolutionary explanation that could explain a sudden upshift in overall population set points of body weight. Finally, the set point theory is entirely physiological and does not take into account any cultural or socioeconomic factors that may impact body weight [10, 11].

An alternative theory for body weight regulation is in many ways the opposite of the set point theory. The settling point theory claims there is no active defense of body weight and that changes in body weight are the result of environmental stimuli. In other words, body weight is the result of a dynamic equilibrium between inputs (food intake) and outputs (energy expenditure). Any imbalance between food intake and energy expenditure results in a change in body weight that stabilizes the original imbalance [11, 12]. The ubiquitous, obesogenic environment that has developed in western societies over the past 50 years consists of larger portion sizes, increased tendency to eat outside the home, and ease of access to highly palatable, high energy density foods [13, 14]. The settling point theory helps to explain the connection between these societal changes and the sudden increase in obesity incidence; however, the theory has its own limitations. For example, energy restriction studies have shown that during weight loss, resting energy expenditure decreases by a magnitude much greater than what would be induced by falling body weight indicating that it

is being actively driven down to counteract the body weight loss [15, 16]. Also, the settling point theory does not explain the large variation in weight gain between individuals subjected to the same environment [1, 17]. Both limitations indicate that there must be a genetic or physiological component that is also contributing to body weight regulation.

The dual intervention point model combines both physiological and environmental influences on body weight regulation into a single theory. In this model, there are two independently regulated body fatness set points that define the boundaries outside of which physiological regulation drives body weight. Between the two set points, environmental factors are the major influence allowing body weight to fluctuate more freely in this region [11, 18]. Evolutionary based theories propose that the lower set point developed to protect against starvation while the upper set point ensures the ability to evade predators [18, 19]. Invention of tools and establishment of community based societies weakened the need for humans to protect against predation perhaps resulting in the development of genetic variations that increased the upper set point. Genetic differences in the distance between the upper and lower regulatory bounds and may help to explain variation in inter-individual susceptibility to environmental influences [11]. High susceptibility to obesogenic, environmental stimuli over most of an individual's body weight range could have the appearance of no physiological control to prevent weight gain. In this way, the dual intervention point model helps to explain the apparent asymmetry in body weight regulation that has been observed [20, 21]. Additionally, increases in the upper set point beyond the socially acceptable limit to body weight may contribute to the perceived severity of the obesity epidemic in some countries. Although 50-70% of variance in BMI is thought to be genetic, it is important to remember that genetic and environmental factors do not impact

physiology independently of each other [22, 23]. Countless combinations of gene-gene and gene-environment interactions create an extremely complex system for body weight regulation that is not yet fully understood.

Hypothalamic regulation of energy homeostasis

These aforementioned theories in body weight regulation are supported by complex, physiological systems centered on the brain's interpretation of periphery signals. The human brain can be divided into three main segments: the hindbrain, midbrain and forebrain the latter of which consists of the cerebellum, thalamus and hypothalamus. The hypothalamus plays an essential role in energy homeostasis due to its ability to sense and process various signals from the body related to energy reserves, translating that information into signals that alter food intake and energy expenditure [24, 25]. The hypothalamus is composed of several functional domains defined by clusters of nuclei. Early research to determine the specifics of hypothalamic processing utilized brain lesion and stimulation techniques, identifying the ventromedial hypothalamic nucleus (VMN) and the lateral hypothalamic area (LHA) as the satiety and hunger centers respectively. VMN stimulation resulted in suppressed food intake while LHA stimulation increased intake. Conversely, induced lesions in the VMN led to hyperphagia and obesity with loss of LHA function driving food intake and body weight down [24, 26]. Although informative, these studies lacked precision, in that stimulation or lesioning of a target area tended to activate or damage nearby areas of the brain as well [27]. Additionally, due to the amount of back and forth signaling between areas of the hypothalamus and other regions of the brain, it is unknown whether the targeted regions are the main source of a response, or if they act as a relay station to other areas of the brain. Redundancy in hypothalamic signaling pathways is also supported by observed regulation

recovery in animals undergoing lesioning procedures [28]. As knowledge about the inner working of the hypothalamus grows, the concept of precise locations for satiety and hunger has been replaced with specific stimulant-response neuronal pathways [27, 29].

The concept of first/second order neuronal signaling in the hypothalamus is one attempt to clarify the pathways involved in energy homeostasis. The arcuate nucleus (ARC) of the hypothalamus is responsible for first order signaling and functions to relay sensory information regarding energy stores and current intake to other areas of the hypothalamus [27, 30]. The pathway begins with stimulation of two distinct cell populations within the ARC. Orexigenic responsive neurons co-express agouti-related peptide (AgRP) and neuropeptide Y (NPY). Anorexigenic responsive neurons co-express proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART). The mechanisms by which POMC/CART and AgRP/NPY exhibit their respective downstream effects is thought to be mediated by second order neuronal signaling in the paraventricular nucleus (PVN) and LHA which further relays information to other parts of the brain and periphery [27, 30-32]. POMC/CART neurons of the ARC signal to the PVN where POMC is converted to a number of neuropeptides including α-melanocyte stimulating hormone (α-MSH) an agonist of the melanocortin 4 receptors (MC4Rs) located in this region of the hypothalamus [31-34]. Activation of MC4Rs results in the suppression of food intake and elevation of energy expenditure [35-37]. CART has also been shown to inhibit food intake in animal models; however, the mechanism of action most likely involves relays through serotonin receptors in the hindbrain rather than the hypothalamus [38-40]. Orexigenic ARC neurons also signal to the PVN within the hypothalamus, however AgRP acts as an antagonist to MC4Rs through competitive binding and inverse agonism of the receptor [41, 42]. Inhibition of MC4Rs

promotes increased feeding and decreased energy expenditure as exemplified by MC4R knockout mice that exhibit an obese phenotype [43, 44]. Finally, NPY is one of the most potent orexigenic molecules known. Part of the pancreatic peptide-fold (PP-fold) family of molecules, this signal exerts its effects through interaction with a family of G-protein receptors (Y1 – Y5) expressed throughout the body including the hypothalamus, thyroid, digestive system and adipose tissue [40, 45]. Orexigenic ARC neurons also project to the LHA where NPY and AgRP stimulate orexins and melanin concentrating hormone (MCH) release, which has been shown to increase food intake in rodent models [31, 38, 46, 47].

The blood brain barrier has a higher degree of permeability near the ARC, which allows neurons in this region of the hypothalamus to receive signals about energy balance directly from the blood. Signals, such as leptin and insulin, provide information on body fat content and current metabolic state. Insulin is a hormone secreted by pancreatic β cells that signals an anabolic state, having downstream effects on carbohydrate, protein and fat metabolism and storage. In terms of body weight regulation, studies have shown that the amount of both basal and post-prandial insulin secretion is highly correlated with body weight and may be dependent on the amount of white adipose tissue in the body [27, 48, 49]. Furthermore, both orexigenic and anorexigenic ARC neurons express a high concentration of insulin receptors. Administration of insulin in the ARC functions to inhibit AgRP/NPY neurons and stimulate POMC/CART neurons resulting in decreased energy intake, increased energy expenditure and net weight loss in animal models [50-53]. While these studies are useful in clarifying signaling pathways, they are an oversimplification of insulin's effect on food intake under physiological conditions. For example, although insulin reaches the brain in concentrations proportional to plasma circulation amounts, increased plasma insulin

typically results in hypoglycemia, which drives food intake up [49, 54, 55]. Additionally, insulin deficiency is not associated with hyperphagia or obesity [10, 27, 52]. In fact, hyperinsulinemia and Type 2 diabetes are common comorbidities of obesity [56-59]. Although a causal link to obesity has not been established, it is possible that the insulin resistance characteristic of Type 2 diabetes also affects body weight regulation pathways making weight loss more difficult.

In addition to its own role in energy homeostasis, insulin also influences the secretion of the adiposity signal leptin. Leptin is an anorexigenic peptide primarily secreted in white adipose tissue [31, 60]. Although the exact mechanisms are still unclear, research has shown that insulin-stimulated glucose utilization in adipocytes may be an underlying factor in stimulation of leptin secretion [61-63]. As is the case for insulin, neurons within the ARC express leptin receptors and administration of leptin to the hypothalamus stimulates POMC/CART and inhibits AgRP/NPY signaling [64-66]. The role of leptin in body weight regulation was solidified through a series of parabiosis experiments using mice with specific genetic deficiencies. The ob/ob mouse does not produce leptin as the result of a gene mutation, but has intact leptin receptors. The db/db mouse, on the other hand, is autosomal recessive for a mutation that makes the leptin receptor inactive although the leptin molecule is still produced normally. Both mouse models have an obese phenotype. However when parabiotically joined to genetically normal mice, the obese phenotype is reversed in ob/ob, but not db/db mice. Additionally, joining ob/ob and db/db mice allows leptin produced in the db/db mouse to circulate to the ob/ob mouse reversing the obese phenotype of ob/ob mouse [67-71]. These results indicated the potential for obesity resulting from a leptin deficiency to be reversed through exogenous leptin supplementation. Despite this seemingly simple

pharmacological treatment for obesity, further research in humans showed that the majority of obese individuals exhibit normal or elevated plasma leptin concentrations identifying leptin resistance as a key concern [72-74]. One potential mechanism for decreased leptin sensitivity is expression of inhibitor molecules in the ARC. Like other cytokine receptors, activation of the leptin receptor triggers release of Suppressor of Cytokine Signaling-3 (SOCS-3), which inhibits further leptin signal transduction [75-77]. Deficiency of SOCS-3 has been shown to improve sensitivity to leptin signaling in the brain, however, it remains unclear whether leptin resistance is a cause or a result of obesity [78-80].

Hindbrain regulation of food intake

The intuitive relationship between food intake and changes in body weight is the basic premise for short-term appetite studies. Throughout much of the 20th century, meal initiation was thought to be controlled by factors related to energy availability. The Glucostatic Theory of food intake for example proposed that reduced plasma concentrations of glucose triggered a response in the brain to increase hunger and initiate food intake [54, 81, 82]. Thus, food intake was a means for maintaining energy homeostasis with meal size being dictated by the amount of energy needed to restore balance [83]. Investigation into this theory showed numerous limitations such as insulin induced, supra-physiological drops in plasma glucose being needed to cause meal initiation in animals and meals being initiated without a corresponding drop in glucose in humans [84, 85]. As a result, similar theories have been proposed with the alternative signals such as hepatic fatty acid utilization, ATP generation, and body heat [10]. One major flaw with all available energy driven models of meal initiation is the timeline over which the proposed signal is responsive to food intake. There is a significant delay between ingestion of food and nutrient absorption. While taste is

a predictor of energy content, modern food additives such as non-caloric sweeteners and monosodium glutamate (MSG) may decrease the reliability between taste perception and actual energy content, making the body dependent on downstream indicators of energy balance [86-88]. Digestion, absorption and metabolism of food are required for changes in energy balance to occur. Therefore, the true energy content of the meal may not be known until hours after meal cessation [27, 89]. Thus, complex system involving signals from different stages of energy consumption and digestion is currently hypothesized to govern meal size and satiety.

While the forebrain is important for long-term energy homeostasis, the hindbrain-gut-axis is important for individual meal size regulation. The hindbrain consists of numerous structures including the medulla, which plays an important role in food intake. The medulla is located on the lower part of the brainstem and controls numerous autonomic functions including those associated with digestion [90]. The term cephalic phase response refers to autonomic, physiological responses to food cues in anticipation of meal initiation that function to increase the efficiency with which the gastrointestinal tract digests and absorbs nutrients. Such responses include salivation, secretion of gastric and pancreatic enzymes and changes in gut motility [91, 92]. Cephalic phase signaling occurs via a cluster of neurons within the medulla known as the solitary nucleus (NTS for the Latin nucleus tractus solitarii). Projections from the NTS innervate the mouth, esophagus, stomach, and intestines with efferents eliciting physiological responses and afferents relaying sensory information back to the brain [93, 94]. Although there is two-way communication between the NTS and various regions of the hypothalamus, physiological response for meal initiation and cessation is largely autonomically controlled and is not thought to require forebrain processing [95, 96].

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Once a meal is initiated, the ability for the medulla to regulate meal size is highly reliant on signals that provide information on the quantity and energy content of food being consumed. In the mouth, afferent taste fibers collect information on the five basic tastes (sweet, salty, sour, bitter and umami) in addition to texture and temperature information. This sensory experience of the meal is transferred to the NTS via the trigeminal nerve, which results in classification of the ingested substance as nutritive or toxic with subsequent reflex to accept or reject that item [97]. Food preferences develop through NTS signaling with downstream nutrient absorption providing positive reinforcement for consumption of similar tasting food. Multiple experiences with the same food create changes in neural pathways of the NTS that more strongly link taste and expected nutritive content [87, 98]. In addition to taste, afferents receptive to touch are also important signals to the NTS. Pressure afferents located throughout the esophagus and mucosa of the stomach function to detect the presence of ingested food signaling that a meal has been initiated [96, 99, 100]. Mechanoreceptors located within the muscle layers of the stomach are responsive to both gastric distension and active contractions and function to relay information regarding the amount of food consumed back to the NTS [101-103]. Previous research has mimicked the gastric distension that occurs during food intake with intra-gastric balloon inflation. Results from these studies show decreased food intake after acute balloon inflation indicating that gastric distension may be a potent signal for meal termination [104-106]. Further investigation however, showed that effects on food intake diminished with chronic distension indicating that adaptation to this signal is likely [107-109].

In order to discern information about the energy content of digested food, chemoreceptor vagal afferents responding to gut peptides are located throughout the gastrointestinal tract. As proposed, gut peptides related to appetite all share certain key characteristics. First administration of the peptide has a predictable effect on food intake that is not the result of illness or abnormal processes. Secondly, administration of an antagonist to the signal produces the opposite effect on intake. Finally, appearance of the signal occurs as part of normal responses to ingested food [27, 83]. For ease of discussion, gut peptides will be separated by the physiological location where they are predominately secreted. However, it is important to keep in mind that these peptides do not act in isolation of each other. Depending on eating rate and meal content, up to 40% of a digesta can reach the small intestine before meal cessation occurs indicating that gastric and upper small intestinal gut peptides may signal energy information concurrently to the NTS [110]. Likewise, even though it takes hours for meal contents to reach the large intestine, colonic gut peptides may be interacting with gut peptides in the upper gastrointestinal tract that are responding to the next meal [111, 112]. Finally, while secreted in the gastrointestinal tract, gut peptides do not exhibit only local effects. As will be discussed below, research has shown changes in plasma concentrations of these peptides with exogenous administration having effects on appetite and long-term energy homeostasis.

In addition to mechanosensory signals, the stomach also generates the gut peptide ghrelin. Ghrelin is a 28-amino acid peptide that is mainly secreted from cells in the mucosa of the stomach. This peptide undergoes post-translational acetylation with medium chain fatty acids which allows it to bind to growth hormone secretagogue receptor 1 (GHS-R1) [113]. To date, ghrelin is the only peripherally secreted hormone shown to increase food

intake in both rodents and humans [114-117]. Ghrelin exerts its orexigenic effects by interaction with vagal afferents as evidenced by the expression of GHS-R1 receptors on a subset of vagal neurons and an abolishment of appetite-stimulating effects in animals that have undergone surgical vagotomy [118, 119]. Unlike satiation peptides, ghrelin plasma concentrations increase before meals and are suppressed by food intake. It has been proposed that pre-prandial surges in ghrelin secretions are a cephalic phase response that may help increase the likelihood of meal initiation [114, 115, 120]. Alternatively, ghrelin induced increases in acid secretion and motility in the stomach suggests that its pre-prandial surges may be a physiological response to help prepare the body for food intake [121]. Interestingly, ghrelin has also been shown to play a role in long-term energy homeostasis. Stimulation of AgRP/NPY neurons in the hypothalamus by ghrelin may help to explain why this gut peptide is inversely correlated with body weight [122-124]. Pharmacological strategies to block ghrelin signaling have been proposed as a viable strategy for weight loss. Targets for these studies include inhibition of ghrelin O-acyltransferase, anti-ghrelin antibodies, and ghrelin-receptor antagonists. Short-term studies in animals demonstrate effectiveness of these methods to decrease food intake and promote weight loss, however successful therapies in humans is still being investigated [114, 125-127].

The small intestine is the site of production for numerous hormones and regulatory factors secreted in response to nutrients passing through the lumen. Enteroendocrine cells, the secretory cells of the intestines, are located throughout the mucosa and are classified by their area of concentration and secretory products. Although they make up less than 1% of the total intestinal epithelial cell population, numerous enteroendocrine secretory products have been proposed to play a role in energy sensing and food intake in humans [128]. For

example, Glucose-dependent Insulinotropic Polypeptide (GIP) is a 42-amino acid peptide secreted from K enteroendocrine cells located primarily in the duodenum and jejunum. Formally known as gastric inhibitory peptide, early research proposed GIP as a satiety hormone due to purported effects to slow gastric emptying and inhibit gastric acid secretion [129-131]. However, these studies were conducted using supra-physiological concentrations of GIP and this function of GIP under normal circumstances is no longer supported [132]. Further research on this peptide demonstrated that normal GIP secretion was closely related to fat and carbohydrate absorption with downstream effects on insulin release [133-135]. This finding supported the potential of GIP to play an important role in NTS nutrient sensing during food intake. However, vagal afferent fibers do not appear to be activated by GIP and there is no evidence of GIP receptors on these nerves [136, 137]. While increased plasma concentrations of GIP have been shown to decrease insulin secretion and may have important effects on postprandial glucose utilization, there is little evidence to support GIP as an important signaling hormone for satiety or food intake.

Eneteroendocrine I cells are also located in the duodenum and jejunum of the small intestine. These cells secrete cholecystokinin (CCK) primarily in response to ingested fat and proteins [138-140]. In humans, proCCK is cleaved by prohormone convertases resulting in the major circulating forms of CCK (CCK-58, CCK-33, CCK-22 and CCK-8) all of which are capable of activating CCK receptors located on vagal afferents (CCKR-1) and in the brain (CCKR-2) [141-145]. CCK signals in tandem with gastric distension through vagal afferents to decrease food intake. The anorexic effects of CCK were elucidated through a combination of CCK blocking experiments and experiments with exogenous CCK. Collectively, results from these studies showed that reduced meal size with CCK

administration was abolished and food intake increased when either CCK receptors are blocked or vagal signaling to the NTS is otherwise impeded [146-151]. CCK for the therapeutic treatment of obesity has been suggested, however experimental results are not encouraging. Chronic administration of CCK is has been shown to be ineffective in reducing long-term body weight. Animals who received exogenous CCK before the start of every meal compensated for decreases in meal size by increasing the number of meals per day [152]. Other studies that attempted to lower body weight through continuous administration of CCK were also ineffective possibly due to the peptide's short half-life of 1-2 minutes [153, 154]. It is possible however, that CCK may play a role in long-term body weight regulation through signaling in the hypothalamus and interaction with adiposity signals [27, 155].

Peptide YY (PYY), named for the two tyrosine (Y) molecules on its carboxy-terminus end, is a 36-amino acid peptide secreted by L cells in the distal ileum and large intestine. PYY contains the same PP-fold structural motif and binds to the same family of receptors as NPY (Y1, Y2, Y3, Y4 and Y5) [156, 157]. The downstream effects of PYY on food intake and body weight depend on the peptide form and location of administration. There are two bioactive forms of PYY: the intact peptide (PYY_{1-36}) and PYY_{3-36}, in which the two amino-terminal amino acids have been cleaved by dipeptidyl aminopeptidase IV (DPP 4) [158]. When injected directly into the brain, PYY_{1-36} has been shown to have preferential affinity for Y1 and Y5 receptors in the hypothalamus and has potent orexigenic effects similar to NPY [159-162]. Alternatively, exogenous administration of PYY_{3-36} has been shown to suppress food intake and promote weight loss in rodent and humans through activation of Y2 receptors [163-166]. This contradictory impact of PYY on appetite is due to differences in receptor effects within the hypothalamus. Y1 and Y5 receptors are stimulatory

of AgRP/NPY neurons while Y2 receptors are presynaptic autoinhibitory receptors for AgRP/NPY neurons [167-169]. The respective orexigenic and anorexigenic activities of PYY_{1-36} and PYY_{3-36} are also reflective of normal changes in plasma concentrations of each form with PYY_{1-36} predominating in the fasted state and PYY_{3-36} being more abundant post-prandially [158].

Glucagon-like peptide 1 (GLP-1) is also secreted by enteroendocrine L cells. GLP-1 is derived from proglucagon, the parent molecule of a number of other hormones and regulatory factors such as glucagon, oxyntomodulin, GLP2, and glycetin [167]. GLP-1 receptors (GLP1Rs) are expressed in in the gastrointestinal tract, pancreas, and vagal afferents indicating potential for GLP-1 to modulate food intake and glucose regulation. In the ARC of the hypothalamus, GLP1Rs are located primarily on POMC/CART neurons. In rats, GLP-1 administration in the brain resulted in substantial decrease in food intake with peripheral administration in rats and humans confirming the anorexic potential of GLP-1 [170-173]. Though consistent, results from these studies are most likely not representative of gut produced GLP-1 impact on satiation. Although GLP-1 plasma concentrations increase post-prandially, GLP-1 has a short half-life being rapidly degraded by DPP 4. It is estimated that only 10-15% of secreted GLP-1 ends up in circulation and it is unlikely that small changes in plasma GLP-1 that occur under normal physiological conditions stimulate the changes on food intake seen in the aforementioned studies [174]. The physiological importance of GLP-1 on satiation is also questioned by research showing GLP1R deficient mice have normal food intake and body weight [175]. Therefore, the most important biological roles for GLP-1 are most likely its downstream effects as an incretin and its role in signaling for the enteric nervous system.

Peak plasma PYY and GLP-1 concentrations occur 1-2 hours after meal ingestion, long before the bulk of nutrients have reached ileum or large intestine [96, 176]. This pattern of secretion suggests the existence of non-nutritive stimulation of L cells through the enteric nervous system that relays nutrient information in the duodenum through vagal afferents to downstream parts of the gastrointestinal tract [177, 178]. While the enteric nervous system may be vital to initial PYY and GLP-1 secretion, L cells are highly responsive to macronutrient content of meals with carbohydrates, lipids and protein respectively eliciting increasingly larger PYY and GLP-1 responses [142, 162, 164, 179]. Vagal afferents in the ileum and large intestine express Y2 and GLP1R receptors providing a mechanism by which these peptides can provide feedback to the enteric nervous system completing the so called "ileal brake" loop. The majority of carbohydrates and protein are usually broken down and absorbed before reaching the ileum. If a significant concentration of nutrients reaches the ileum, nutritive stimulation of L cells increases PYY/GLP-1 secretion that in turn delays gastric emptying and slows gastrointestinal motility [180]. Slower gastric emptying lessens the digestive load on the upper small intestine allowing for increased absorptive efficiency. In turn, fewer nutrients reach the ileum, PYY/GLP-1 secretion is decreased and inhibition on gastric emptying is removed thus completing the loop [181-183].

In addition to the aforementioned peptides, there are a number of other gut derived signals, including apolipoprotein A-IV (Apo-AIV), gastrin-relasing peptide (GRP), somatostatin, oxymtomodulin, and enterostatin, that have been reported to reduced meal size [83, 167]. Additionally, it has also been proposed that signals from the microbiota of the large intestine play a role in communicating between the gut and brain [184]. The numerous

overlapping signals produced within the gut create a complex regulatory system. In upcoming sections, the impacts of fiber on this system and resulting effects on appetite and food intake will be discussed.

Non-homeostatic influences on appetite and food intake

In addition to regulatory factors for homeostatic control of body weight, other physiological processes that integrate sensory perceptions and host of social and environmental factors also influence human eating behavior. One of the biggest motivators of food selection and meal size is the hedonic reward system. Neurological pathways throughout the brain gather sensory information about the taste as smell of food and connect that information to the secretions of opioids such as dopamine [185]. Palatability ratings increase for those foods that have a higher reward value. Highly palatable foods are often characterized by high fat or sugar content and increased energy density [186]. As a result of increased meal size and energy density, studies have shown increased energy intake with increased palatability in both animal and human models [187-189]. Sensory-specific satiety is important to the hedonic reward system as the pleasant responses to food ingested decreases the more that food is consumed [190, 191]. Numerous studies have shown that increased variety in the meal helps to limit dampening effects of sensory specific satiety leading to increased intake [192-194]. Interestingly, perceptions in variety are not limited to differences in food orosensory qualities. Changes in visual perception of calorie-matched foods have also been shown to increase intake [195, 196].

Cultural and societal standards also have strong influences on food intake and body weight. Cultural norms influence food preferences, eating rate and expectations of

appropriate portion size. For example, portion sizes in restaurant and pre-packaged foods have increased dramatically in the U.S. and other western countries [197, 198]. Research has shown that larger portions lead to increased intake in both meals and snacks [199-202]. Meal size has also been shown to increase with increased social interaction, possibly as the result of social cues and increased meal duration [203]. Environmental factors impact meal initiation as much or more than biological factors. People eat when they are not hungry if the social setting dictates its appropriateness or don't eat when they are hungry for reasons such as time constraints, lack of availability of food or self-restriction. Although discussed individually, social, hedonic and homeostatic influences on appetite occur simultaneously in free-living conditions. In appetite research, these factors are rarely studied together with two of the three categories being controlled for. For the research conducted in this paper, social and hedonic influences on appetite will be controlled for in an attempt to focus on the influence of fiber on homeostatic influences on appetite and food intake.

<div align="center">Fiber Definition and Classification</div>

The term fiber was first used by Australian scientist Eben Hipsley in 1953 as a short hand description for the molecules that make up the plant cell wall. Plant cell walls are composed of carbohydrates such as cellulose, hemicellulose, and pectin, held together in complex structures along with lignin and phenolic acids. This structure-based definition of fiber was updated in the 1970s to include a human physiological component: resistance to enzymatic digestion [204, 205]. In normal carbohydrate metabolism, starches are broken down by alpha amylase, an $\alpha(1-4)$ glycoside hydrolase which is secreted by the pancreas and salivary glands in humans. This calcium dependent metalloenzyme acts at random locations

along long-chain polysaccharides yielding maltotriose, maltose, glucose, and limit dextrins from amylose and amylopectin in starches. These carbohydrate metabolites are further digested to monosaccharaides by isomaltase and disaccharidases then absorbed in the small intestine. Unlike digestible carbohydrates, fiber monomeric units are joined by $\beta(1-4)$ glycosidic bonds which are not substrates for alpha amylase. As a result, fibers are able to pass through the early digestive tract undigested [206].

Throughout the 20th century, one main goal of fiber research was to assess potential health benefits of consumption. In 2000, the American Association of Cereal Chemists (AACC) assessed the existing literature and concluded that three physiological effects of increased fiber consumption were well substantiated. These impacts were included in their official definition of fiber, which states that dietary fiber "promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" [207]. The impact of fiber on these endpoints was supported by numerous epidemiological and clinical trials where fiber was consumed from whole foods or isolated from foods where they naturally occur [208-211]. In addition to including health effects of fiber, the AACC 2000 definition also clarified information related to fiber digestibility to state that while resistant to enzymatic digestion, dietary fibers are partially or completely fermented in the large intestine. Fermentation is enzymatically-controlled, anaerobic breakdown of an energy-containing compound. In humans, this occurs in the large intestine through a symbiotic relationship with colonic bacteria [207, 212].

Adequate intake recommendations for dietary fiber intake are 38 g/day for men and 25 g/day for women [213, 214]. However actual consumption is much lower at 18 and 15

g/day respectively for men and women according to the most recent NHANES data [215]. The low intake of natural high fiber foods relative to recommended levels created a strong interest in development of foods that contained added fiber. The definition of fiber continued to evolve as scientific advancements led to the development of synthetic, enzymatically resistant, carbohydrates. In 2001, the Institute of Medicine (IOM) proposed a separation of endogenous food fiber (dietary fiber) and fiber that had been isolated or synthesized (functional fiber) [216]. The motivation to distinguish the sources of fiber was based primarily on a desire to distinguish the health benefit differences between the two. Dietary fiber, as defined by the IOM, was correlated with lower risk of type 2 diabetes and cardiovascular disease [217-222]. However, because interventions were run with whole foods, it is difficult to distinguish a unique effect of fiber from an effect of high fiber foods where fiber content is usually associated with vitamins and minerals that also may impact the measured outcomes. Functional fibers on the other hand are studied in the absence of these confounding factors and present an opportunity to investigate the physiological role of fiber on its own. The proposed two-pronged IOM definition also requires that novel synthetic fibers be shown to have a physiological benefit to humans before being classified as functional fiber. This change represents a shift in the concept of fiber, such that its resistance to enzymatic digestion is secondary to its ability to impact human health.

By the mid 2000's, world-wide agencies attempting to define fiber generally fell into two camps: those focused on analytical methods of measuring fiber and those focused on physiological health benefits of fiber. This difference in approach was especially confusing for starch derived functional fibers such as resistant starch and resistant dextrins. Some analytical methods for measuring fiber eliminate all starches. As a result, starch derived

functional fibers were not recognized in some agency definitions of fiber [205, 223]. In an effort to standardize the definition and labeling of fiber globally, in 2009 the Codex Alimentarius Commission (CODEX) gave the following definition: *Dietary fibre means carbohydrate polymers with 10 or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:*

1. *Edible carbohydrate polymers natural occurring in the food as consumed*

2. *Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities*

3. *Synthetic carbohydrate polymers, which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities*

The most notable change made by the CODEX from the IOM definition is to include non-endogenous sources under the heading of dietary fiber once they are shown to have health benefits in humans. Other changes include the limitation of the term fiber to "carbohydrate polymers", although a footnote does extend the definition to include lignin and other compounds when associated with, but not isolated from, complexes of plant origin, and specification that polymers be at least 10 units in size. The size limitation was the result of solubility differences for smaller oligosaccharides that made them more difficult to accurately measure [205]. However, no digestive physiological differences exist and a second footnote leaves the decision to include non-digestible carbohydrates 3 to 9 units in length as dietary fiber up to national authorities [223]. Although the U.S. currently does not

have a formal definition of fiber, the Food and Drug Administration (FDA) is set to adopt the CODEX definition of fiber effective in 2016 [224].

The CODEX's focus on health benefits for non-endogenous fiber sources presents a need for research to thoroughly investigate potential claims for novel, man-made fibers. While there may be benefits to disease risk, this dissertation will focus on the potential satiation effects of such fibers and their impact short-term energy intake. In order to more easily understand the various impacts of fiber on appetite, it is best to first subdivide fiber into meaningful categories. The most widely accepted classification of fiber is based on its solubility in water creating two main groups: insoluble and soluble fiber. This classification, however, is based on analytical methods of measuring fiber in foods and is not always descriptive of differences in physiological impact between fibers [225]. Insoluble fibers, for instance, vary greatly in the degree they are fermented in the colon and soluble fibers differ in their ability to change the viscosity of the digestive fluid. Furthermore, the impacts of soluble and insoluble fibers on human physiology are inconsistent within these classifications [226]. For these reasons, instead of discussing the impacts of soluble and insoluble fibers on appetite, fiber will be discussed in terms of their viscosity and ability to be fermented.

Physiological Effects of Fiber

Viscosity and gastric motility

Fiber's potential impact on appetite begins in the mouth. Many naturally occurring high fiber foods require increased chewing to create particle sizes small enough to swallow. This increased mastication increases oral processing time and may help to decrease caloric

intake by slowing consumption rate and increasing satiety hormones [227-229]. In addition to this mastication effect, some fibers increase the viscosity of chyme in the stomach and small intestine. Dynamic viscosity is defined as the resistance of a fluid to flow and is measured in pascal-seconds. Studies have shown that increased meal viscosity is associated with increased satiety and decreased energy intake [230-232]. Although a recent systematic review of short-term feeding studies did not support the theory that viscous fibers had enhanced satiety effects over other fiber types, changes in viscosity represents a potential mechanism through which fiber can influence appetite [233]. It is hypothesized that potential satiating effects of viscous fibers are mediated by physiological changes on the gut including increased gastric distension and decreased gastric emptying rate [234, 235].

Gastric distension is one of the first satiation signals that occur during food intake. Chewing and swallowing activate mechanoreceptors in the mouth and pharynx, which elicit a vagal nerve reflex in the stomach to relax to accept the food bolus. Initial reflexive relaxation is supported by adaptive relaxation in the stomach as the gastric reservoir fills to allow for storage and digestion of the bolus by gastric juices. Distension in the reservoir stimulates contractions that move some of the bolus to the distal compartment, which functions in grinding and mixing of intragastric contents as well regulation of nutrient delivery to the duodedum. As chyme begins to fill the antrum, distention in this region of the stomach initiates inhibitory signals to the reservoir to promote relaxation and prolonged storage [236, 237]. As described earlier, sustained gastric distention is an important contributing signal for meal termination as mechanoreceptors in the stomach signal to brain via vagal nerve afferents to the NTS [102, 104].

Gastric emptying is the passage of chyme from the stomach to the small intestine and is regulated by contractions of the pyloric sphincter. The rate of gastric emptying differs for liquid and solid components of the gastric digesta. Liquids are emptied very quickly, followed by solid particles, which first must be sufficiently ground to smaller pieces before passing through the pyloric sphincter [236, 238]. The pylorus prevents the passage of particles greater than 2mm in diameter with larger particles spending more time in the stomach [239]. It stands to reason that the longer particles stay in the stomach, the greater impact they can have on gastric physiology. Studies have shown an inverse relationship between fiber particle size and rate of gastric emptying [240]. Fiber, therefore, can decrease gastric emptying rate by increasing particle sizes in the solid phase or by increasing the viscosity of the liquid phase.

Increased viscosity of the liquid phase increases gastric emptying time by a number of mechanisms. First, viscous fluids are more difficult for muscle contractions to penetrate resulting in weakened propulsion and slower movement between gastric compartments and down the gastrointestinal tract. Decreased propulsion combined with an innate resistance to flow results in overall increased motility time [237]. Viscous fibers such as gums and pectins have been shown to slow gastric emptying rate of the liquid phase in pig models [241, 242] although these effects have not been consistently replicated in humans [243-245]. The magnitude and stability of viscosity change varies greatly with the structure, molecular weight and chemical composition of the fiber being studied [246, 247]. Inconsistencies in observed effect of fiber on gastric motility may be due in part to the stability of gel formation under the acidic conditions in the stomach. Acid stable gels have been shown to stay much longer in the stomach and delay gastric emptying more effectively than non-acid stable gels

[248, 249]. Acid stable gel formation may be key to slowed gastric emptying and increased gastric distention and may help to explain feelings of increased satiety in some [244, 250-252] but not all [253] appetite studies using viscous fibers.

Glycemic Control

In addition to potential satiating effects, viscosity also alters nutrient digestion, absorption and subsequent hormone signaling in the small intestine. Viscous fibers form a gel matrix around undigested nutrients, which is more difficult for both pancreatic enzymes to penetrate and digested nutrients to dissolve out of. As a result, digestion and absorption occur more slowly and a greater concentration of nutrients reaches the distal small intestine increasing secretion of incretin hormones GIP and GLP-1 [246, 247]. GIP and GLP-1 have both been shown to stimulate insulin secretion through binding with their respective receptors on pancreatic β cells [254]. Furthermore, as described earlier, GLP-1 functions to slow gastric motility via the ileal brake loop thereby decreasing the rate at which carbohydrates are digested and absorbed into the blood. Ingestion of viscous fiber therefore potentially lowers plasma glucose concentrations through increased incretin secretion and decreased gastric motility. While some studies have confirmed an effect of viscous fiber on glycemic response [255, 256], others have found no response or overall decreases of plasma incretin concentrations [257, 258]. Depending on the dosage and the chemical properties of the viscous fiber used, decreased glucose absorption and increased incretion secretion are two mechanisms by which viscous fibers, more than non-viscous fibers may help to attenuate the glycemic response of a meal [259, 260].

While viscous fibers may have a stronger impact on postprandial blood glucose concentrations, epidemiological data correlates non-viscous cereal fiber intake with a decrease in type 2 diabetes incidence [222, 261]. This correlation is seen even after the correction for potential confounders including body weight and antioxidant consumption. Non-viscous cereal fibers do not have the same physiological impacts on nutrient absorption and gastric motility as viscous fibers and thus a definite mechanism behind their potentially beneficial glycemic effects has not been elucidated [259, 260]. However, some proposed underlying mechanisms include impacts on overall insulin sensitivity [262, 263], fermentation product regulation of glucose production [264] and long-term changes in gut microbiota [265]. A more detailed explanation of the physiological effects of fiber fermentation will be discussed in upcoming sections.

Fermentation

One of the most well known qualities of fiber is its ability to be fermented. Unlike ruminants whose digestive physiology begins with fermentation, humans undergo post-gastric fermentation [266]. While digestible carbohydrates are metabolized and mostly absorbed before leaving the small intestine, fiber polysaccharides remain intact as part of the chyme that passes through the ileum of the small intestine into the large intestine. In humans, the large intestine consists of four parts: the cecum, colon, rectum and anal canal. Chyme passes from the ileum and enters the cecum of the large intestine. Though in other species the cecum is robust and a highly integrated part of digestion, in humans it is very rudimentary and serves merely as a connecting point for the ileum and the appendix. Chyme then passes into the colon. The colon also consists of four major sections: the ascending colon, transverse colon, descending colon and sigmoid colon. In terms of functional division, the proximal

colon contains the ascending and transverse colon and the distal colon contains the descending and sigmoid colon [267, 268]. The proximal colon is highly populated with saccharolytic bacteria and receives the highest concentration of undigested polysaccharides. As a result, it is the site of the greatest carbohydrate fermentation and short-chain fatty acid (SCFA) production. Any remaining fibrous material in the chyme then passes to the distal colon. Due to a higher pH, the environment is more favorable for proteolytic bacteria rather than saccharolytic bacteria. The change in microbiotic composition coupled with a depleted substrate supply results in much lower carbohydrate fermentation and SCFA production [269, 270]. Any unfermented fibrous materials become a part of the feces in the rectum, passes through the anal canal and is expelled from the body.

Although the population of saccharolytic bacteria present in the colon is highly diverse, three phyla, Bacteroidetes, Firmicutes, and Actinobacteria predominate [270, 271]. Though differing in end products, the fermentation process starts the same. First, fiber polysaccharides bind to the bacterial surface where they are hydrolyzed by bacterial enzymes to smaller oligosacchardies. These oligosaccharides are transported into the periplasm where they undergo further enzymatic degradation to yield monosaccharides, which are then transported into the bacterial cytoplasm [272]. Bacteria are able to metabolize these sugars for ATP generation via the Embden-Mayerhof-Parnas pathway and the pentose-phosphate pathway ultimately yielding pyruvate [269, 273]. Because colonic bacteria are anaerobic prokaryotes, they lack the enzymes and organelles necessary for cellular respiration. Fermentation is therefore an alternative mechanism to capture some of the remaining energy from the original carbohydrate substrate and SCFAs are the byproducts of this activity [269]. The degree of fermentation and type of SCFAs produced varies based on the substrate and

the particular bacteria doing the fermenting. For humans, the main fermentation end products are acetate, propionate and butyrate produced in a 60:20:20 ratio along with gasses such as carbon dioxide and hydrogen and expelled from the bacteria into the lumen of the colon [267, 274, 275]. These end products are the basis for the symbiotic relationship that bacteria have with other microbiota in the colon and of course, the host organism.

Colonic epithelial cells absorb SCFAs through a number of mechanism including passive diffusion, carrier mediated transport and active transport. As indicated by their name, SCFAs do not have long, hydrophobic areas, which means they do not form aggregates in the digestive tract as long-chain fatty acids do. These smaller, more soluble SCFAs are rapidly absorbed, in part, through passive diffusion into the colonocyte [276]. Passive diffusion requires that SCFAs be in their protonated form in order to pass through the lipid bilayer. Under physiological conditions, it has been suggested that SCFAs exist primarily in their acid form as their pKa is lower than the pH of the colonic lumen [277]. However, this theory does not take into account the large influx of hydrogen ions near the apical membrane sustained by a number of ion exchange pumps that promotes protonation of SCFAs [278, 279]. It is therefore proposed, that passive diffusion accounts for more than half of SCFA absorption in the colon [280]. A second mechanism by which SCFAs are absorbed is through carrier-mediated exchange for bicarbonate with an as of yet unidentified antiporter [269]. Bicarbonate may serve a role in maintaining the pH at the mucosal epithelial surface and support mucous formation [281-283]. Finally, monocarboxylate transporters (MCTs) are also involved in SCFA absorption. There are over 15 isoforms in the human colon and function as proton-coupled, electroneutral cotransporters for SCFA anions. They are of

greater concentration in the distal colon and can also exist in a sodium-mediated form (SMCTs) [284, 285].

Fiber Measurement and Effects on Satiety

Short chain fatty acids and appetite

Once absorbed, colonocytes readily metabolize SCFAs, especially butyrate and acetate for energy. *In vitro* studies show that up to 70% of colonocyte energy needs are met by SCFA oxidation. It is thought that colonocytes metabolize most if not all of the butyrate absorbed. Those SCFAs that escape digestion are transported out of the colonocyte and into portal circulation [269, 274]. Because the intracellular pH is much higher than the lumen of the colon, all SCFAs are thought to exist in their anion form. Therefore, there is very little passive diffusion across the basolateral membrane and transport is heavily reliant on MCT transporters [285]. From portal circulation, SCFAs are transported to the liver where any remaining butyrate, propionate and approximately 70% of acetate are absorbed and metabolized [280, 286]. Propionate is readily converted to succinyl CoA and enters the citric-acid-cycle where it is further metabolized to oxaloacetate and used as a substrate for gluconeogenesis. Acetate is metabolized by hepatocytes for energy as well as used as a substrate for cholesterol and long-chain fatty acid production [287]. Acetate not absorbed by the liver are transported along with other trace SCFAs to other tissues such as muscle and adipose tissue where they are absorbed and metabolized. Though significantly less efficient than in other species, in humans, bacterial fermentation and subsequent SCFA metabolism may contribute 5-10% of daily energy [288].

SCFAs don't just impact energy balance through direct metabolism. Research in the last decade has uncovered Free Fatty Acid Receptors (FFARs) that paint the picture of SCFAs as important signaling molecules for regulation of appetite, fatty acid metabolism and glucose metabolism. Formerly GPR43 and GPR41, FFAR2 and FFAR3 are G-protein coupled receptors that bind SCFAs such as acetate, propionate and butyrate. Both FFA2 and FFA3 are co-localized with enteroendocrine L-cells of the gastrointestinal tract [289, 290, 291]. Increased plasma PYY and GLP-1 levels following fermentable fiber ingestion have been seen in many [292, 293-295], but not all studies [296], suggesting that SCFA stimulates release of these hormones from L cells.

Outside the gut, it is thought that SCFAs play a more direct role in glucose and fatty acid metabolism through influence in adenosine monophosphate-activated protein kinase (AMPK) pathways. SCFAs have been shown to increase AMPK activity in liver, adipose and muscle tissue [297, 298]. Activation of AMPK begins a metabolic response that results in decreased fat storage in adipose tissue and increased fatty acid oxidation and glucose oxidation in other tissues [269, 299]. SCFAs have also been showed to increase leptin expression via a FFA2 – dependent pathway [300, 301]. In addition to receptor-mediated impacts on satiety and metabolism, acetate may itself act as an anorectic signal in the brain. Studies have shown that acetate crosses the blood-brain barrier where it provides energy to glial cells [302]. Recent research has also that acetate preferentially accumulates in the hypothalamus where it is rapidly converted to acetyl-CoA and enters the TCA cycle. Downstream impacts on AMPK and molonyl-CoA concentration are associated with increased expression of POMC and suppression of both NPY and AgRP [303].

Fermentation measurement

Due to the inaccessibility of the site of fermentation, direct measurement of SCFA production is extremely difficult. Most *in vivo* research has used stable and radioisotope techniques in animal models (pigs, cows and rats) to assess production rates of specific SCFAs [304-306]. One limitation to these studies is the questionable relatability of animal model SCFA production to humans due to differences in gastrointestinal physiology. A second limitation is that by design, these studies require the limitation of response measurement to one or two specific SCFAs which may not capture the full range of SCFAs produced from a given food source. Finally, *in vivo* measurements of SCFA production are highly invasive, time consuming and expensive and are therefore rarely done in in humans [307]. *In vitro* techniques using human fecal inoculum show significant variation in SCFA production based on the fiber source utilized [308, 309]. While these data provide some indication on the relative fermentability of various fibers, they are not necessarily representative of what happens *in vivo* as the processes of isolating microbiota for these studies alters the diversity of from that what is naturally seen. Additionally, fermentation end products accumulate during in vitro studies and may reduce the rate of production over time. This artificial reduction in SCFA production may not be representative *in vivo* processes where continuous absorption occurs [269].

Indirect measurement of SCFAs in blood and urine are common, however due to the extremely low concentration of SCFAs in these biological fluids, complicated extraction techniques are required to accurately analyze the samples. Extraction and subsequent analysis requires specific equipment and expertise making the analysis expensive and highly sensitive to variation and inaccuracies resulting from novice analyzers [307, 310]. Additionally, the

value of such data is questionable given that most of the produced SCFAs would have been metabolized by other organs before reaching the blood or being expelled in the urine. SCFA concentrations can also be measured in the feces and data used as an indicator of colonic fermentation. However, it is estimated that only 5-10% of produced SCFA are expelled in the feces and that changes in fecal concentration of SCFA may be more indicative of changes in absorption, rather than changes in production [311].

An alternative approach to direct measurement of SCFAs is to measure other byproducts of fermentation such as gasses. As described earlier, hydrogen, carbon dioxide and methane are also produced by colonic fermentation of fiber. Archaea and other phyla of bacteria use carbon dioxide and hydrogen as substrates for their own metabolism with the excess gasses being excreted in the breath or in flatus [269]. Depending on the microbiota present, some individuals may not produce substantial concentrations of methane. Additionally, methane concentrations may not be sensitive to changes in meal size or composition [312, 313]. Carbon dioxide concentrations in the breath are of course confounded by non-colonic fermentation sources. Hydrogen, therefore, is the best candidate for a fermentation proxy in expired air. Although some hydrogen is lost through other routes, the only source of hydrogen gas in the breath can be from bacterial fermentation in the bowel. Additionally, breath hydrogen concentration has been shown to correlate well with the hydrogen concentration produced in the colon [314, 315]. In a practical sense, the test is non-invasive, participants can collect the samples themselves and samples can be stored for up to two weeks at room temperature. Furthermore, the biochemical analysis is simple, reliable, and inexpensive making it a good choice for large, human studies [316].

Caloric dilution

In population studies, low fiber diets have been associated with increased incidence of chronic disease including obesity [317-319]. Likewise, high fiber diets have been associated with lower waist circumference and BMI in both children and adults [320-323]. Reviews of interventions studies on the effects of fiber on weight loss have found improved weight loss in high fiber vs. low fiber intervention [235, 324]. This trend is true for whole food and supplemental fiber interventions investigating both viscous and non-viscous, fermentable fibers. In addition to previously described impacts on satiety, fiber is thought to decrease energy intake and aid in weight management through energy dilution of carbohydrate based foods.

Short-term feeding studies have shown that people are generally more sensitive to the weight or volume of food consumed and not the amount of energy consumed [201, 325, 326]. Because fiber is not readily digested, the overall energy density of the food is diluted with increased fiber content. Consumption of a consistent weight of a lower energy dense food results in decreased overall energy intake and may promote weight loss. Due to energy losses associated with fermentation, fiber does not provide the 4kcal/g of energy expected from readily digestible carbohydrates. However, as described earlier, SCFA metabolism resulting from fiber fermentation means that some energy is recovered from fiber and in some respects, fiber is in fact metabolized. Research determining the caloric value for different fibers shows a typical range of 0 -3 kcal/g for fiber depending on the chemical composition and properties of the fiber tested [327, 328]. It is estimated that non-viscous, fermentable fibers such as resistant starch and resistant dextrin provide about 1.5-2.0 kcal/g and are therefore likely to have meaningful caloric dilution effects depending on dosage [328].

Appetite Measurement

Although the concept of appetite is intuitive and simple it is actually the sum of three distinct components: hunger, satiation and satiety. Hunger is the motivation to eat and is often measured in sensory terms through cognitive reflection by participants. Once food intake is initiated, satiation determines meal size and duration and is measured by collecting data on caloric intake. After cessation of an eating event, satiety is defined as the period before the next meal starts [329]. In its purest form, satiety can be measured in terms of the amount of time taken between meals; however, in research the timing of meals is often set by the researcher. Decreased hunger and changes in biomarkers of appetite are associated with increased satiety and can be used as a satiety proxy in research where meal times are fixed.

Questionnaires

Appetite research is heavily reliant on human introspection about habits and sensory information related to food intake. Questionnaires are an important tool for gathering and organizing this information in meaningful ways. Two common approaches to the design of appetite questionnaires are fixed point and visual analogue scales (VAS). Fixed-point scales come in a variety of forms including Likert-type scales (strongly agree/disagree), frequency (times/week, never, sometimes, often) and preference (e.g. palatability on a scale of 1-10). Fixed-point scales are excellent screening tools as they allow for quick categorization of potential participants concerning normal eating patterns, food preferences and motivations to eat [330, 331]. The points on such questionnaires are often ordinal in nature, though it is difficult to quantify the meaning of category intervals [332]. Additionally, fixed-point scales

may not be sensitive to acute changes in sensory perception as, with repeated measurement in a short time period, it is easier for participants to remember earlier responses.

VAS questionnaires use a 100 or 150mm line anchored with extremes of the sensation being measured (e.g. 0mm = not hungry at all and 100 mm = as hungry as I have ever felt). Participants are instructed to mark the line that corresponds to their current state. For analysis, the response is quantified by measuring the distance of the participants mark from the left end of the line [333]. Any number of appetite related questions can be asked using VAS; however future food intake seems to be most highly correlated with the following questions: How hungry do you feel right now? How full do you feel right now? What is your desire to eat right now? What is your prospective consumption right now? [334, 335]. Questions about preoccupation with food and desire to eat foods with specific sensory qualities (i.e salty, fatty, sweet) help to provide a more thorough view of appetite changes, however their sensitivity is highly dependent on participants' ability to distinguish those perceptions from similarly phrased questions. Thirst ratings are also commonly collected to help the participant separate what is thought to be two concurrent sensations [336]. Overall VAS is an easy tool to capture changes in appetite over time though the ability to separate out treatment effects may be dampened by subjects' reluctance to make full use of the scale (either sticking to or staying away from extremes) which has been shown to be more of a problem from electronic VAS systems then traditional pen and paper models [337].

Biomarkers of satiation and satiety

There are two main approaches to measuring biomarkers of satiation and satiety: biomarkers in the brain and biomarkers in the periphery. Measurement of central nervous

system biomarkers of appetite is commonly done through positron emission tomography (PET) or function magnetic resonance imaging (fMRI). In PET, intravenous ^{15}O is administered and used to measure cerebral blood flow. Sites of brain activity have increased blood flow and will therefore uptake more ^{15}O then surrounding areas and emit more gamma rays for detection [338]. In fMRI, the subject is placed within a strong magnetic field and radiofrequency pulses are applied to excite hydrogen atoms throughout the brain. When these excited protons return to their original energy state, they emit radiowaves for detection. Increased blood flow in activated brain areas decreases the concentration of deoxygenated hemoglobin in these areas. Deoxygenated hemoglobin locally distorts the magnetic field and affects the relaxation process of activated hydrogen atoms in the brain. Less distortion results in small increases in the fMRI signal [339]. Both PET and fMRI are geared towards measuring hedonic appetite and sensory-specific satiety and have indicated differences between lean and obese subjects relative to these measures [340]. While suitable for hypothesis building, these scans cannot be used to establish a causal relationship between stimulus and response due to the indirect nature of the data. Use of PET and fMRI are also limited by the high cost, specialized equipment and training needed to administer and analyze the data. Furthermore, due to the extreme physical nature of these techniques (participants must be lying down with their head restrained) the types of manipulations that can be measured are highly restricted and results may not have much external validity [333].

Changes in plasma concentration of gut peptides and glycemic response are the main peripheral biomarkers of appetite and physiological control of meal size. Plasma concentration of hormones such as CCK, PYY, ghrelin, insulin and GLP-1 are commonly quantified using radioimmunoassay (RIA) techniques. In RIAs, a sample of human plasma

containing the molecule of interest is incubated with a known quantity of antibody for that molecule. A radioactive version of the target molecule, known as the tracer, is then added to the mixture. Unbound antibody binds to the tracer and forms a pellet whose radioactivity can be measured using a gamma counter. There is an inverse relationship between the radioactivity measured and the concentration of the target molecule in the original sample. A binding curve is established for each assay using known quantities of the target molecule. The concentration of the target molecule in the original sample is quantified by plotting its radioactivity on the binding curve [341, 342]. Although the method is theoretically simple, RIAs are not without their limitations. Most importantly, RIAs are an indirect measure of concentration and changes in radioactivity counts are not solely an effect of changes in concentration of the target molecule. Non-specific interference with antigen-antibody binding and quality control of reaction ingredients both influence final readings and will be misinterpreted as changes in hormone concentration. These effects are of particular importance for target molecules whose physiological serum concentrations fall within a tight range [343]. Secondly, it is important to keep in mind that plasma concentrations of these hormones may not accurately reflect the local concentrations in the gut. Results investigating the correlation between plasma concentration of gut hormones and appetite are mixed [344, 345].

Food intake

In research, food intake is measured by two main methods: direct measurement and self-report. Direct measurement of food intake is possible when participants are kept in the laboratory and food selections are limited by the researcher. By weighing portioned food before and after consumption, the researcher is able to measure the weight of food consumed

during the meal and use that information to calculate caloric intake. While highly repeatable and accurate, in laboratory food consumption may not have high external validity [346, 347]. The amount of calories consumed at a given meal in free-living conditions is influenced by food choice, meal timing and social context. These environmental cues are often purposefully controlled in research; therefore food intake changes seen in the lab may not represent intervention effects in a free-living environment.

There are a number of techniques to collect food intake data when participants are not in the laboratory. Among the most commonly assessment tools used are 24-hour diet recalls, food frequency questionnaires and diet diaries. Inaccuracies in self-reporting of food intake have been widely reported [348-350] with particular emphasis on underreporting. The ability for participants to provide an accurate accounting of intake is heavily reliant on each individual's knowledge about the food being consumed (difficulty with restaurant and homemade recipes not prepared by the participant), familiarity with estimating portion sizes (a piece of cake, a slice of pizza), and completeness with reporting all intake details (added oils in cooking or use of condiments). Research into the nature of reporting inaccuracies has revealed gender and body weight differences in reporting accuracy with women underreporting more than men and overweight and obese individuals underreporting more than lean individuals [351, 352]. Additionally, other studies have shown that the quality of reporting improves with training, repeated measurement and decreased time delay between consumption and recording [334, 353]. Despite this information, there are still difficulties in assuring the quality of food intake data in research studies. Furthermore, collection of food intake data without information about the environmental context in which it occurred

complicates interpretation of the data and is a major confounder in many short-term appetite studies.

Overall Hypothesis

The overarching goal of this research is to explore the relationship between fiber consumption, appetite and food intake in healthy adults to provide a mechanism to support epidemiological correlations with lower body weight. A series of experiments were conducted to test the following hypotheses concerning non-viscous, fermentable fibers consumed as part of a mixed meal:

(1) RS4/resistant dextrin consumption promotes satiation and decreases *ad libitum* food intake at the next meal and lower intake over a 24-hour period.

(2) RS4/resistant dextrin consumption promotes postprandial satiety as measured by subjective appetite ratings.

(3) RS4/resistant dextrin fermentation modulates satiety and satiation through changes in plasma biomarkers of appetite.

As described earlier, fiber replacement of rapidly digestible carbohydrate also has the potential to change glycemic response to test foods. Therefore, another objective of the current research is to observe the effect of resistant starch/dextrin consumption on post-prandial glucose and insulin responses.

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CHAPTER 3

EFFECT OF RESISTANT WHEAT STARCH ON SUBJECTIVE APPETITE AND FOOD
INTAKE IN HEALTHY ADULTS

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Abstract

Purpose. The effect of replacing wheat flour with resistant wheat starch in a breakfast meal was investigated in this randomized, cross-over design. **Methods**. Following an overnight fast, 27 healthy adults (aged 23±2 years with a BMI of 23.0±3.0 kg/m^2) consumed standard muffins or muffins where 40% of the wheat flour was replaced with resistant wheat starch. Appetite questionnaires and plasma samples were collected before the test meal and at ten time points following meal consumption. An *ad libitum* lunch meal was provided 240 minutes after breakfast, and the amount eaten recorded. Food intake was measured over the remainder of the day using a diet diary and appetite was measured hourly using an appetite questionnaire. Plasma was analyzed for markers of satiety and glycemic response. **Results.** Replacing wheat flour with resistant wheat starch reduced plasma insulin (p<0.05) but had no effect on plasma concentration of glucose CCK, GLP or PYY concentration (p>0.05). Moreover, there was no effect on appetite ratings, energy consumption at the lunch meal (p>0.05). Total daily energy intake (including the breakfast meal) was reduced by 179 kcal

when participants consumed the resistant starch muffins for breakfast (p=0.05). **Conclusion**. Results indicate that replacing wheat flour with resistant starch decreases plasma insulin concentration and reduces energy intake over a 24 hour period.

Keywords: Dietary Fiber, Fibersym, Appetite, Food Intake

Introduction

The increase in the number of overweight and obese individuals is a leading public health concern as these conditions are associated with an increased risk of chronic disease [1-4]. The recent and rapid increase in the number of overweight and obese people suggests that environmental changes are a key etiological factor. Population studies indicate that a low-fiber diet is a risk factor for weight gain [5-7]. Consumption of dietary fiber is currently below recommended levels in many countries and this may contribute to the overweight/obesity epidemic [8-9]. Increasing the amount of dietary fiber may be a useful dietary strategy to reduce the number of overweight and obese individuals. However, public health efforts to increase fiber consumption have only had limited success [10-11]. A different approach to increasing fiber consumption is to add functional fibers, such as resistant starch to frequently consumed foods.

Resistant starch (RS) is a starch that is not digested in the small intestine and passes into the colon to be fermented by the microbiota [12]. There are five classifications of RS. RS1 is physically inaccessible to digestion and found in whole grains, seeds and legumes. RS2 is innately resistant, granular type found in high amylose corn and unripe bananas. RS3

is retrograde starch that becomes resistant through the cooking and cooling process. Finally RS4 is chemically modified to create structures that are resistant to digestion [13-14]. A further category of resistant starch, RS5, which is a starch-lipid complex has also been proposed [15]. Studies indicate that RS provides fewer calories per gram than rapidly digestible starch [16].

Resistant starch flours have been developed that can replace flour in commonly eaten products. Replacing rapidly digestible starch (RDS) with RS would reduce the caloric content of a meal that could result in weight loss due to an energy dilution effect. However, it is not clear if the reduction in energy intake would elicit a compensatory appetite response. Several gut derived hormones that are related to feelings of satiety are secreted in response to nutrients in the small intestine [17]. Consequently, a reduction in available carbohydrates would presumably reduce the stimulation of some satiety-related peptides resulting in a reduced appetitive response leading to a compensatory response where individuals consume more at the next meal. On the other hand, it has been proposed that humans eat by weight [18-19]. So, maintaining the weight of the food but reducing the energy content would lead to lower overall energy intake.

While RS is not digested and absorbed in the small intestine it may be fermented in the colon to produce short-chain fatty acids (SCFA) [12]. Emerging evidence from cell models indicates that SCFA can stimulate the secretion of the putative satiety hormones GLP-1 and PYY from colonic cells [20-22]. If this effect were to hold in humans this may provide a mechanism for RS to promote satiety although the effect may only become apparent several hours following consumption. Short term feeding studies investigating RS2

and RS3 type starches have found beneficial effects on glycemic response, but weak or no effect on subjective appetite or short term food intake [23-25]. Studies using RS4 type starches, however, have shown greater glucose lowering capacity [26-28]. However, there is little known about the effects of RS4 on appetite and food intake in humans.

The primary aim of this present study was to test the effect of a breakfast meal containing 26g fiber from RS4 type resistant starch on same day caloric intake. Additionally, impacts on subjective appetite, biomarkers of appetites and glycemic response were also measured. We hypothesized that participants consuming resistant starch would have a reduced caloric intake over a single day due to increased satiety and that the glycemic response would also be muted.

Methods

Potential participants were invited to attend a screening session where they completed a questionnaire that posed questions about their general health and dietary habits. Their height and weight were measured to determine if they met the study inclusion/exclusion criteria. Inclusion criteria were: body mass index (BMI) between $18.5 - 29.9$ kg/m^2, aged between 18-35 years, regular breakfast consumers (>5 days each week). The exclusion criteria were: not weight stable (> 3kg weight change in previous 3 months), use/used tobacco products, had the presence of acute or chronic illness or were restrained eaters (>13 on the restraint section of the three-factor eating questionnaire) [29]. During the screening session, potential participants also tasted samples of all test foods to be used in the study and to rate their palatability. Participants were excluded from study participation if their rating for

any test food was less than 6 on a 9 point scale. The protocol was approved by the Iowa State University Institutional Review Board and all participants signed an informed consent form before being enrolled in the study.

Protocol

This study used a single-blind, cross-over design and randomized participants to treatment order. Participants were asked to report to the laboratory on two separate occasion separated by at least one week. The day before each test session, participants were asked to refrain from alcohol consumption and strenuous exercise. They were also asked to refrain from drinking caffeinated beverages for 12 hours before the test session and to not eat or drink anything except water starting at 10:00 pm the night before the test session. Participants were asked to arrive at the Nutrition Wellness Research Center (NWRC) at 8:00 am the following morning. At the start of the test session, an indwelling catheter was placed into the participant's non-dominant arm by the study nurse, and the participant allowed to rest for 30 minutes to acclimatize to the catheter. A baseline blood sample was collected and participants were asked to complete a baseline appetite questionnaire. The participant was then provided with a breakfast meal of muffins and orange juice.

The test muffins were either control made with all purpose flour or resistant starch (RS) muffins containing Fibersym® flour. Participants were asked to eat and drink all food and beverage provided within 15 minutes. After the breakfast meal, participants completed an appetite questionnaire and another blood sample was collected (t=0). Over the next 4 hours, blood samples were collected and appetite questionnaires completed at regular intervals (t = 0+15, 30, 45, 60, 90, 120, 150, 180 and 240 minutes). After the final blood

sample was collected the indwelling catheter was removed from the participants arm and they were allowed to rest for five minutes before being served an *ad libitum* lunch meal. The lunch meal was weighed without the participant's knowledge before and after consumption and the amount eaten recorded. After finishing lunch, participants were allowed to leave the laboratory. They were asked to complete hourly appetite questionnaires and to keep a log of all food and beverages consumed for the remainder of the day. Data from the diet diaries was analyzed using Nutritionist Pro™ Diet Analysis Software (version 2.1.13; First DataBank, San Bruno, CA)

Test foods and beverages

The test breakfast used in this study included Tropicana® orange juice (PepsiCo, Purchase, NY) and in house made breakfast muffins. The control recipe for the muffins included all-purpose flour (Gold Medal, General Mills Inc., Minneapolis, MN) eggs (Great Value, Wal-Mart Stores Inc., Bentonville, AR), whole milk (Great Value), granulated white sugar (California & Hawaii Sugar Company, Crockett, CA), canola oil (Great Value), baking powder and salt. For the RS muffins, 40% by weight of the all-purpose flour was replaced with Fibersym® flour. Egg whites were also added to the RS batter to match the protein and fat content of the control meal. In order to keep the fiber dosage consistent, participants all received three muffins for breakfast. Each RS muffin contained 8.7g fiber for a total dosage of 26g in the breakfast meal.

Including the beverage, the control breakfast provided 777 kcal (16g protein, 122g available carbohydrate, 24g fat, 2g fiber). The resistant starch breakfast provided 693 kcal (16g protein, 102g available carbohydrate, 24g fat, 26g fiber). For the ad libitum lunch meal,

participants were served a meal of pasta and tomato sauce (Barilla Group, Parma, Italy) with shredded parmesan cheese (Kraft Food Groups Inc, Northfield Il) and asked to eat until comfortably full.

Subjective appetite measurements

Subjective appetite was measured using a standard appetite questionnaire [30] that posed the following questions: How hungry do you feel right now? How full do you feel right now? What is your desire to eat right now? What is your prospective consumption right now? How thirsty are you right now? Responses were measured using a visual analogue scale anchored with opposing statements at each end (e.g. not hungry at all or as hungry as I have ever felt) stored on a PalmPilot with a time and date stamp to check for protocol compliance. For data collected outside the laboratory only time-points where 75% of the participants responded within 10 minutes of the correct time were used in the analysis.

Glucose and hormone analysis

Blood was drawn into 4ml EDTA-coated vacutainer tubes, mixed with aprotinin, centrifuged, then divided into aliquots before being stored at -80°C for further analysis. Plasma samples were assayed for glucose using a biochemical analyzer (YSI Life Sciences, Model 2700 select) while all other analyses (insulin, GLP-1, PYY_{3-36}, ghrelin and CCK-8) were completed using established radioimmunoassay (RIA) procedures [31-32]. Plasma samples were ethanol extracted [33] prior to analysis for GLP-1, CCK-8 and PYY_{3-36}. All samples were run in duplicate and all samples from a given participant were analyzed within the same batch. Insulin antibodies and tracers were made in Dr Hsu's laboratory. All other ^{125}I-Tracers used were purchased from PerkinElmer (Waltham, MA). Ghrelin analysis was

done using T-4747 (Bachem, Bubendorf, Sui) antibodies. Antibodies for PYY_{3-36} and GLP-1 were purchased from Bachem (T-4090 & T-4056) while CCK-8 antibody C2581 was purchased from Sigma Aldrich (St. Louis, MO). Detection limits and coefficient of variations (CV) for each of the RIA measured hormones are as follows: Insulin: 3.1 – 400 µU, interassay CV 8%, intra-assay CV 7%; Ghrelin: 50 – 1600 pg/mL, interassay CV 11%, intra-assay CV 7%; GLP-1: 3.6 – 500pg/mL, inter-assay CV 10%, intra-assay CV 8%, PYY_{3-36}: 5 – 640 pg/mL, inter-assay CV 8%, intra-assay CV 3%, CCK-8: 0.6 – 80pg/mL, interassay CV 10%, intra-assay CV 9%.

Statistical analysis

All data is show as means ± standard deviations. Area under the curve (AUC) for blood responses and subjective appetite was calculated using the trapezoid method [34]. Treatment differences for each outcome measured were assessed using a one-way, repeated measures ANOVA. Statistical analysis was conducted using SPSS for Windows or Mac (version 16.0; SPSS, Chicago, IL, USA) with statistical significance set at $p < 0.05$, two-tailed.

Results

Participant demographics

Thirty-one participants were randomized to the study. Four participants (3 male and 1 female) dropped from the study due to schedule conflicts. Twenty-seven participants, 12 female and 15 male, completed the study. The participant's mean age was 23 ± 3 years and mean BMI was 23.45 ± 2.4 kg/m^2.

Blood measures

Figure 1 shows time response and AUC data for glucose and all hormones assessed for the study. Each measure demonstrated the expected post-prandial responses following breakfast consumption with decreased ghrelin and increases in all other measures. Statistical analysis of results revealed lower insulin following RS consumption ($p < 0.05$). No other statistically significant treatment effect was found ($p > 0.05$). F-values for non-significant data are as follows: glucose ($F(1, 26) = 3.43$; $p > 0.05$), GLP-1 ($F(1, 26) = 0.077$; $p > 0.05$), ghrelin ($F(1, 26) = 2.272$; $p > 0.05$), CCK-8 ($F(1, 26) = 0.005$; $p > 0.05$) and PYY_{3-36} ($F(1, 26) = 0.073$; $p > 0.05$).

Subjective appetite

Figure 2 shows time response and AUC data for hunger, fullness, desire to eat and prospective consumption. There was a no treatment effect observed for any subjective appetite measures (data not shown for thirst). F-values for non-significant data are as follows: hunger ($F(1, 26) = 0.119$; $p > 0.05$), fullness ($F(1, 26) = 0.027$; $p > 0.05$), desire to eat ($F(1, 26) = 0.332$; $p > 0.05$), prospective consumption ($F(1, 26) = 0.023$; $p > 0.05$) and thirst ($F(1, 26) = 0.002$; $p > 0.05$).

Food intake

Before analysis, food intake data was normalized by taking the natural log. Table 1 shows mean intakes by treatment along with F and p values for normalized data. Food intake measures consisted of in laboratory, breakfast, *ad libitum* lunch intake and evening food intake measured from participant diet diaries. There was no statistically significant difference in lunch intake or food intake over the remainder of the test day. However, when the

breakfast meal was included in the analysis energy intake was lower on the RS test day (p = 0.05).

Discussion

The results of this study showed that replacing RDS with RS in muffins resulted in reduced energy intake over the test day. This reduction in energy intake did not result in a compensatory appetite response and there was no statistically significant effect on several satiety related hormones in the four hours following consumption of the test meal. There was a reduction in plasma insulin but no effect on plasma glucose. However, it is not clear if this effect would persist over a longer period of time. Longer-term studies to determine the effect of resistant starch on body weight are required.

In this present study, we did not observe a treatment effect on appetite ratings in the four hours post-consumption of resistant starch possibly due to the high satiety value of the vehicle used. In an effort to keep the fiber dosage consistent, all participants were asked to consume the same quantity of food regardless of body weight or BMI. Hunger, desire to eat, and prospective consumption ratings for the control breakfast were reduced to 45% of baseline measures for the first three hours after eating. This was coupled with more than a five-fold increase in fullness ratings over the same time period. The large magnitude and persistence of increased satiety for the control meal may have masked any potential satiating effects of the resistant starch meal resulting in no treatment differences in subjective appetite ratings or satiety hormones.

Although there was no effect of treatment on the amount eaten at lunch or intake for the remainder of the test day, overall daily energy intake (including breakfast) was lower on the day that the resistant starch breakfast was eaten. This indicates that the reduction in the energy content of the muffins caused by replacing rapidly digestible starch with resistant starch was not compensated for by eating more at subsequent meals. This finding is interesting as it provides a potential mechanism to explain population studies which show that high fiber consumption is associated with decreased BMI and body weight [35-37]. Also it provides evidence against complete caloric compensation, which suggests that replacing foods with low-calorie versions will result in the missing calories being consumed later in the day [38-39]. While the breakfast meals differed by over 80kcal, intake at the lunch meal was nearly identical. However there was over 100kcal difference between treatments after participants left the laboratory with participants eating less on days when the resistant starch muffins were consumed. The decreased food intake from diet diaries may be due to in part to physiological changes resulting from colonic fermentation of resistant starch [40-41]. However, future studies would be needed to elicit exact mechanism behind this observed effect.

This study found lower plasma insulin following RS consumption, but there was no effect on plasma glucose concentration. These results are broadly in line with those reported by Bodinham et al [26] but conflict with other studies that have found replacing RDS with RS reduces plasma insulin and glucose [24, 42]. In this present study the available carbohydrate was lower in the RS muffins than the RDS muffins although the amount of available carbohydrate was relatively high in both meals. Differences in the type of foods

used, the type or dose of resistant starch used or the test food used to provide the RS may also explain the different results.

This study has a number of limitations that must be considered when interpreting the data. First the caloric value for the resistant starch breakfast meal is based on manufacturer specifications for Fibersym® which ignores any calories due to insoluble fiber content [13]. It is estimated that non-viscous, fermentable fibers such as resistant starch provide 1.5-2.0 kcal/g as a result of colonic fermentation and subsequent short chain fatty acid production and metabolism [16]. It is likely that the caloric value for the resistant starch breakfast meal is higher than the reported value. Secondly, the observed reduction in total daily caloric consumption is also dependent on self-reporting of food intake from diet diaries. The accuracy of self-reported diet diary intake has long been questioned, with error rates of 20% and greater being estimated [43-45]. However, diet diaries from this study were checked for validity using established methods [46] and no trends in over or under reporting of caloric intake were observed. Finally, this was a single dose, single day feeding study and results cannot be extrapolated to conclude that RS would have long-term effects on body weight. Future research should be conducted to elucidate physiological mechanisms behind the potential satiating abilities of RS and to assess the long-term effects of RS on energy intake and body weight.

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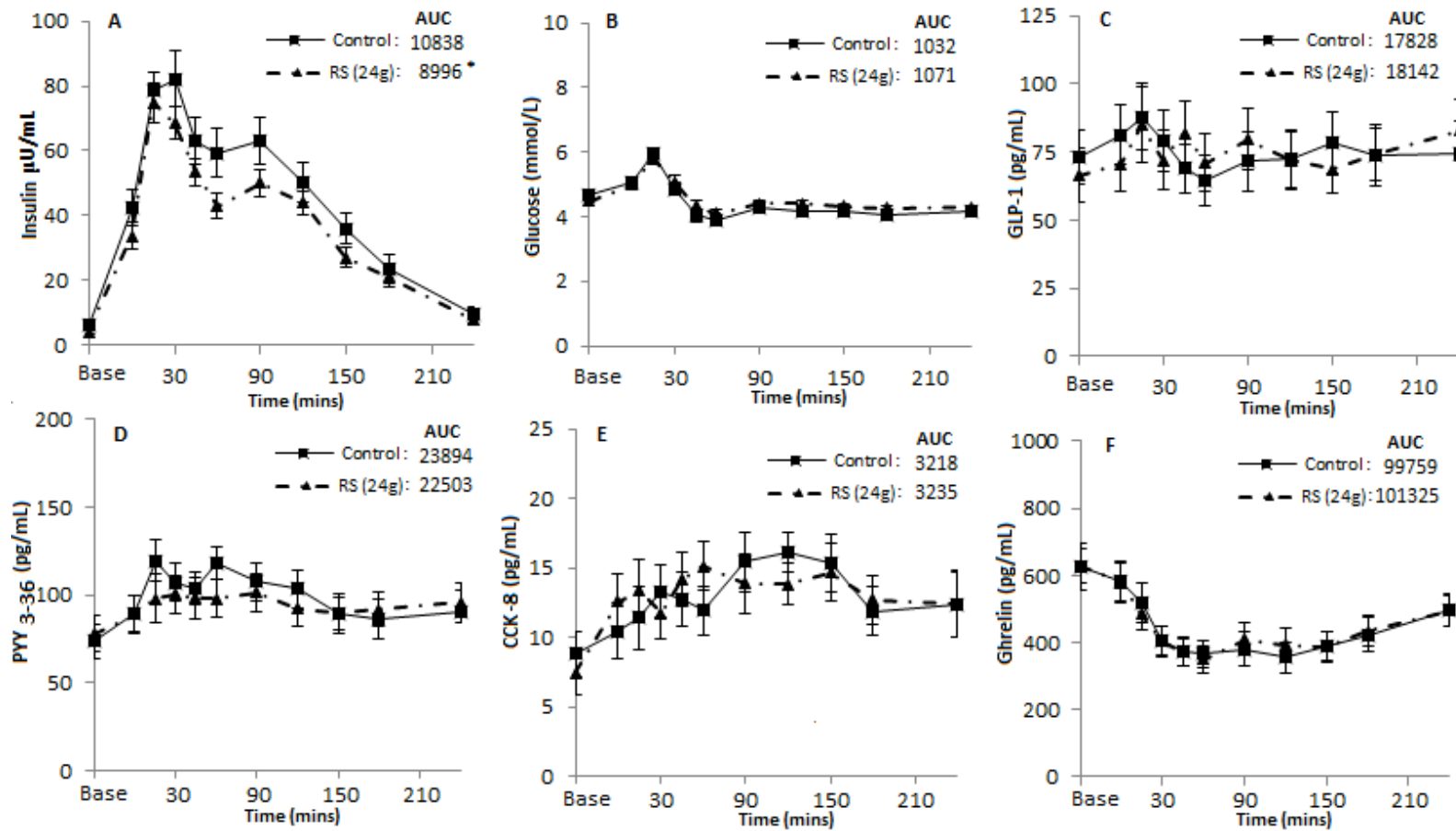
Fig. 1 Plasma hormone responses for insulin (**a**), glucose (**b**), GLP-1 (**c**), PYY$_{3-36}$ (**d**), CCK-8 (**e**), and ghrelin (**f**) measured from baseline through 240 minutes post consumption of a breakfast meal containing 0 g (solid line) or 24g (squares) of resistant starch. * Indicates a statistically significant treatment difference (p < 0.05).
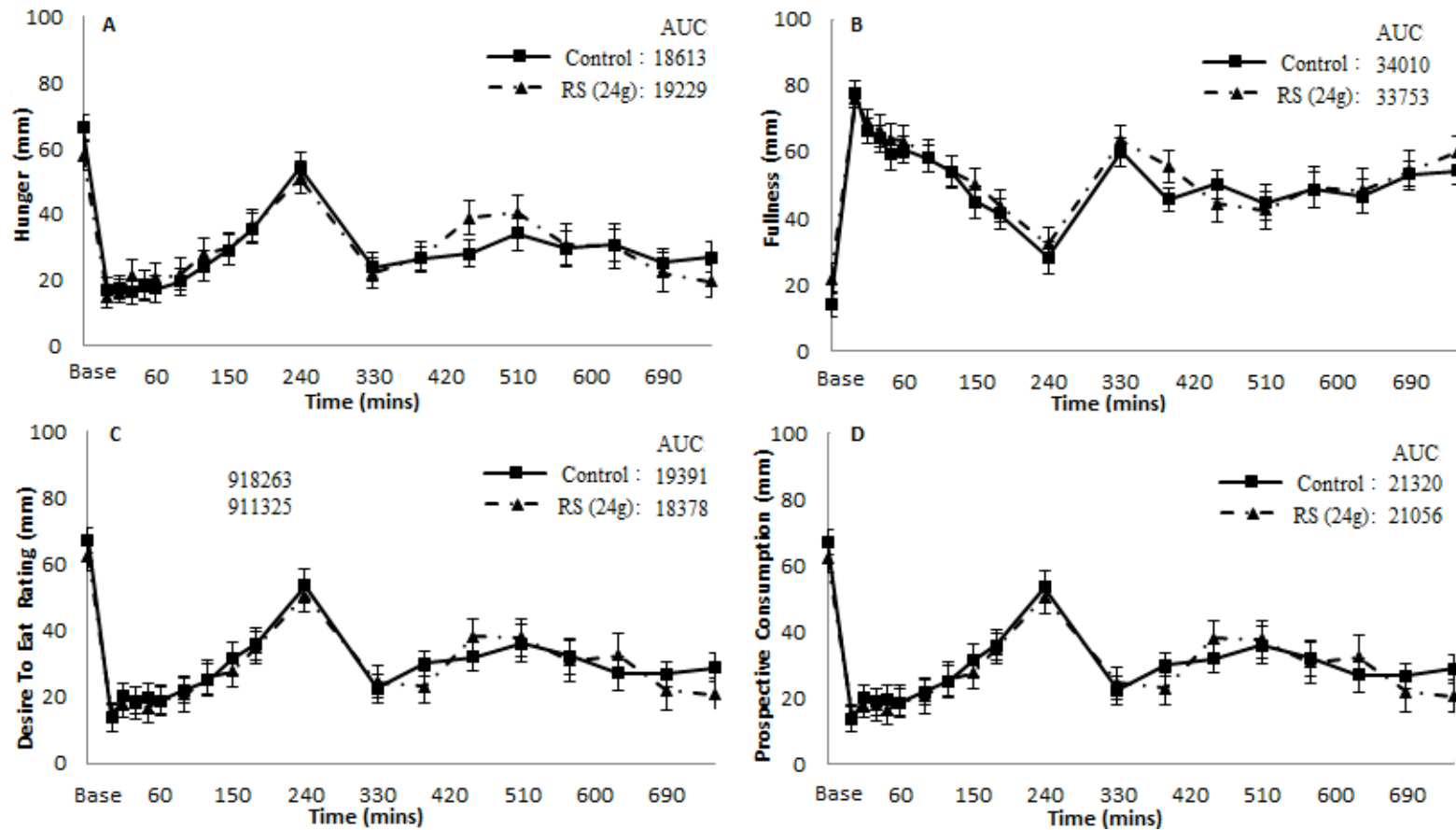
Fig. 2 VAS scores of hunger (**a**), fullness (**b**), desire to eat (**c**), and prospective consumption (**d**) rated from baseline through 240 minutes post consumption of a breakfast meal containing 0 g (solid line) or 24g (dashed line) of resistant starch. There was no statistically significant treatment differences observed (p > 0.05).

Table 1 Food Intake Data – Means (kCal) ± SE

Breakfast Meal	Lunch	Diet Diary	Total (Including Breakfast
Control (2g fiber, 777kcal)	752 ± 63	1418 ± 119	2947 ± 152
Resistant Starch (26g fiber, 693kcal)	783 ± 72	1292 ± 103	2768 ± 153
$F_{(1, 26)}$	1.089	1.998	2.357
Main Effect P-Value	0.306	0.1609	0.05

CHAPTER 4

THE EFFECT OF SOLUBLE FIBER DEXTRIN ON POSTPRANDIAL APPETITE AND
SUBSEQUENT FOOD INTAKE IN HEALTHY ADULTS

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Abstract

Purpose: The aim of this present study was to determine the effect of consuming a beverage containing soluble fiber dextrin (SFD) on subjective appetite, the plasma concentration of selected hormones and metabolites related to appetite, the glycemic response, and food intake in healthy adults. **Methods**. In a double-blind, randomized, cross-over study, forty-one participants consumed a standardized lunch meal with a test beverage containing 0g, 10g or 20g fiber from SFD. Appetite questionnaires were completed and blood samples collected immediately before lunch and at regular intervals for 150 minutes. Then, an afternoon snack was provided and the amount eaten recorded. The participants were then allowed to leave the laboratory, but were asked to complete hourly appetite questionnaires and to keep a diet diary for the remainder of the day. **Results.** Consuming the test beverages containing SFD had no effect on subjective appetite over the 150 minutes

following consumption (p>0.05). After the participants left the laboratory (210 minutes – 510 minutes post consumption), mean values for hunger and desire to eat were significantly lower and fullness higher following consumption of the test beverage containing 20g fiber from SFD (p<0.05). There was no statistically significant effect of consuming SFD on food intake at the test snack or during the evening period. Plasma GIP was lower following consumption of the 20g fiber from SFD test beverage (p<0.05), but there was no treatment effect on the plasma concentration of GLP-1 ghrelin, CCK-8, PYY$_{3-36}$, insulin or glucose (p>0.05). **Conclusion**. The consumption of SFD may reduce subjective appetite several hours after consumption although this did not translate into an effect on food intake.

Keywords: Dietary Fiber, Dextrin, Appetite, Food Intake

Introduction

The rapid rise in the number of overweight and obese individuals has increased efforts to identify foods or food ingredients that promote satiation or satiety and reduce energy intake. Dietary fiber has attracted considerable interest because increased fiber consumption is associated with a lower body weight [1-4] or a lower body mass index (BMI) [5-8]. It is possible that the effect of fiber on body weight is due to increased satiation and decreased short-term energy intake [9-13] although a recent systematic review has questioned the satiating effect of some dietary fibers [14]. The intake of dietary fiber by consumers is well below recommended levels in many countries [15-16] and the development of dietary strategies to increase the consumption of fiber may be useful in reducing the number of overweight and obese individuals.

While strategies for increasing fiber intake have generally focused on increasing the consumption of high fiber foods such as fruits and vegetables, these interventions have had limited success in changing eating habits and therefore little impact on overall fiber intake [17-18]. An alternative strategy to increase the consumption of fiber is to develop functional fibers that provide similar physiological benefits to naturally occurring high fiber foods but that can be incorporated into a wide array of commonly eaten foods. One such functional fiber ingredient is soluble fiber dextrin (SFD); a type of dietary fiber that completely dissolves in water without noticeably changing its appearance, taste or viscosity [19]. SFD is resistant to digestion in the small intestine but may be fermented in the colon [20]. To date, only a limited number of studies have been conducted regarding the impact of SFD on appetite or food intake. These studies have found that adding SFD to a beverage reduces appetite and food intake at a subsequent test meal [19, 21]. This effect on food intake may be explained, in part, because SFD suppresses the post-prandial ghrelin response [22]. The influence of SFD on other hormones related to appetite such as cholecystokinin-8 (CCK-8), glucagon like peptide 1 (GLP-1), peptide YY_{3-36} (PYY_{3-36}), ghrelin, and glucose-dependent insulintropic polypeptide (GIP) has not been investigated. In addition, SFD may also alter the post-prandial glycemic response. For example, studies have shown that dietary fiber reduces the post-prandial plasma concentration of glucose and insulin [23]. This effect may be due to the replacement of available carbohydrate with fiber [24], a change in glucose absorption kinetics due to a slower gastric emptying rate [25], or the fermentation of fiber to produce short-chain fatty acids (SCFAs) that improve glycemic control [26]. A change in the postprandial glycemic response may also influence appetite by reducing the glycemic index of a meal [27].

The primary aim of this present study was to test the effect of a beverage containing SFD on subjective appetite in healthy adults. Additionally, the effect of SFD on food intake, glucose, insulin, CCK-8, GLP-1, PYY_{3-36}, ghrelin, and GIP was determined. Based on previous work [19], we hypothesize that SFD will increase feelings of satiety and decrease energy intake at subsequent meals and that these effects will be modulated, in part, by changes in the plasma concentration of putative satiety hormones.

Methods

This study was conducted with the approval of the Iowa State University Institutional Review Board and signed informed consent was obtained from all participants prior to enrolling in the study.

Participants

Potential participants were informed about the study via a mass email sent out to Iowa State University faculty, students and staff and flyers advertising the study posted in the local community. Individuals interested in the study were invited to a screening session where they completed a questionnaire that posed questions about their general health. In addition, the participant's height and body weight was measured using a calibrated stadiometer (Model S100, Ayrton Corp., Prior Lake, MN) and clinical weighing scales (Detecto 758C, Cardinal Scale Manufacturing Company, Webb City, MO). The participants were asked to taste samples of all the test foods and rate each of them on a 9-point scale. Participants were only included in the study if they were aged between 18 – 40 years, had a BMI between 19.9 and 29.9 and were willing to eat the test foods. Participants were excluded from the study if they:

had a presence or history of gastrointestinal disease, were a restrained eater (≥14 on the restraint section of the three-factor eating questionnaire) [28], used tobacco products, did not find the test foods palatable (< 5 on a 9 point scale), had an acute or chronic disease, were using medication that lists an effect on appetite as a side effect, or did not regularly consume an afternoon snack (< 4 times a week).

Protocol

This study used a double-blind, randomized, cross-over design with each participant completing three separate test sessions. There was a washout period of at least one week between each test session. Participants were asked to avoid alcohol consumption and strenuous physical activity for the 24 hours before each test session and to refrain from eating or drinking (except water) after 10:00 pm on the evening before each test day. On the morning of each test session the participants were required to eat a standardized breakfast that was provided by the research team and then fast until reporting to the test facility four hours later.

On arriving at the test facility, an indwelling catheter was placed into the participant's non-dominant arm. Participants were then allowed to rest for 30 minutes before a baseline blood sample was collected and appetite questionnaire completed. Then, the participants were served lunch with one of three test beverages and asked to consume the test meal in its entirety within 15 minutes. On completion of this meal, participants were asked to fill out an appetite questionnaire and a blood sample was collected (t0). Further appetite questionnaires and blood samples were collected at t0+15, 30, 45, 60, 90, 120 and 150 minutes. Following the 150 minute measurement, subjects were served a snack and asked to eat until comfortably

full. The snack meal was weighed before and after serving, without the participant's knowledge, and the amount consumed recorded. The indwelling catheter was then removed and participants were instructed to complete hourly appetite questionnaires contained on a hand-held computer (PalmPilot) and record all foods and beverages eaten during the remainder of the day using a diet diary.

Test foods and beverages

The participant's daily energy expenditure was calculated using validated equations to estimate basal metabolic rate [29] and multiplying this figure by a physical activity level of 1.5. The standardized breakfast meal consumed on the morning of each test session provided 25% of the participant's estimated daily energy requirement. The macronutrient composition of the breakfast meal was 60% carbohydrate, 14% protein and 26% fat and consisted of Honey Nut Cheerios® (General Mills Inc., Minneapolis, MN), Great Value whole milk (Wal-Mart Stores Inc., Bentonville, AR), wheat bread (Sara Lee Corp., Chicago, IL), and strawberry jelly (J.M. Smuckers Comp., Orville, OH). The lunch meal of chicken salad was prepared using Swanson® canned chicken (Campbell Soup Comp., Camden, NJ), fat free ranch dressing (Kraft Food Groups Inc., Northfield, Il), Kraft® light mayonnaise, iceberg lettuce, Great Value butter and dried cranberries (Ocean Spray ®, Lakeville, MA) and was served with Wonder Bread® white bread (Flower Foods, Thomasville, GA). Excluding the beverage, the test meal provided 25% of the participant's total daily energy requirement and had a macronutrient profile of 55% carbohydrate, 15% protein and 30% fat. The *ad libitum* snack provided at the end of each test session consisted of sliced apples with a caramel dip (Crunch Pak ®, Cashmere, WA).

The test beverage served with the lunch meal provided 0, 10 or 20 grams of fiber from SFD. The SFD ingredient (Tate & Lyle Ingredients Americas LLC, Hoffman Estates, IL) contained 50% fiber and 50% digestible carbohydrate. Maltodextrin, a rapidly digestible carbohydrate, was added to match the beverages for available carbohydrate. Treatment 1 contained 10g fiber (20g SFD) plus 10g of maltodextrin and provided 20g total digestible carbohydrate. Treatment 2 contained 20g fiber (40g SFD) and provided 20g total digestible carbohydrate. The control beverage contained 20g of maltodextrin and provided 0g of fiber and 20g total digestible carbohydrate. In addition to being matched for available carbohydrate, all beverages were approximately matched for energy (80, 83, and 85 kcal for the control, treatment 1 and treatment 2 respectively). To create the test beverages, each treatment mixture was dissolved in 355ml of water and flavored with Crystal Light (Kraft Food Groups Inc, Northfield, Il)

Measurement of food intake

Data from diet diaries were analyzed using NutritionistPro© software and checked for validity using the McCrory method [30]. No trends in over or under reporting of caloric intake were observed.

Subjective appetite measures

Subjective appetite was measured using a standard appetite questionnaire [31] that posed the following questions: How hungry do you feel right now? How full do you feel right now? What is your desire to eat right now? What is your prospective consumption right now? How thirsty are you right now? Responses were measured using a visual analogue scale anchored with opposing statements at each end (e.g. not hungry at all or as

hungry as I have ever felt). Responses to the appetite questions were captured and stored on a PalmPilot hand-held that placed a time and date stamp on each entry to check for protocol compliance. For all appetite measurements collected outside the laboratory, compliance was defined as completion of the appetite questionnaire within 15 minutes of the designated time. Only time points with a minimum of 70% of participants with compliant responses were included in the final analysis.

Glucose and hormone analysis

Blood was drawn into 4 ml EDTA-coated vacutainer tubes and mixed with 400 µl of 10 000 KIU (1·4 mg)/ml aprotinin. Samples were centrifuged for 15 minutes at 3000 g and 4°C then divided into aliquots before being stored at -80°C for further analysis. Plasma samples were assayed for glucose using a biochemical analyzer (YSI Life Sciences, Model 2700 select) while all other analyses (insulin, GLP-1, PYY$_{3-36}$, GIP, ghrelin and CCK) were completed using established RIA procedures [32-33]. All samples were run in duplicate and all samples from a given participant were analyzed within the same batch. Insulin antibodies and the insulin and GIP tracers were made in Dr Hsu's laboratory. All other ^{125}I-Tracers used were purchased from PerkinElmer (Waltham, MA). The ghrelin and GIP analysis were done using T-4747 (Bachem, Bubendorf, Sui) and H-027-02 (Phoenix Pharmaceuticals Inc., Burlingame, CA) antibodies. Prior to RIA analysis for CCK, GLP-1 and PYY$_{3-36}$, samples were ethanol extracted. Briefly, 0.5 mL of plasma was mixed with 1mL of 96% ethanol and allowed to stand for 10 minutes at room temperature. Incubated samples were then centrifuged at 3000rpm and 4°C for 15 minutes. After centrifuging, the supernatant was decanted into a clean microcentrifuge tube and dried in a Jouan RC 10.10 vacuum concentrator (Thermo Fisher Scientific, Waltham, MA) at 37°C. Dried extracts were then

reconstituted using 0.5mL of assay buffer and stored at -20°C for further analysis. Antibodies for PYY_{3-36} and GLP-1 were purchased from Bachem (T-4090 & T-4056) while CCK-8 antibody C2581 was purchased from Sigma Aldrich (St. Louis, MO. Detection limits and coefficient of variations (CV) for each of the RIA measured hormones are as follows: Insulin: $1.5 - 200$ µU, interassay CV 13%, intra-assay CV 7%; Ghrelin: $50 - 1600$ pg/mL, interassay CV 15%, intra-assay CV 7%; GLP-1: $7.5 - 1000$pg/mL, inter-assay CV 12%, intra-assay CV 8%, PYY_{3-36}: $5 - 320$pg/mL, inter-assay CV 15%, intra-assay CV 8%, GIP: $25 - 2500$pg/mL, inter-assay CV 9%, intra-assay CV 6%, CCK-8: $0.5 - 80$pg/mL, interassay CV 11%, intra-assay CV 7%.

Statistical analysis

SPSS for windows (version 20, 2012, IBM, Armonk, NY) was used to perform all statistical analysis and data are presented as means ± standard error. A mixed model, repeated measures analysis of covariance (ANCOVA) was used to test overall treatment effect on plasma parameters. Baseline values were included as a covariate and participants were added as random variables in the model. The appetite data was split into two periods: in-laboratory data and free-living data. This was due to a change in the protocol and a reduction in compliance after the participants had left the laboratory. For the in-laboratory data, a mixed model, repeated measures ANCOVA was used to test overall treatment effect on plasma parameters and subjective appetite measurements. Baseline values were included as a covariate and participants were added as random variables in the model. For the free-living appetite data, the mean rating for each of the subjective appetite questions was calculated and analyzed using a one-way, repeated measures analysis of variance (ANOVA). Differences in food intake were also analyzed using one-way, repeated measures ANOVA.

For all measures, post-hoc analysis was performed by Bonferoni adjusted pairwise comparison of treatment effects. Statistical significance was set at p<0.05.

A power calculation indicated that a sample size of 38 would allow the detection of a 10% difference in subjective appetite measures (the primary outcome measure) with $\alpha=0.05$ and $\beta= 0.9$. Forty five participants were recruited to allow for attrition.

Results

Participant demographics

Forty-five participants were recruited and randomized to a treatment order. Two males and two females dropped from the study due to scheduling conflicts. Forty-one participants, 19 females and 22 males, completed the study. The participant's mean age was 24 ± 4 years and mean BMI was 23.4 ± 2.5 kg/m^2.

Hormones and glucose

Figure 1 shows the data for the plasma concentration of GIP, ghrelin, CCK-8 and PYY$_{3-36}$. Statistical analysis of results revealed a significant main effect of treatment on GIP $(F(2, 179) = 7.101, p = 0.001)$. Post hoc analysis showed that the plasma concentration of GIP was lower following consumption of the 20g fiber from SFD beverage as compared to the control (p=0.001). No statistically significant main effect of treatment was found on ghrelin $(F(2, 294) = 2.663; p=0.071)$, CCK-8 $(F(2, 200) = 2.496; p=0.085)$, or PYY$_{3-36}$ $(F(2, 11) = 2.091; p=0.172)$. Additionally, there was no observed treatment effect on plasma glucose $(F(2, 147) = 1.250; p=0.290)$, insulin $(F(2, 177) = 1.121; p=0.328)$, GLP-1 $(F(2, 246) = 0.028; p=0.973)$ (data not shown).

Subjective appetite

Data collected in the laboratory

Data for subjective appetite ratings Hunger, Fullness, Desire to Eat and Prospective Consumption that were collected in the laboratory are shown in Figure 2. There was no statistically significant effect of treatment on: hunger (F (2, 139) = 0.324; p=0.724), fullness (F(2, 178) = 1.351; p=0.262), desire to eat (F (2, 118) = 1.054; p=0.352), prospective consumption (F (2, 54) = 0.843;p=0.436), or thirst (F(2, 112) = 0.977; p=0.379 data not shown.

Free-living appetite data

Figure 3 shows mean appetite ratings of the free-living data subset for hunger, fullness, desire to eat and prospective consumption. These time points correspond to 3.5 – 8.5 hours (210 – 510 minutes) post consumption of test beverages. Repeated measures ANOVA revealed significant main treatment effects on hunger (F(2, 39) = 4.291; p=0.021), fullness (F(2, 39) = 4.145; p=0.023), and desire to eat (F (2, 39) = 6.459; p=0.004). Post-hoc analysis showed lower hunger and desire to eat in addition to higher fullness following consumption of the 20 g fiber from SFD beverage as compared to the control. There was no significant main treatment effect for prospective consumption (F(2,39) =3.018; p=0.060) or thirst (F(2,39)=2.780; p =0.068), data not shown.

Food intake

Table 1 shows mean intakes for each treatment. Statistical analysis revealed no main effect of treatment on calories consumed at the snack, consumption over the rest of the day (diet diary intake), or the combined total energy intake.

Discussion

This study found that consuming a beverage containing 20g fiber from SFD reduces the plasma concentration of GIP during the first 150 minutes post consumption and reduces feelings of hunger and desire to eat while increasing fullness several hours after consumption. However, there was no effect on other biomarkers of appetite during the first 150 minutes after consumption or food intake throughout the test day.

There was a statistically significant reduction of the plasma concentration of GIP during the first 150 minutes post-consumption. GIP is secreted by K cells of the small intestine in response to food intake and functions to stimulate insulin secretion [34-37]. High fiber, low glycemic foods are generally associated with a decreased postprandial glucose and insulin response, in part regulated by changes in plasma GIP concentration [38-40]. Although this study confirmed previous research showing a reduction in plasma GIP concentration following a meal with a higher fiber content, [39] we did not detect a significant treatment effect on plasma glucose or insulin concentration. Despite similar fiber doses being used in the two studies (27g vs 20g), there were differences in the characteristics of the test meal and fiber source used. Additionally, unlike previous studies, the present study matched treatments for available carbohydrate.

Although we did not detect a significant differences in subjective appetite measures while participants were in the laboratory, participants reported increased fullness, reduced hunger and reduced desire to eat following the 20g fiber from SFD treatment 3.5 to 8.5 hours after the beverage was consumed. It is not clear why this effect was observed, but one possible explanation may be due to the fermentation of SFD in the colon. Ingestion of

fermentable carbohydrates has been shown to produce short-chain fatty acids (SCFAs) and increase satiety in both rodents and humans models potentially through regulation of satiety hormones GLP-1 and PYY_{3-36} [41-44]. However, it could take several hours for the SFD to reach the large intestine to be fermented, which may explain why no effect was observed in the laboratory. In this present study, we cannot confirm that the SFD was fermented or that this fermentation was temporally related to changes in appetite or biomarkers of appetite such as GLP-1 or $PYY_{3-36.}$ Further studies are required to investigate the mechanisms through which SFD may influence appetite over a longer time-period.

Despite the observed changes in appetite 3.5 – 8.5 hours after consuming the SFD containing beverages, this did not translate into a reduction in *ad libitum* food intake as measured using diet diaries. These results differ from those provided by previous studies that found reduced appetite and food intake after consuming SFD [19, 21]. However, these prior studies provided the SFD in multiple servings with the initial dose of SFD being given following an overnight fast. In this present study, a single preload was used and SFD was provided with lunch rather than breakfast. Regarding the reduction in appetite observed during the evening period, it is possible that this decrease in appetite was not sufficiently large to robustly influence food intake in a free-living situation where participants are more likely to be exposed to factors that stimulate food intake [45-47]. In addition, it is possible that errors in recording food intake using diet diaries could mask a small effect on food intake.

Overall this study's results did not show a strong effect of SFD on appetite, food intake and plasma markers of appetite for the first 150 minutes post-consumption. However,

the beneficial changes on subjective appetite observed during the evening hours warrants further attention. It is not clear why SFD would promote changes in appetite several hours after consumption but one possible explanation is that SFD may promote increased satiety through colonic fermentation and production of SCFAs. One previously mentioned limitation of this study is that breath hydrogen measures were not collected during the time over which significant effects on appetite were observed to confirm fermentation occurred. Follow up studies should consider a longer test day in order to fully capture the relationship between colonic fermentation of SFD, biomarkers of appetite, and subjective appetite ratings under a controlled environment. A second limitation of this study is that this was a single dose, single day feeding study and results cannot be extrapolated to conclude that SFD would have long-term effects on body weight. Future research should be conducted to elucidate physiological mechanisms behind the potential satiating abilities of SFD and to assess the long-term effects of SFD on energy intake and body weight.

Acknowledgements

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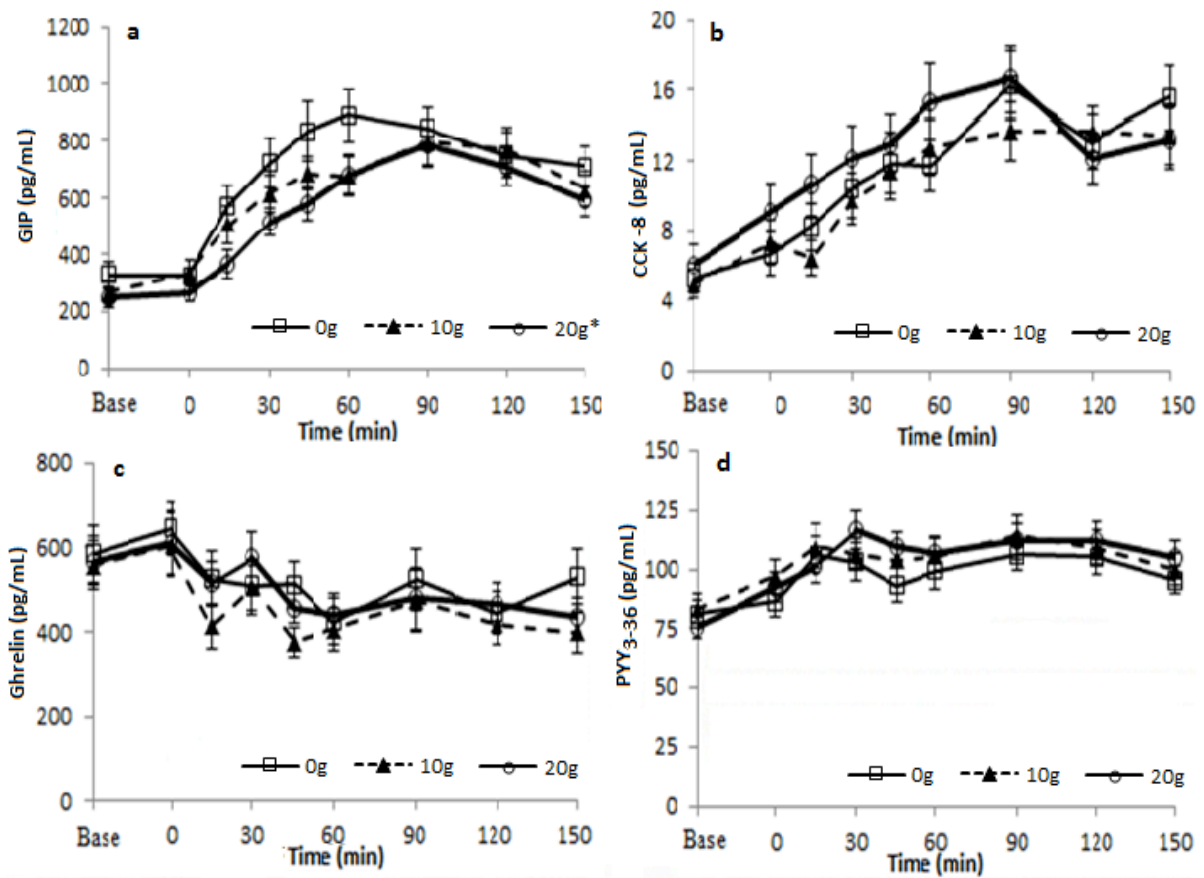
Tables and Figures

Fig. 1 Plasma hormone responses for glucose-dependent insulintropic polypeptide (**a**), cholecystokinin (**b**), ghrelin (**c**), and peptide YY_{3-36} (**d**) measured from baseline through 150 minutes post consumption of a test beverage containing 0 g (squares), 10 g (triangles) or 20g (circles) of fiber from SFD. * Indicates a statistically significant decrease in plasma concentration as compared to control ($p < 0.05$).
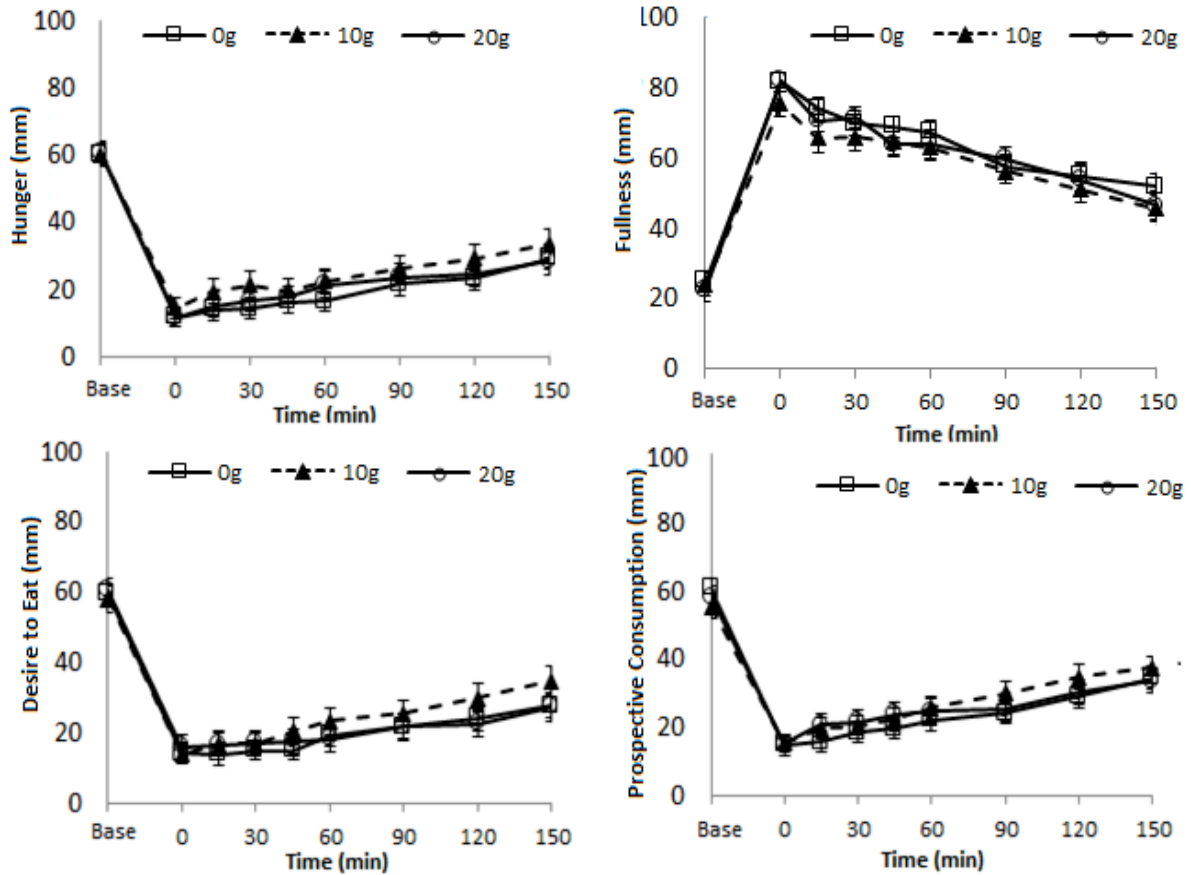

Fig. 2 VAS scores of hunger (**a**), fullness (**b**), desire to eat (**c**), and prospective consumption (**d**) rated from baseline through 150 minutes post consumption of a test beverage containing 0 g (squares), 10 g (triangles) or 20g (circles) of fiber from SFD. There were no statistically significant treatment differences observed ($p > 0.05$).
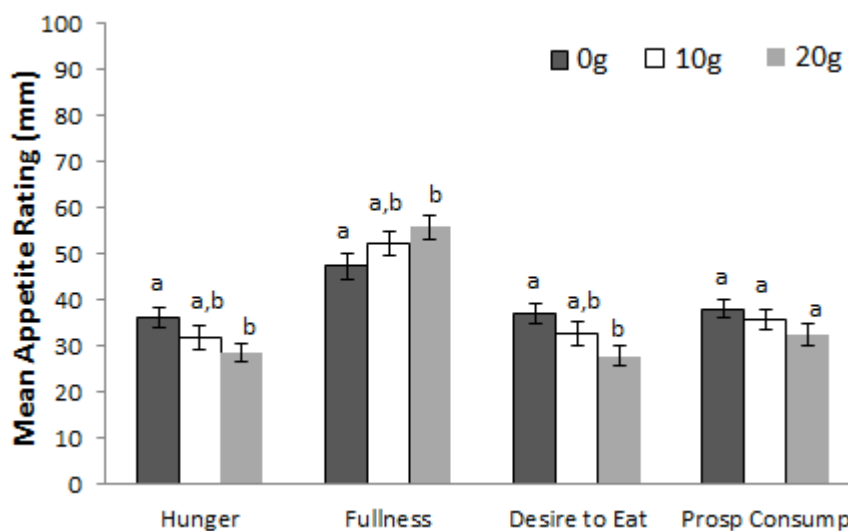
Fig. 3 Mean ± SE for subjective appetite measures hunger, fullness, desire to eat, and prospective consumption collected 3.5 – 8.5 hours post consumption of test beverages. Different letters for a given appetite response indicates statistically significant difference (p < 0.05)

Table 1 Food Intake Data – Means (kCal) ± SE

Treatment Beverage (g fiber from SFD)	Snack	Diet Diary	Total (Including Breakfast and Lunch)
0	168 ± 15	1381 ± 93	2857 ± 100
10	152 ± 13	1257 ± 83	2718 ± 95
20	171 ± 15	1365 ± 97	2845 ± 108
F (2, 39)	1.141	0.723	1.005
Main Effect P-Value	0.330	0.492	0.375

CHAPTER 5

THE EFFECT OF SOLUBLE FIBER DEXTRIN ON SUBJECTIVE AND
PHSYSIOLOGICAL MARKERS OF APPETITE

A paper to be submitted to *Physiology and Behavior*

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Abstract

Purpose. The aim of this study was to determine the effect of consuming soluble fiber dextrin (SFD) on subjective appetite, breath hydrogen, the plasma concentration of hormones related to appetite, and food intake in healthy adults. **Methods**. Forty-three participants completed a double blind, randomized, cross-over study. Two sources of SFD (corn and tapioca) at two doses (10 and 20g) were tested in this study along with a control treatment. For each treatment, 50% of the SFD was provided in liquid form as part of the breakfast meal and 50% in solid form for the mid-morning snack. Appetite questionnaires, blood samples and breath hydrogen samples were collected immediately before breakfast and at regular intervals during the 10 hour test session. Additionally, participants were fed an ad libitum lunch meal, afternoon snack and dinner meal and the amount eaten recorded. Following the dinner meal, participants left the laboratory but were required to keep a diet

diary for the remainder of the day. **Results.** Breath hydrogen concentrations were significantly higher following SFD consumption as compared to control (p <0.05). There was no observed overall treatment effect of consuming SFD on the plasma concentration of GLP-1, ghrelin, CCK-8 or PYY_{3-36}, (p>0.05). Consuming SFD also had no effect on subjective appetite or food intake during the test day (p>0.05). **Conclusion**. Consuming SFD increased breath hydrogen indicating that fermentation occurred. However, this study does not support an effect of SFD on food intake, subjective appetite ratings or biomarkers of appetite during a single day.

Keywords: Dietary Fiber, Dextrin, Appetite, Food Intake

Introduction

Throughout the developed world, the number of overweight and obese adults has risen markedly over the past few decades. This is of concern as these conditions are associated with increased risk of developing chronic diseases such as type 2 diabetes [1-3], cardiovascular disease [4], or cancer [5-7]. Consequently, reducing the number of overweight or obese individuals is a leading public health goal in developed countries.

Resistant starch is a type of fiber that can be isolated from foods and incorporated into a wide variety of food products. As resistant starch provides fewer calories per gram than rapidly digestible starch it could aid weight management through an energy dilution effect. Accumulating evidence from studies using rodent models indicate that replacing rapidly digestible starch with resistant starch reduces weight gain in obesity resistant and obesity prone rats [8-9]. Additionally studies conducted in humans suggest that resistant

starch may also aid weight management by increasing satiety and decreasing short term food intake [10-12].

Soluble fiber dextrin (SFD) is a type of dietary fiber derived from starch. Like resistant starches, data from previous studies suggest that consuming soluble fiber might influence appetite several hours after consumption [13-15]. A potential explanation for this delay in an observed effect is that it takes several hours for the SFD to reach the large intestine. Here, the SFD undergoes bacterial fermentation to produce short-chain fatty acids (SCFA). Recent studies using colonic cells report that SCFA trigger the release of the hormones GLP-1 and PYY_{3-36} [16-17]. As these hormones are related to physiological control of food intake [18], a possible explanation for the results obtained in the study of SFD is that colonic fermentation of SFD produced SCFAs that in turn increased secretion of GLP-1 and PYY_{3-36}.

The primary outcome of this present study was to determine the effect of consuming different doses of SFD on food intake over a single day. The secondary outcomes were to determine the effect of SFD on subjective appetite, satiety-related hormones and breath hydrogen as these may provide a mechanistic explanation for any effect on food intake. Additionally, it was determined if SFD produced a dose response effect and if there were any differences between the SFD derived from corn and tapioca starch. We hypothesized that participants consuming SFD would reduce food intake over a single day due to increased satiety. We also hypothesized that the increased satiety would be explained by increased breath hydrogen, higher CCK-8, GLP-1 and PYY_{3-36} and lower ghrelin. In addition, we hypothesized that the effect on appetite would be dose dependent (increasing the SFD dose

will have a larger effect on the outcome measures) and that there would be no difference between the sources of SFD (corn vs tapioca).

Methods

Participants

Healthy adults aged 18-45 years with a BMI of $19.9 - 29.9$ kg/m^2 were recruited via a mass email sent to Iowa State University faculty, students and staff. Individuals interested in the study were invited to a screening session where their height and weight were measured and they were asked to complete a questionnaire that posed questions about their general health and attitudes to food. Additionally, during the screening, participants were asked to taste test foods used in the study (SFD beverages, bars, and foods used for ad libitum test meals) and to rate the palatability of each item on a scale from 1 (least palatable) to 9 (most palatable). Participants were excluded from the study if they: were outside the target age or BMI range, were not weight stable (weight change of 3kg or more in the past 3 months), did not regularly consume breakfast and afternoon snacks, had a presence or history of gastrointestinal disease or food intolerance, were a restrained eater ($\geq$14 on the restraint section of the three-factor eating questionnaire) [19], did not find the test foods palatable (< 5 on a 9 point scale), or were using medication that lists a side effect on appetite. This study was approved by the Iowa State University IRB and all participants signed an informed consent form prior to being enrolled in the study.

Protocol

This study used a double-blind, randomized, cross-over design. All participants reported to the laboratory on five separate occasions with at least one week between each test session. Participants were instructed to avoid consuming alcohol or conducting strenuous activity in the 24 hours prior to each test session. On the evening before each test session, participants were asked to consume a standardized evening meal that had been provided by the research team. The participants were asked to finish eating the meal by 9:00pm and to refrain from consuming any further foods or beverages, except water, until reporting to the laboratory at 7:30am the following morning.

On reporting to the laboratory, the participant's body weight was measured using clinical weighing scales (Detecto 758C, Cardinal Scale Manufacturing Company, Webb City, MO). They were then taken to a quiet room where an indwelling catheter inserted into their non-dominant arm by a registered nurse. The participant was allowed to acclimatize to the indwelling catheter for 30 minutes before a baseline blood draw and breath sample were taken. A baseline appetite questionnaire was also completed. The participant was then provided with a breakfast meal that provided 20% of their estimated daily energy requirements which they were required to eat in its entirety within 15 minutes. The breakfast included one of the five SFD test beverages. On completion of this meal, another blood draw and breath sample were collected and an appetite questionnaire was completed (t=0). At 10:15am (t=120), one of five SFD test bars was served as a mid-morning snack which the participant was required to eat in its entirety within 5 minutes of serving. An ad libitum lunch meal in excess of what would reasonably be expected to be consumed was served at 12:30pm (t=240). Participants were instructed that they had 15 minutes to eat until

comfortably full, after which, the meal was withdrawn. A mid-afternoon snack, in excess of what could reasonably be eaten, was provided at 3:00pm (t=390). Participants were instructed that they had for 10 minutes to eat until comfortably full. After the final blood draw was taken (t=600), the indwelling catheter was removed from the participant's arm. An ad libitum evening meal was served and the participant was instructed to eat until comfortably full. Following this meal, the participant was allowed to leave the laboratory.

Participants were asked to keep a diet diary to record all food and beverage intake for the remainder of the day. These diaries were analyzed to determine energy and macronutrient intake using Nutritionist Pro™ Diet Analysis Software (version 2.1.13; First DataBank, San Bruno, CA). During the test day, participants were asked to complete twenty-four appetite questionnaires at regular intervals throughout the test day (t=15, 30, 45, 60, 90, 120, 135, 150, 165, 180, 240, 255, 270, 285, 300, 360, 390, 405, 420, 435, 450, 480, 540, and 600 minutes after breakfast consumption). Breath samples were collected in two hour intervals (t=120, 240, 360, 480 and 600 minutes after breakfast consumption). Additionally, blood samples were collected twelve times following the breakfast meal (t=60, 120, 180, 240, 270, 300, 260, 290, 420, 480, 540 and 600 minutes after breakfast consumption).

Test foods and beverages

The SFD used in the present study was derived from two different sources: corn and tapioca. Both sources of SFD provide 50% fiber and 50% digestible carbohydrate, therefore the two doses (20 and 40g SFD) tested provided 10 and 20g fiber respectively. Five treatments were used in this study: control, corn10 (20 g SFD to provide 10g fiber), corn20 (40g SFD to provide 20g fiber), tapioca10 (20g SFD to provide 10g fiber) and tapioca20 (40g

SFD to provide 20g fiber). For each treatment, half of the SFD dose was provided with breakfast and half as part of a mid-morning snack. The SFD was consumed as a milk-based beverage with breakfast and as an ingredient in a snack bar product at the mid-morning snack.

The standardized dinner meal consumed on the evening before test session included chicken nuggets (Tyson Foods Inc., Springdale AR), barbeque sauce (Kraft Food Groups Inc, Northfield Il), mashed potatoes (Idahoan Foods, Idaho Falls, Idaho), Great Value mixed vegetable (Wal-Mart Stores Inc., Bentonville, AR), and chocolate chip cookies (Nabisco®, East Hanover, NJ) for dessert. This meal provided 814 kcal calories and had a macronutrient profile of 15% protein, 46%carbohydrate and 39% fat.

For the breakfast meal, participants' basal metabolic rate was estimated using validated equations [20]. This figure was multiplied by 1.3 to estimate total daily energy (TEE) requirements. Not including the test beverage, the breakfast meal provided 20% of the calculated TEE with a macronutrient profile of 13% protein, 61% carbohydrate and 26% fat. Foods included in the meal were hard-boiled egg (Crystal Farms, Minnetonka, MN), pineapple chunks (Dole Food Company, Westlake Village, CA), cinnamon raisin bagel (Bimbo Bakeries, Fort Worth TX) and salted butter (Land O Lakes®, Arden Hills, MN).

Following the mid-morning snack bar, which provided the second half of the daily SFD dose, *ad libitium* meals were served for lunch, afternoon snack and dinner. The lunch meal consisted of pasta and tomato sauce (Barilla Group, Parma, Italy) with Kraft® shredded parmesan cheese. The afternoon snack was Classic Lay's® potato chips (PepsiCo Inc., Purchase NY) and dinner was chicken fried rice (Kahiki® Foods Inc., Colombus, OH). All

meals and snacks were served with an 8 oz bottle of Dasani® water (The Coca-Cola Company, Atlanta, GA). Participants were not allowed to eat or drink outside of the test foods and drinks provided.

Subjective appetite and food intake

Participants completed a standard appetite questionnaire contained on a PalmPilot. The following questions were asked: How hungry do you feel right now? How full do you feel right now? What is your desire to eat right now? What is your prospective consumption right now? Responses were measured using a visual analogue scale anchored with opposing statements at each end (e.g. not hungry at all or as hungry as I have ever felt). Answers were captured and stored with a time and date stamp so compliance to the study protocol could be determined.

Hormones and breath hydrogen

Breath samples were collected using a custom sampler kit (Quintron Inc., Milwaukee, WI) and analyzed using a Quintron MicroLyzer Model SC. Blood was drawn into EDTA coated vacutainers, mixed with a relevant preservative and centrifuged. The plasma was collected and stored at -80C until being assayed. Plasma samples were ethanol extracted [21] before being analyzed for GLP-1, CCK-8 and PYY_{3-36} using established RIA procedures [22-23]. Ghrelin was also measured via radioimmunoassay but used unextracted blood samples. For all analyses, samples were run in duplicate and all samples from a given participant were analyzed within the same batch. All ^{125}I-Tracers used were purchased from PerkinElmer (Waltham, MA). Antibodies for Ghrelin, PYY_{3-36} and GLP-1 were purchased from Bachem (T-4747, T-4090 & T-4056 respectively) while CCK-8 antibody C2581 was purchased from

Sigma Aldrich (St. Louis, MO). Detection limits and coefficient of variations (CV) for each of the RIA measured hormones are as follows: Ghrelin: 50 – 3200 pg/mL, interassay CV 13%, intra-assay CV 10%; GLP-1: 7.5 – 1000pg/mL, inter-assay CV 12%, intra-assay CV 9%, PYY_{3-36}: 3.7 – 250 pg/mL, inter-assay CV 14%, intra-assay CV 8%, CCK-8: 0.62 – 80pg/mL, interassay CV 13%, intra-assay CV 8%.

Statistical analysis

A power calculation indicated that a sample of 43 participants would be sufficient to detect a 150 kcal difference in food intake at $p<0.05$ and beta = 0.9. Forty eight participants were recruited to allow for attrition. Means and standard error were calculated for all study variables. Treatment effects of SFD on subjective appetite measures, hormone response and breath hydrogen data were analyzed with a mixed model ANCOVA using treatment and time point as repeated measures and baseline as a covariate. Treatment effects on food intake were analyzed using a one way, repeated measures ANOVA. All post-hoc, pairwise comparisons were performed using Bonferroni adjustments. Statistical analysis was conducted using SPSS for Windows or Mac (version 16.0; SPSS, Chicago, IL, USA).

Results

Participant demographics

In total, 68 people completed the screening process for this study. Twenty were found to be ineligible with the two most common disqualifying factors being BMI outside of the target range (6 people) and low palatability ratings for the test foods (12 total: 2 for test beverage, 9 for test bar, 1 for pasta lunch). Forty-eight participants were randomized into the

study. One male participant dropped after completing two sessions due to an unrelated health issue. Four participants (3 female, 1 male) dropped after four sessions due to schedule conflicts. Forty-three participants completed the study (22 male and 21 female). Average age was 25 ± 3.6 years with a BMI of 23.9 ± 2.9 kg/m^2.

Breath hydrogen

Figure 1 shows mean breath hydrogen results by treatment. Mixed-model ANCOVA revealed a significant overall treatment effect ($F_{(4, 527)} = 17.0$, $p < 0.001$) with all SFD treatments having significantly higher breath hydrogen than control ($p < 0.05$). Post –hoc analysis showed a dose response for tapioca based SFD with Tapioca20 having significantly higher breath hydrogen than both Tapioca10 and Corn10 ($p < 0.001$ for both). Corn based SFD showed a trend towards a dose response with Corn20 having slightly higher breath hydrogen response when compared to Corn10 and Tapioca10 ($p=0.066$ and 0.052 respectively).

Hormones

Figure 2 shows data for the plasma concentration of GLP-1, ghrelin, CCK-8 and PYY_{3-36}. Statistical analysis did not reveal an overall treatment difference for any hormone measures: GLP-1 ($F_{(4, 683)} = 1.478$; $p>0.05$), ghrelin ($F_{(4,571} = 0.372$; $p>0.05$), CCK-8 ($F_{(4, 659)} = 0.851$; $p>0.05$) and PYY_{3-36} ($F_{(4, 659)} = 2.262$; $p>0.05$). Post-hoc analysis however showed significantly higher PYY_{3-36} concentrations for the Corn20 treatment as compared to control ($p=0.043$).

Subjective appetite

Figure 3 shows mean appetite ratings for hunger, fullness, desire to eat and prospective consumption from baseline through 10 hours (600 mins) post-consumption of the treatment beverage. Repeated measures ANCOVA showed no significant main treatment effects on hunger ($F_{(4, 603)} =$

2.722;p>0.05), fullness (F(4,853) = 1.720; p>0.05), desire to eat (F (4, 679) = 0.909; p>0.05) and prospective consumption (F(4,766)=1.247;p>0.05).

Food intake

Food intake measures consisted of in laboratory, *ad libitum* lunch, afternoon snack and dinner intake as well as evening food intake measured from participant diet diaries. Table 1 shows mean intakes for each treatment. Statistical analysis revealed no main treatment effect on calories consumed from the lunch, snack, evening meal or consumption over the rest of the day (diet diary intake). Additionally, when the breakfast and mid-morning snack were factored in and total caloric intake for the test day was analyzed there was no treatment differences observed.

Discussion

This present study investigated the effect of consuming SFD on food intake, subjective appetite, plasma concentration of several hormones related to satiety and breath hydrogen (an indirect marker of colonic fermentation of carbohydrates). We hypothesized that SFD would be fermented to produce SCFAs, which would stimulate the secretion of PYY_{3-36} and GLP-1 from colonic cells, resulting in reduced appetite and food intake. While the consumption of SFD increased breath hydrogen, there were no statistically significant overall effects of treatment on subjective markers of appetite, hormone concentrations, or food intake. Consequently, these data do not support an effect of SFD on food intake or appetite.

The results obtained by this present study are in contrast to previous studies that have found that consuming SFD reduces appetite [12]. While there are several possible explanations for these discrepant results (e.g., differences in the study group or experimental design) a significant difference is the vehicle used to deliver the SFD. Previous studies have used a beverage as the vehicle for delivering SFD whereas this present study used a beverage and a solid food. However, a number of studies have found that beverages are less satiating than solid foods [24-27] and it is not clear why using a solid food rather than a beverage vehicle would have a seemingly opposite effect on appetite. One possibility is that dietary fiber influences appetite through different mechanisms than the other macronutrients rendering the form in which it is consumed to be less important. Another possibility is that the solid food used in this present study had a strong satiating effect independent of the resistant dextrin content, which acted to mask an effect of SFD on appetite. In addition to vehicle influences, participants were required to spend the entire day in the laboratory and the change in their activity levels or eating patterns may have contributed to an attenuated appetite response [28-29]. Further research is warranted to understand how the form in which SFD is consumed influences appetite and to determine the optimum vehicle for its delivery.

We hypothesized that SFD would be fermented in the colon. Indeed, breath hydrogen was higher following consumption of SFD providing evidence that SFD was fermented. A dose response relationship was observed but there was no statistically significant difference between the sources of SFD (tapioca or corn). SFD appears to be readily fermented and the peak breath hydrogen concentration was higher following consumption of the SFD compared to recent studies of gel-forming pectin [30] or fructo-oligosaccharides [31]. However, we

failed to observe any statistically significant correlation between breath hydrogen and any questionnaire responses or biochemical markers of appetite. This finding is in agreement with a recent study that found no relationship between breath hydrogen and subjective appetite although a statistically significant correlation between breath hydrogen and GLP-1 was observed [32].

Previous studies found that the plasma concentration of GLP-1 and PYY_{3-36} were increased when rodents ate a diet enriched with resistant starch [33]. By contrast, plasma concentration of GLP-1 was reduced in a human study following the consumption of a test food containing resistant starch [34]. In addition, cell studies have shown that SCFA stimulate the release of PYY_{3-36} and GLP-1 from colonic cells [35-37]. This present study found no effect of consuming SFD on plasma concentration of CCK, ghrelin, PYY_{3-36} or GLP-1. These discrepant results may be due to differences in the type of fiber used and the dose provided, differences in the study group or differences in the experimental design. Further research is warranted to confirm that these biomarkers of appetite respond to the consumption of SFD through increased SCFA production resulting from colonic fermentation. Moreover, the dose of SFD required to robustly elicit an effect is required.

This present study has a number of limitations that must be considered when interpreting the data. The participant's food choices and meal times were dictated by the research team. Consequently, this may have interfered with the normal expression of appetite. Moreover, free food was supplied which may have stimulated overconsumption thereby masking an effect of SFD [38-39]. These issues are common to laboratory-based studies where external validity is low [40-41]. It is possible that a study of free-living

individuals may highlight other effects of SFD on appetite (e.g., extending time between meals, reduced snacking, and reduced intake at self-selected meal times). This study was also a single exposure study and it might take repeated exposures to a food for individual's to 'learn' its effect on appetite and alter their food intake. In conclusion, this study does not support an effect of SFD, when consumed in two doses split between milk and brownies, on food intake, appetite or biomarkers of appetite over a single day.

Acknowledgements

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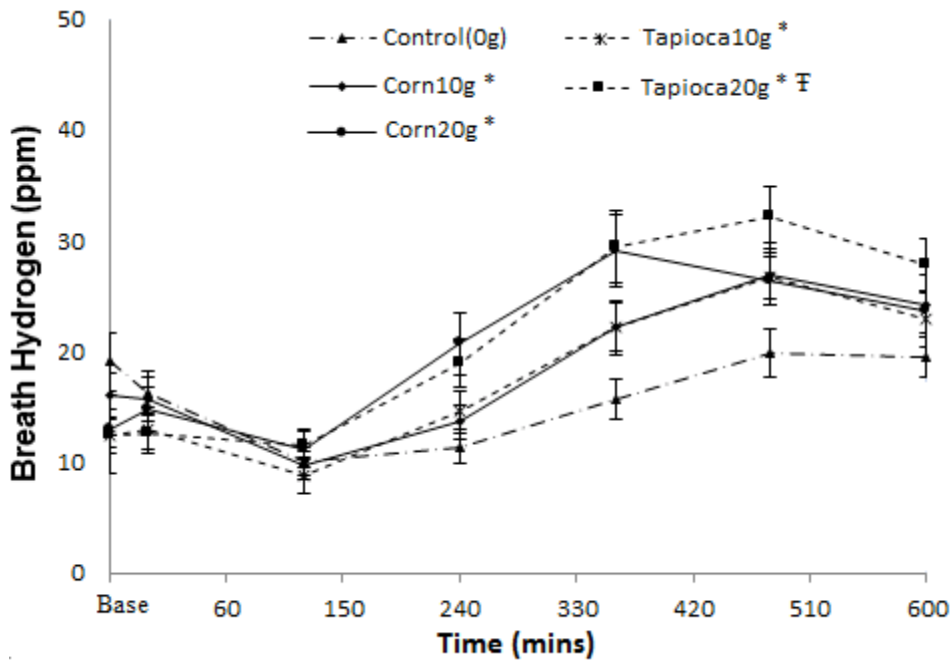
Tables and Figures

Fig. 1 Mean ± SE for breath hydrogen rated through 600 minutes following control (triangles), Corn10 (diamonds), Corn20 (circles), Tapioca10 (star) and Tapioca20 (square) treatments. *Indicates statistical significance from control (p <0.05). [F] Indicates statistical significance from both Corn10 and Tapioca10 treatments (p<0.05).
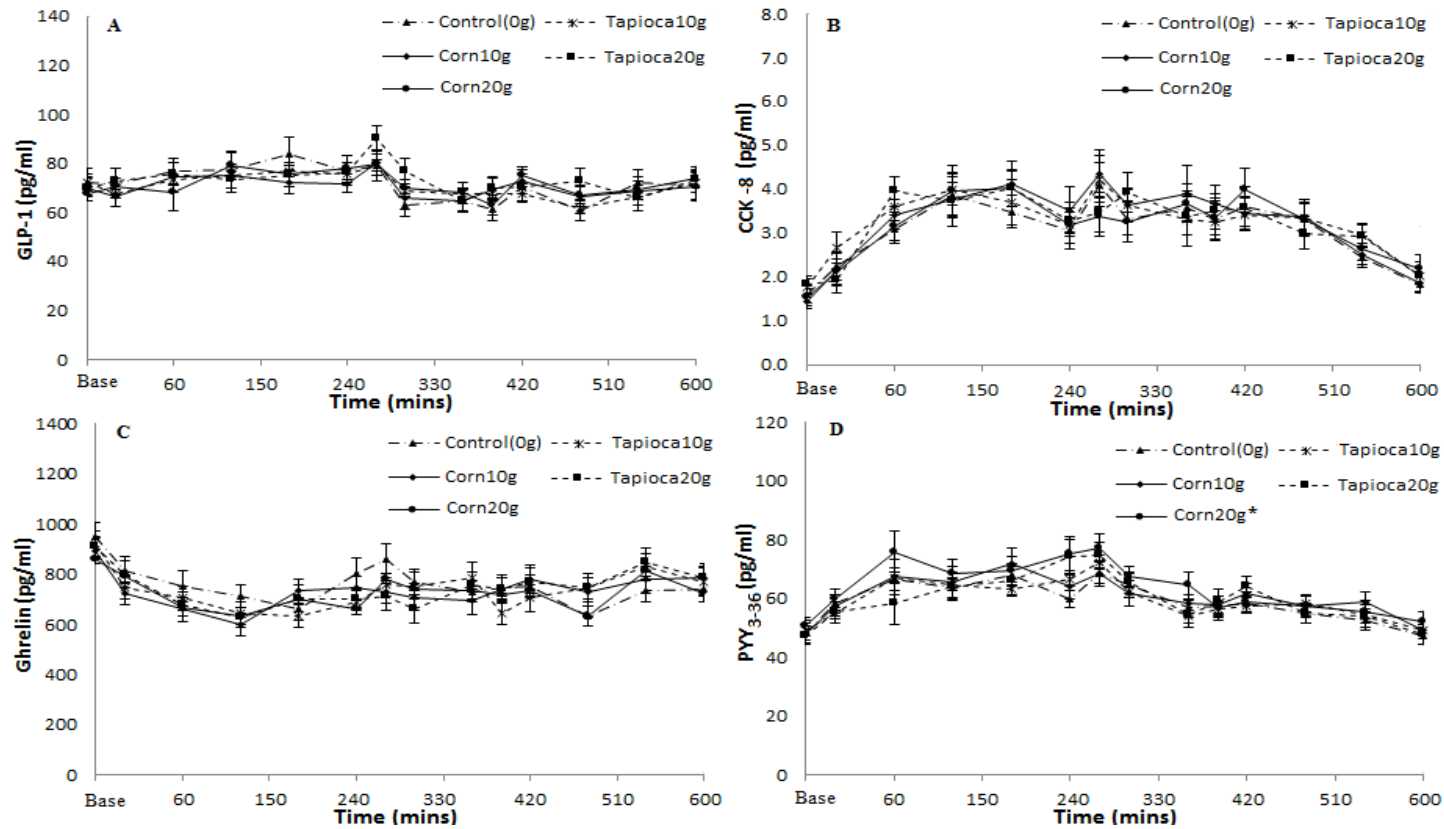
Fig. 2 Plasma hormone responses for GLP-1 (**A**), cholecystokinin (**B**), ghrelin (**C**), and peptide YY (**D**) measured from baseline through 600 minutes following control (triangles), Corn10 (diamonds), Corn20 (circles), Tapioca10 (star) and Tapioca20 (square) treatments. *Indicates statistical significance from control ($p < 0.05$).
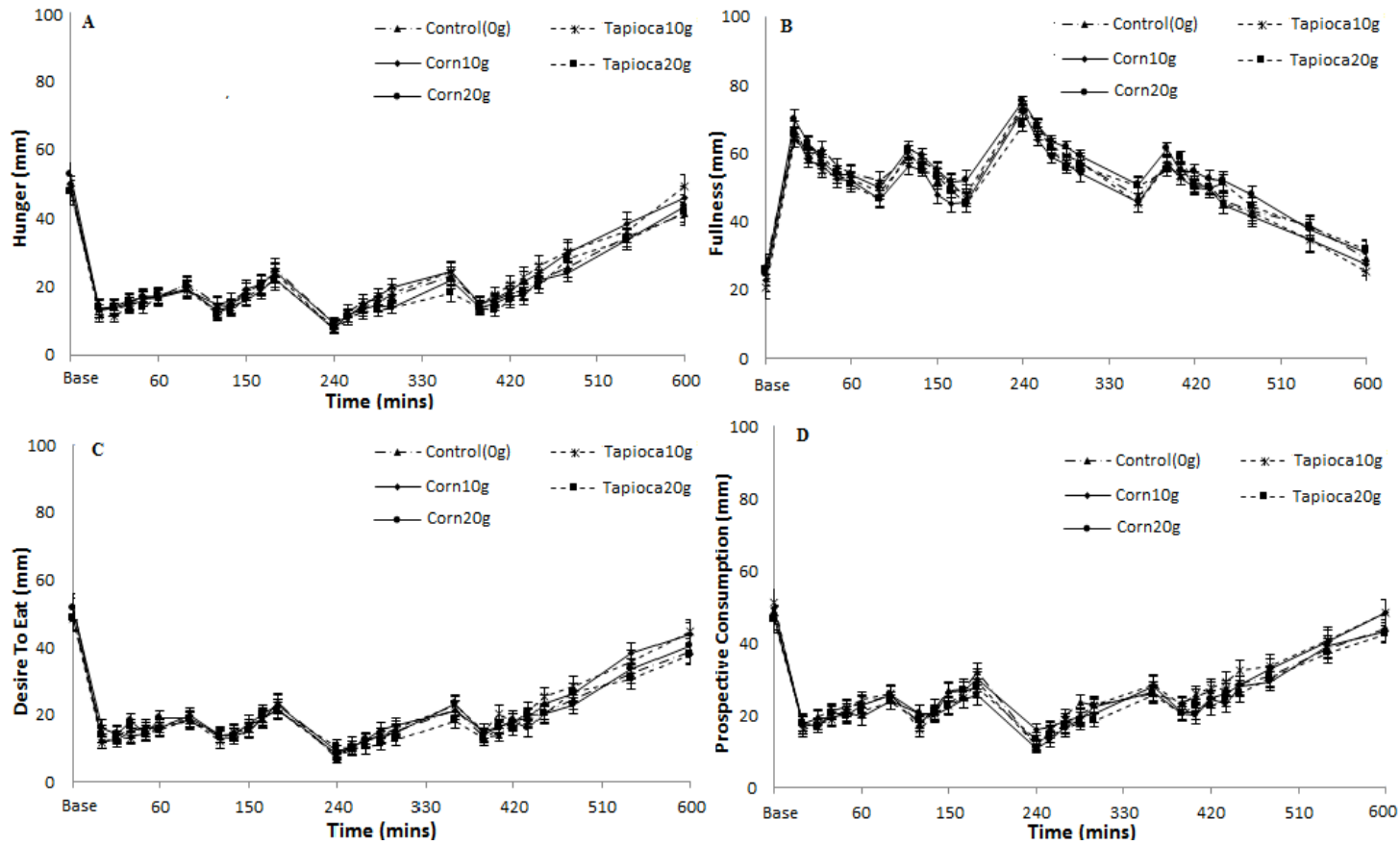
Fig. 3 VAS scores of hunger (**A**), fullness (**B**), desire to eat (**C**), and prospective consumption (**D**) rated from baseline through 600 minutes following control (triangles), Corn10 (diamonds), Corn20 (circles), Tapioca10 (star) and Tapioca20 (square) treatments. There was no statistically significant treatment differences observed (p > 0.05).

Table 1 Food Intake Data – Means (kCal) ± SE

Treatment	Lunch	Afternoon Snack	Dinner	Food Logs	Total (including breakfast & morning snack)
Control	441 ± 33	263 ± 18	499 ± 38	290 ± 44	2342 ± 90
Corn10	452 ± 33	282 ± 20	534 ± 38	358 ± 60	2489 ± 94
Corn20	457 ± 27	268 ± 19	511 ± 36	337 ± 60	2440 ± 85
Tapioca10	444 ± 31	264 ± 18	481 ± 29	360 ± 51	2412 ± 84
Tapioca20	422 ± 30	239 ± 18	502 ± 36	276 ± 48	2316 ± 95
F (4, 39)	0.808	1.583	0.798	0.741	2.040
Main Effect P-Value	0.528	0.198	0.534	0.570	0.108

CHAPTER 6

GENERAL CONCLUSIONS

General Conclusions

Results from the previously described studies have demonstrated the following:

1) RS4/resistant dextrin consumption did not have an effect on next meal *ad libitum* intake.

2) RS4/resistant dextrin consumption did not have an effect on subject appetite ratings in the laboratory.

3) In free-living conditions, appetite and food intake changes were observed, but inconsistent between studies.

4) Resistant dextrin is likely fermented in the healthy young adults with peak breath hydrogen occurring 6-8 hours post consumption

5) There is no robust effect of RS4/resistant dextrin on biomarkers of satiety nor glycemic response

Overall, the results presented in this dissertation are consistent with the majority of short-term appetite studies investigating resistant starch or resistant dextrin in humans. When data on biomarkers, food intake and subjective appetite ratings are all collected, these studies often find significant impact in only one of those three categories. As explained in Chapter 2, each measure is evaluating a different aspect of overall appetite. As food intake is not strictly a result of physiological signals or subjective ratings of appetite, results across these categories will not always point in the same direction. The interesting thing in the presented work, as well as outside literature, is the inconsistencies in which commercially available

resistant starches are reported to impact these categories. When multiple measures are taken, there is evidence to support claims that resistant starches are fermented only [Chapter 5, Reference 1] have effects on appetite only [Chapter 4, References 2, 3], impact short-term food intake only [Chapter 3, References 4, 5], or only change glycemic response [6, 7]. Other studies testing multiple sources of fiber have found only minor effects between different fiber sources [8, 9] with no effects relative to rapidly digestible starch.

Multiple hypothesis testing in short-term feeding studies is a common practice and is the most efficient way to collect data on closely related topics. However, incomplete reporting of such data can further confuse a topic already complicated by variation in fiber types, fiber amount and dose timing. Although limited registries, such as clinicaltrial.gov, track some human studies from start to finish, in the aforementioned studies, it is unclear as to which of the reported measures was the primary outcome of the study and if that outcome was set before data observation. It is therefore likely that the inconsistent effects of resistant starch/resistant dextrin on the human appetite system reported in recent reviews [10, 11] are due in part to type I errors in the reporting of post-hoc analyses. Moreover, due to publication bias, the published literature may not fully represent the body of work that has been conducted on this topic. Publication pressures may result in both selective outcome reporting in published papers and not publishing null findings [12, 13].

Regardless of reported primary outcomes, short-term feeding studies investigating the effects of resistant starch/resistant dextrin are nearly exclusively framed around helping to fight the obesity epidemic. Furthermore, there is no significant change in methodology associated with differences in reported primary outcomes (subjective appetite, biomarkers or

food intake). While changes in biomarkers and subjective measures of appetite are proxies of motivation to eat, food intake is the only measured outcome that has a direct impact on energy balance. Although the reliability of short-term feeding study results being representative of longer-term eating patterns is questionable, the greatest mechanistic evidence short-term studies can provide to explain the correlation between fiber consumption and lower body weight is to demonstrate changes in food intake.

Overall, the studies presented in this dissertation are consistent with previous studies' observations of no robust effect of RS4 or resistant dextrin on short-term food intake [1, 5, 8]. For the studies in which statistically significant treatment effects were observed [Chapter 3, References 5], the magnitude of the decreased food intake was fairly small (~50 kcal for single meal effects or 200kcal for single day effects). To our knowledge the study described in Chapter 3 is the first to show an impact of RS4 on short-term energy intake. The small magnitude of RS4/resistant dextrin purported effect on food intake is not entirely unexpected given the numerous physiological and environmental stimuli that drive food intake. As a concept, it may be unreasonable to expect a small change in the consumption of any single nutrient to have sweeping effects on appetite or short-term food intake.

The modest effects of commercially available RS4 and resistant dextrins on appetite and short-term food intake demonstrate that chemically modified fibers may not be as effective as naturally occurring fiber types on these measures. Much of the research that links fiber consumption with increased satiety has been conducted with whole foods and fibers in their naturally occurring food matrix [14]. These results call into question the assumption that fiber fortification of low-fiber foods will have the same physiological and behavior benefits

observed with increased consumption of naturally occurring high fiber foods. While the uneven effectiveness of naturally occurring versus chemically modified fibers may be attributable in part to differences in chemical structure of the fibers, differences in the food matrix most certainly contribute. Fiber fortification of often targets snack foods usually with high fat or high sugar content while naturally occurring fibers are found in staple foods such as grains and produce. It is possible that any modest effects of RS4/resistant dextrin on short-term food intake are nullified by the high caloric content of the vehicle food. Therefore increased consumption of fiber-fortified foods may actually lead to greater overall energy intake and a positive energy balance over time.

Finally, the lack of robust effects of RS4 and resistant starch on appetite and single day food intake shows that the correlation between fiber consumption and lower body may not be mediated through changes in these short-term outcomes. As it is it unclear if fiber impacts short and long-term food intake in the same way, research studies investigating effects of long-term fiber supplementation should be considered separately. Studies in which resistant starch was supplemented for at least one week show mixed impacts on longer-term food intake and body weight [15-17]. As is true for short-term feeding studies, the reported effects of fiber on total day energy consumption in these longer-term studies are partially confounded by the assumption that fiber provides no calories. Further research into the energy gained through fiber fermentation and SCFA production should be researched and incorporated into studies investigating the impact of fiber on food intake and body weight.

Recommendations for Future Research

Continued research in the short-term effects of commercial fiber ingredients like those used in these studies should pay particular attention to the qualities of the test vehicle being used. The general importance of using test foods that make sense for the time of day being administered has been previously discussed as a consideration for all short-term appetite studies [17]. Specific to fiber research, the satiation characteristics of the test food should also be evaluated so as to not mask potential fiber effects. The work presented in this dissertation, along with outside work collectively indicates increased potential to identify an effect of fiber when a low satiety vehicle such as beverages or yogurt is used [5, 18-20]. To more easily evaluate the efficacy of new fibers, it may be prudent to identify a set of standardized test vehicles to use in short-term appetite studies.

As part of choosing an optimal test vehicle, changes in orosensory characteristics between the control and test products should also be considered. There are few studies that rigorously evaluate orosensory changes of test foods being provided. As discussed in the literature review of this dissertation, orosensory characteristics play a significant role in appetite and short-term energy intake. Replacement of rapidly digestible flour with resistant starch flour in bread type foods often leads to a softer or unrecognizable texture as compared to the control food. Other fibers may create more dense or dry textures when used in recipes. Future work should deliberately investigate the orosensory qualities of test and control vehicles and work to match them as closely as possible.

In addition to vehicle considerations, there should also be more attention paid to the target population studied. The results from the presented work along with systematic reviews

conducted of fiber's potential effects on appetite and satiety [10, 11] show great inconsistencies in fiber treatment effects in a young, healthy, adult population. While methodology difference certainly plays a role in the variation between studies, the non-specific nature of the target population may also contribute. Identification of those individuals who would benefit most from a fiber intervention such as habitual low fiber consumers or obese individuals may prove beneficial in truly evaluating the efficacy of a fiber intervention. Low fiber consumers may be more sensitive to increases in fiber consumption, while obese individuals have been shown to respond differently than lean individuals in appetite and short-term energy intake studies [21-23]. To date, not much research has been conducted to assess the effects of fiber on these specific populations.

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