

**EFFECTS OF FIBER ON SATIETY, FOOD INTAKE, GLUCOSE,
INSULIN, AND GUT HORMONES IN HEALTHY HUMAN
SUBJECTS**

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Abstract

Epidemiologic studies suggest that people who eat more fiber have a lower body weight than people who eat less fiber. Potential mechanisms for this relationship may include greater feelings of satiety, reductions in food intake, or changes in blood glucose, insulin, or gut hormone levels. The following work describes two unique intervention studies designed to help explain this relationship.

In the first study we hypothesized that certain *types* of fiber would enhance satiety more than others when consumed in muffins for breakfast. Healthy men and women participated in this randomized double-blind, crossover study comparing satiety after subjects consumed four different fibers and a low-fiber control. Subjects used 100 mm visual analog scales (VAS) to rate satiety for 3 hours after muffin consumption. Satiety differed among treatments. Resistant starch and corn bran had the most impact on satiety, while polydextrose had little effect and behaved like the low-fiber treatment.

In the second study we hypothesized that increasing *doses* of fiber would increase satiety and decrease food intake in a dose-dependent manner. We also hypothesized that glucose, insulin, ghrelin, GLP-1, and PYY₃₋₃₆ would change in proportion to fiber dose. Healthy men and women participated in this randomized double-blind, crossover study. Subjects consumed muffins with 0, 4, 8, and 12 g of mixed fibers and proceeded to rate satiety with VAS over a three-hour period. Blood was drawn at regular intervals and *ad libitum* food intake was assessed at two different time points. The 12 g fiber muffin was consistently and significantly more satiating than the 0 g muffin; however, food intake did not differ among treatments. Glucose, insulin, ghrelin, and GLP-1 differed among treatment doses, but not in the manner we expected. Glucose and insulin did not

correlate with each other or with appetite. Ghrelin was significantly higher after 12 g of fiber than after all other doses, and GLP-1 decreased consistently with fiber dose. PYY₃₋₃₆ did not differ among treatments.

Results from these studies indicate that certain types and doses of fiber positively influence satiety. However, caution should be used when making blanket statements about fiber as a generic substance; this research suggests that some types and doses are not as effective as others. Furthermore, feelings of satiety may not be consistently linked to food intake or other commonly accepted physiologic measures for satiety.

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Chapter One
LITERATURE REVIEW

Defining Fiber

In the 1950s, fiber was loosely described as any non-digestible portion of a plant cell wall ¹. Through the years this definition has evolved to include a much wider range of substances. In 2002, the Institute of Medicine (IOM) stated that *total fiber* is determined by the sum of *dietary fiber* plus *functional fiber* ². *Dietary fiber* consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants, while *functional fiber* is isolated, non-digestible carbohydrates that have beneficial physiologic effects in humans. Similar fiber definitions have also been proposed by the American Association of Cereal Chemists, 2001; the Codex Alimentarius Commission, 2006; and the Health Council of the Netherlands, 2006. Each definition agrees that fiber is not digested or absorbed in the small intestine. These organizations, however, continue to debate about which food sources can be called fiber, which analytical methods are best, and the level of detail needed to describe fiber's physiologic benefits ³.

In the United States fiber is described on food labels as soluble or insoluble ³. These properties are determined by methods from the Association of Analytical Communities (AOAC). These analytical methods are important; however, solubility represents only one part of fiber. At least two key factors are not captured through standard AOAC analysis: 1) certain fibers, like some resistant starches or oligosaccharides, might be incorrectly identified or misrepresented, with the solubility analyses and 2) the physiologic properties, or benefits, of various fiber types cannot be assigned based solely on solubility.

When it comes to health benefits, it is somewhat inaccurate to make blanket statements based on the categories of soluble and insoluble fibers. Instead, health benefits and fiber properties are likely better discussed in terms of specific fiber type (i.e. psyllium, pectin, etc.) and not as a group of fibers. For example, the Dietary Fiber Position Paper from the American Dietetic Association suggests that it is unfair to classify all soluble fibers as cholesterol-lowering or all insoluble fibers as stool-size promoters ⁴. Instead, specific health benefits should be ascribed to a specific fiber. For example, psyllium is a soluble fiber that has been associated with decreasing cholesterol levels ⁵.

In most cases, the physiologic benefits of a fiber may be further defined when considering solubility along with viscosity and fermentability.

Viscosity is evaluated by how much a fiber thickens when it is added to fluid; it is also associated with water-holding properties ⁶. Determining the viscosity of a liquid product is relatively simple, while methods to determine the viscosity of a fiber as part of a food/diet matrix are difficult and inconsistent. It is unlikely that determining the viscosity of a fiber in liquid will translate into how the fiber behaves when it is added to a food product. For example, a particular fiber may be very viscous in water, but when baked into a food with other ingredients it will likely behave quite differently. Animal studies have attempted to determine intestinal content viscosity after an animal has eaten various fibers ⁷. However, it is extremely difficult to extrapolate these findings since viscosity is not static; viscosity likely: changes at different points along the digestive tract, depends on the other components of a meal, and varies with gut motility.

Of note, viscous fibers are often deemed equivalent to soluble fibers, but this is not accurate. For example, some soluble fibers can be highly viscous (i.e. native guar gum), while others are not (i.e. partially hydrolyzed guar gum) ⁸.

Fermentability of a fiber is also difficult to assess and discuss. Fiber is not digested in the small intestine; and therefore, arrives at the large intestine intact and available for fermentation by the resident microflora ⁹. The fermentation process yields short chain fatty acids (SCFA), which are available for uptake by colonocytes. Certain types of fibers are more fermented than others. For example, pectin is generally accepted as a fermentable fiber, while cellulose is deemed non-fermentable ⁹. However, current assessment methodology has many limitations. Neither in vitro nor in vivo measurements give a clear representation of how a particular fiber would be fermented in a particular individual. In vitro methods attempt to determine fermentability by inoculating various fibers with human feces samples; however, this closed, static system does not represent the dynamic and changing environment of the human colon ¹⁰. Meanwhile, in vivo measurements of fiber fermentation are impossible to extrapolate since every individual colonizes different types and amounts of microflora.

Unfortunately, there are no “gold standards” for measuring the viscosity or fermentability of a particular food product. Both of these characteristics will likely vary from product to product (i.e. in bread versus in a cracker) even if the fiber source is the same for both. As well, these properties may also vary from one digestive tract to another. In other words, what is viscous for one person may not be for another, and what one person ferments, another may not.

Ultimately, discussing fiber is challenging. Each type of fiber has distinct physical and chemical characteristics and will impart unique physiologic benefits. This makes it extremely difficult to develop definitions and classification systems that will accurately predict specific physiologic responses. The current classification by solubility is helpful, but would be strengthened if accurate and replicable methods were available for assessing viscosity and fermentability.

Specific Types of Fiber

In this section I will briefly discuss the generally recognized analytical and physiological characteristics of eight specific fibers: β -glucan, corn bran, polydextrose, resistant starch (RS), pea fiber, pectin, guar gum (GG), and methylcellulose. These are the eight types of fiber used in my research (chapters 2 and 3); the brief descriptions below shed light on the diversity within the fiber family. These fibers were chosen for a number of reasons: 1) a previous literature review suggesting the potential for improved satiety ¹¹ 2) ability to be baked uniformly into muffins, and 3) their broad representation of soluble, insoluble, and resistant starch characteristics.

β -glucan

β -glucan is a glucose polymer with variable linkages and a branched structure ¹². It is considered a soluble fiber and is known to form viscous gels and slow gastric emptying. Some studies of β -glucan suggest this fiber may be beneficial for lowering both cholesterol ¹³⁻¹⁵ and post-prandial blood glucose and insulin levels ¹⁶⁻¹⁹.

β -glucan has also been studied for its effects on satiety. One study found that β -glucan from barley was significantly more satiating than a white flour control ¹⁹. However, Peters et al found that satiety was indistinguishable among a meal replacement bar with β -glucan, without β -glucan, or a bar with another type of fiber (fructooligosaccharide) ²⁰. Similarly, another study reports fermentable β -glucan fiber (with added pectin) was less satiating than the non-fermentable fiber, methylcellulose (this study did not include a no-fiber control for comparison) ²¹. β -glucan is found naturally in whole oats and barley.

Corn Bran

Corn bran is an insoluble, non-viscous fiber ⁸. This fiber is poorly fermented in the large intestine and is associated with increased laxation ⁹. Recent studies on the physiologic benefits of this fiber are limited. An older study suggests corn bran significantly increases stool weight compared to control, and that it may improve glucose and cholesterol levels in healthy men ²². This was not confirmed in another study, which found 26 or 52 g of corn bran per day had no effect on blood glucose, insulin, or cholesterol levels ²³. Corn bran has not been studied for its effects on satiety. However, some studies have evaluated how corn bran blended with other fibers, such as wheat bran, influences satiety ^{24, 25}.

Polydextrose

Polydextrose is formed when glucose in the presence of acid and sorbitol is subjected to heat polymerization ¹². Because this soluble compound is poorly digested

in the small intestine and incompletely fermented in the large intestine, it is considered a functional fiber under the IOM definition. Some research suggests polydextrose may increase stool weight and may help alleviate constipation^{26,27}. Polydextrose has been studied for its influence on satiety; however, published results show no benefit when consuming this fiber versus control²⁸. Polydextrose is unlikely to lower post-prandial glucose and insulin levels, or to alter cholesterol levels²⁹. The key role for polydextrose is likely its ability to act as a low-calorie sugar replacement in baking.

Resistant Starch

There are four classes of RS (physically inaccessible starch, RS1; native starch granules, RS2; retrograded starch, RS3; and chemically modified starch, RS4). Resistant starch has physiologic effects similar to fiber. Many resistant starches are currently manufactured and added to foods. Some carbohydrate-rich foods contain natural RS; however, the proportion is largely dependent on cooking, cooling, food storage, and food ripeness. Digestion of RS varies across individuals, and therefore the physiologic benefits of this fiber remain unclear. However, a broad review paper states that RS may improve post-prandial glucose and insulin levels, improve SCFA production (fermentation in the large intestine), and reduce cholesterol levels³⁰. It also may increase fecal weight and stool frequency. Data on the relationship between RS and satiety are limited, though some studies suggest RS has little or no satiating effect

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Pea Fiber

There are many types of pea fiber, but in general, they are all considered about half soluble and half insoluble³⁴⁻³⁶. They are also recognized as moderately viscous with limited fermentability³⁷. Some studies indicate that pea fiber may positively influence satiety^{36,38}. When added to usual dietary intake, pea fiber has been found to increase frequency of bowel movements, but it does not alter overall transit time compared to other fibers^{35,39}. The impact of pea fiber on glucose, insulin, and cholesterol levels is inconsistent. Interestingly, pea fiber can be added into baked goods more easily than other fibers (this is due to lower viscosity).

Pectin

Pectin is found in the cell walls of many fruits and vegetables. This fiber is recognized as highly soluble and viscous; it is also largely fermented in the colon^{9,12,37}. Pectin is believed to delay gastric emptying and increase feelings of satiety^{40,41}. Research also suggests large doses of pectin may have cholesterol-lowering properties⁵.

Guar Gum

Guar gum is fermentable, highly soluble, and viscous⁴². Native GG may delay gastric emptying and has been correlated with increased satiety^{42,43}. However, Kovacs et al found no difference in 16-hour satiety ratings when comparing a solid meal to a semi-solid meal with or without GG⁴⁴. Data on how GG affects glucose and insulin levels are inconsistent. GG is a galactomannan and comes from the seeds of guar beans.

Many studies use fully or partially hydrolyzed guar gum (PHGG) instead of native GG, since hydrolyzed forms are less viscous and dissolve more easily in food and beverages. PHGG, when added to a fixed diet, has been inconsistently associated with satiety. One study found PHGG favorably influenced satiety⁴⁵, while another study showed a non-viscous PHGG had no effect on satiety compared to a control⁴⁶.

Methylcellulose

Methylcellulose is a synthetic fiber is derived from cellulose (a glucose polymer with a methyl-ester substitution). This fiber is generally described as highly soluble, viscous, and non-fermentable. Consumption of this fiber is associated with increased stool frequency and fecal weight^{47,48}. Few studies have evaluated the effectiveness of methylcellulose on satiety.

One study found methylcellulose given as a preload before a meal had no effect on appetite ratings one hour later⁴⁹; another study also found no effect on appetite when methylcellulose was given as part of a meal⁴⁰. However, results of a three-week crossover study, found that daily satiety was higher after subjects consumed methylcellulose than after consumption of pectin with added β -glucan²¹.

Methylcellulose is the predominant ingredient in the fiber supplement Citrucel®; it is also used as a bulking agent and emulsifier in many food products.

Fiber Intake in the United States

The Dietary Reference Intakes (DRIs) established by the IOM recommend that adult men and women should consume 38 and 25 g of fiber per day, respectively².

These recommendations are based on epidemiologic evidence that cardiovascular protection is seen when fiber intake meets or exceeds 14 g of fiber per every 1,000 kilocalories consumed.

Fiber intake is low in the United States. It is estimated that the average American consumes only 15 g of fiber per day². Of note, this is difficult to assess since objective biomarkers for fiber intake do not exist; instead, estimated intake is often based on 24-hour food recall, food frequency questionnaires, or food disappearance.

Flours, grains, and potatoes are the most popular sources of fiber in the United States diet; while fruits, legumes, and nuts are the least popular sources⁵⁰. Many food manufacturers are adding fiber to foods that would not normally contain fiber (this is functional fiber)⁵⁰; however, the American Dietetic Association position paper suggests that the addition of functional fiber to foods is likely less beneficial than eating whole foods that are naturally high in dietary fiber⁴.

Fiber and Health

In recent years fiber intake has been increasingly linked to positive health outcomes. For example, some studies report high fiber intake is associated with lower risk for cardiovascular disease, diabetes, and possibly certain cancers⁵¹⁻⁵³. Significant research also suggests there is a correlation between fiber intake and body weight or body mass index (BMI)⁵⁴⁻⁵⁷. The exact mechanism of this relationship, however, remains unclear.

Fiber and Energy Intake

Fiber intake may modify body weight through its influence on overall energy intake. For example, Pasman et al reported 20% lower energy intake when subjects consumed 40 g of PHGG daily for a week compared to a fiber-free control⁴⁵. In a crossover study, energy intake over 11 days decreased by an average of 135 calories per day when subjects consumed either psyllium gum crackers or psyllium gum plus wheat bran crackers before meals⁵⁸. In the same study wheat bran crackers and a control cracker had no effect on energy intake. Another study found subjects ate 24% fewer calories at a buffet lunch after eating 15 g fiber bread compared to 3 g fiber bread for breakfast⁵⁹. Similarly, Samra and Anderson reported significantly less food intake at an *ad libitum* meal when male subjects ate 33 g of insoluble fiber cereal compared to an iso-caloric 1 g fiber cereal²⁴.

In contrast, Freeland et al showed no difference in *ad libitum* food intake 120 minutes after subjects consumed 41 g of wheat bran fiber (Fiber One® cereal) or a control cereal with 1 g of fiber (Wheatlets®)⁶⁰. Additional studies also report no association between fiber consumption and energy intake^{44, 61, 62}.

Fiber and Body Weight

More than three decades ago, Heaton described how fiber may reduce energy intake, which theoretically could lead to weight loss⁶³. He proposed that fiber may displace calories from other foods, it may reduce absorption of certain nutrients from the small intestine, and it may increase mastication and saliva production, which could lead to greater satiety. Years later Pereira and Ludwig also described how fiber intake

might relate to body weight ⁶⁴. They suggested that fiber affects a combination of intrinsic, hormonal, and colonic controls. For instance, the following is an example of a hormonal control. A viscous fiber might slow gastric emptying, regulate glucose and insulin response, and subsequently lead to greater satiety.

A large review, summarizing the effects of fiber on satiety, energy intake, and body weight in healthy people, estimated that increasing fiber intake by 14 g per day was associated with a 10% decrease in energy intake and a 2 kg weight loss over about a 4-month period ⁶⁵. This review also reported the results were more pronounced in obese individuals.

Satiety Overview

Satiety is a feeling that influences the length of time between one meal (or snack) and the next. Satiation is the satisfaction of appetite that is achieved during a meal, and ultimately determines cessation of the meal. In the casual setting, both of these words are often used interchangeably with fullness.

Satiety and appetite are commonly assessed by a combination of subjective and objective markers. Subjective analysis often includes use of visual analog scales (VAS). VAS ask a series of questions about hunger, desire to eat, satisfaction, fullness, nausea, and/or prospective food intake. VAS have been validated using multi-point ratings ^{66,67}, or using 100 mm lines with opposing anchors on either end ^{68,69}. For example, the question, “How hungry do you feel?” would be anchored with “Not at all hungry” at 0 mm, and “I have never been more hungry” at 100 mm (samples of the

VAS we used in our research are shown in Appendix A). Original VAS used a pen and paper method; however, electronic versions have recently been validated⁷⁰⁻⁷².

Objective markers for satiety may include hormone levels, gastric distention, gastric emptying, brain activity on MRI, or short and long-term food intake. However, these are only *markers*; truly objective tests for satiety do not exist (i.e. there are no definitive blood tests that reveal a person's specific satiety level).

Many factors influence satiety and food intake, including: calorie load, time since last meal or snack, or activity level. Additionally, some data suggest satiety is influenced more by the weight or volume of a food than by its macronutrient content or caloric density⁷³⁻⁷⁶. Similarly, the volume of a food at one meal has been shown to affect food intake at the next meal⁷⁷. Other data report that people are more satiated by certain macronutrients. For example, some research suggests people are less sensitive to fat intake than other macronutrients—and that 'calorie for calorie' fat may be the least satiating⁷⁸⁻⁸⁰. Other studies demonstrate that the moisture content of food significantly influences satiety and food intake; and that higher moisture foods may be more satiating^{77,81}. Conversely, completely liquid foods tend to be less satiating than a macronutrient-matched solid food^{82,83}. Finally, Howarth et al suggested food intake is controlled by at least four factors: food form (raw or cooked), palatability, energy density, and variety⁶⁵.

Palatability of a food or meal plays a controversial role in perceptions of hunger, satiation, and satiety. A food's palatability is defined by hedonic perceptions of taste, visual appeal, smell, aftertaste, texture, etc. A recent review suggests that palatability and food intake increase proportionally⁸⁴. However, the same review reports mixed

results when it comes to the influence of palatability on appetite. (Thus, indicating that palatability may influence satiation and satiety differently.) In some cases highly palatable foods can increase feelings of hunger and decrease feelings fullness, whereas the opposite can also be true.

Freeland et al published two experiments in the same paper⁶⁰. In study 1, pizza intake was directly correlated with palatability ($r = 0.41$, $p=0.001$). In other words, the more pleasant the pizza was, the more the subjects ate. In study 2, pizza intake was not correlated with palatability ($r = 0.09$, $p=0.5$). It is difficult to explain such conflicting results when the study protocols were quite similar.

Despite abundant research, and a number of satiety-analyzing tools, we must not forget that people eat for many reasons unrelated to level of hunger or appetite. Food is part of social gatherings, holiday traditions, religious ceremonies, and business meetings. In the United States, food is inexpensive, palatable, and convenient—and this often translates into unplanned meals or snacks. People eat under stress, because something smells good, or because someone else is eating. The reasons why a person chooses to eat are varied and complex. Overall, it is clear that environment and circumstances play a powerful role in the decisions people make about when, what, and how much food to eat. It is impossible to control for the myriad of psychological and situational factors that influence a person's food intake. Clinical studies of satiety attempt to control for these factors by comparing treatments in a randomized manner, having subjects arrive at study visits in the fasted state, and using crossover designs where each subject serves as his or her own control.

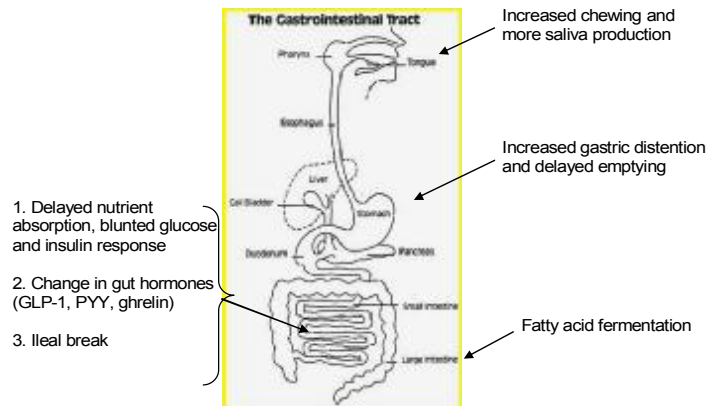
Satiety and Fiber

Many review papers support a direct relationship between fiber intake and satiety^{11, 64, 65, 85, 86}. However, this relationship depends on numerous factors, including but not limited to: the type of fiber (soluble, insoluble, viscous, fermentable), the dose of fiber (1 g versus 25 g), dietary fiber versus functional fiber, the individual (man, woman, obese, lean, young, old), and the duration of fiber intake (one dose at lunch versus daily for one year).

Mechanisms for the Fiber and Satiety Relationship

Multiple mechanisms have been used to describe how fiber influences satiation and satiety (Figure 1 below).

Figure 1. Fiber and Satiety: Proposed Mechanisms



Adapted from Howarth et al. Nutr Reviews 2001; 59(5).

Greater satiation may be partly achieved because of increased time required to chew fiber-filled foods^{65, 86}. Intake of some fiber-containing foods may become self-limiting because they take longer to eat than other foods. Increased time chewing promotes saliva and gastric acid production, which may increase gastric distention.

(Some soluble fibers bind water and also increase distention.) Gastric distention and stretch receptors have been shown to play a role in hunger and fullness^{42, 87-89}.

Furthermore, certain fibers may slow gastric emptying and decrease the rate of glucose absorption in the small intestine. For example, highly viscous fibers (like guar gum) can “trap” glucose in a gel-like matrix; because of this, glucose may appear in the blood stream more slowly. When glucose is released slowly, the insulin response may also be blunted. Slow, steady post-prandial glucose and insulin responses are sometimes correlated with satiation and satiety (detailed discussion below). Research also suggests that satiation is enhanced when the intestinal tract senses nutrients^{65, 90}.

The ileal brake may also influence satiety. This is an inhibitory feedback mechanism that controls transit of a meal through the gastrointestinal (GI) tract⁹¹. As food is pushed out of the stomach and into the small intestine, distal messengers dictate how quickly food will traverse the GI tract. By controlling the speed and movement of an ingested food, nutrient digestion and absorption is optimized. The types and amounts of nutrients consumed may influence ileal brake action (i.e. fat likely triggers the ileal brake most potently)⁹². Certain gut hormones, like polypeptide YY (PYY) and glucagon-like peptide-1 (GLP-1), may also stimulate the ileal brake and overall satiety (detailed discussion below). Finally, certain types of fiber are largely fermentable in the colon. Fermentable fibers produce SCFAs, which have the potential to modify satiety-related pathways⁹³⁻⁹⁵.

Intervention Studies

Intervention studies support the general hypothesis that certain fibers influence satiety. Samra and Anderson found that average appetite area under the curve (AUC) was significantly suppressed after 300 calories of an insoluble fiber cereal compared to an iso-caloric amount of fiber-free white bread²⁴. Similarly, Levine et al reported subjects felt significantly less hungry after eating either 18 g or 35 g of fiber from bran cereal than after a fiber-free control cereal²⁵. (Interestingly, the same study showed no difference in hunger when comparing the fiber-free control to cereals with either 11 g of wheat fiber or 39 g of wheat plus corn bran fiber.) Raben and colleagues found subjects felt significantly more full and had less desire to eat after consuming 25 g of pea fiber baked into wheat bread, than after 9 g of fiber from plain wheat bread; however, no differences in the subjects' hunger or satisfaction level were noted³⁶. Similarly, Burley et al found a small but significant difference in fullness between a 12 g and 3 g fiber breakfast, but no differences in subjects' perception of hunger or desire to eat⁹⁶. Other acute studies have found no relationship between fiber intake and satiety^{61,97,98}.

Differences in study design (i.e. different fiber types, different evaluation periods, different styles for assessing satiety) likely explain these conflicting results. Palatability of the fiber-rich foods may also play a role. If palatability differs between a fiber-rich food and a fiber-free control food, satiety responses and the desire for subsequent food intake could also differ. It is important to consider the effect of palatability ratings in fiber and satiety studies.

Glucose, Insulin, and Satiety

The glucostatic theory states that low blood glucose equates to hunger, while elevated blood glucose stimulates fullness. A recent review strongly supports this theory, stating: when the body senses low blood glucose, hunger and food intake increase so much that weight gain ensues⁹⁹. Similar theories exist for insulin. If insulin is low feelings of hunger may be enhanced, and if insulin is elevated then hunger may be suppressed.

In contrast, some people believe that lower, more sustained blood glucose and insulin levels are the key to satiety. This is often described when talking about foods with a low “glycemic index”. Many low glycemic index foods are rich in fiber. Several studies have discussed the association between fiber-rich foods, improved satiety, and low levels of glucose and insulin.

Unfortunately, neither the glucostatic nor the glycemic index theory is straightforward. Studies have shown that it is difficult to conclude whether glucose or insulin levels are associated with appetite regulation.

A 2007 meta-analysis evaluated the relationship between glucose, insulin and satiety for normal weight and obese individuals¹⁰⁰. The analysis included data from 7 randomized human feeding trials with more than 130 subjects; each study used mixed macronutrient meals to stimulate change in glucose and insulin response. The authors concluded that glucose levels were not significantly associated with hunger, satiety, or energy intake. However, insulin levels were related to appetite regulation in normal weight—but not overweight—subjects. Their analyses suggested that increased insulin

was related to greater satiety, less hunger, and less energy intake for the normal weight subjects only.

Similarly, Holt et al analyzed the relationship between post-prandial satiety and glucose and insulin levels after providing subjects with 38 different foods¹⁰¹. Results of this study found no significant relationship between satiety and glucose or insulin response for any of the foods. However, insulin was negatively correlated with *ad libitum* food intake 2 hours later, which suggests *lower* (not higher) insulin levels might be more likely associated with satiety.

Granfeldt et al reported that 4 barley products (14-18 g of fiber) were more satiating than a white-bread control and that each of them evoked a significantly lower AUC glucose and insulin response¹⁹. Similarly, Lavin and Read found that, compared to a control, 5 g of GG added to a caloric beverage significantly lowered post-prandial glucose and insulin levels, while at the same time increasing satiety⁴³. Adam et al also showed higher satiety scores significantly correlated with lower insulin levels in normal and obese subjects¹⁰². Results of these 3 studies are more likely explained by the glycemic index hypothesis than by the glucostatic theory—since lower glucose and insulin levels were associated with greater satiety.

Many studies also report no relationship between satiety, glucose, or insulin levels. Holt et al compared the differences in plasma glucose between 7 iso-caloric breads with varying fiber content (2-33 g)⁷⁶. They found satiety was not correlated with glucose levels for any of the treatments. However, glucose and satiety were only measured for 2 hours after bread consumption. In this study glucose levels never returned to baseline, so it is possible more time would have been required to see a

potential association. Another study reports no relationship between glucose or insulin levels and a series of hunger and satiety related questions²⁴. Consumption of cereals and bread elicited significantly different glucose and insulin curves, but no difference in the subjects' reported appetites. Furthermore, intravenous insulin infusion studies do not support the relationship between satiety and elevated insulin levels^{103, 104}.

To summarize, it seems unlikely that glucose and insulin play a key role in appetite regulation. Both probably contribute to the bigger pictures of hunger and satiety, though their influence is likely confounded by a number of metabolic or situational factors (i.e. release of gut hormones, time between meals, activity level, insulin resistance). Nonetheless, both glucose and insulin should remain under investigation in feeding studies—especially with regard to changes in anorexigenic and orexigenic hormones.

Gut Hormones

In addition to changes in glucose and insulin levels, food intake also provokes release of hormones from the gut. Many gut hormones were originally studied for their role in regulating the GI tract. Today it is clear that these hormones play a large (and possibly more significant) role in the brain. Therefore, a gut-brain axis surely exists. The extent to which this axis controls specific food intake behaviors is unclear—and likely is not uniform across all people. The complex circuitry regulating food intake, appetite, and/or energy expenditure are beyond the scope of this research and therefore will be discussed with brevity.

Ghrelin, PYY, and GLP-1 are three important appetite-regulating gut hormones; however, more than 20 additional gut hormones have been recognized for their connection with appetite and food intake. For example, cholecystokinin (CCK) is another important appetite-regulating hormone that has been shown to have an inhibitory effect on food intake in animals and humans⁷⁹. The effect of CCK, however, appears to be short-lived and is believed to have a greater effect on meal size and duration, than on appetite sensations in between meals²¹³. Some studies also suggest that CCK release is different between men and women¹⁷². The combination of these factors makes CCK a less favorable choice for some study designs.

The following sections provide information on how appetite and food intake are affected by the presence of ghrelin, GLP-1, and/or PYY; as these are the gut hormones I evaluated in my research (chapters 2 and 3). When data are available, the relationship between fiber intake and these gut hormones is also discussed.

Ghrelin

Ghrelin, a 28 amino-acid peptide, was originally identified as a ligand for the growth hormone secretagogue receptor in the stomach¹⁰⁵. Ghrelin is produced by endocrine cells in the fundus of the stomach—these are called X/A-like cells or ghrelin cells. It is estimated that 75% of ghrelin is of gastric origin and that only small amounts are produced and released from the small intestine (smaller amounts are released from the hypothalamus and pituitary, as well)¹⁰⁶. Ghrelin circulates in the blood as acylated and desacylated forms—acylated ghrelin is believed to be the most active. However, acylated ghrelin circulates bound to large molecules (proteins) and may not always be

fully represented with standard assays¹⁰⁷. Total ghrelin levels are commonly evaluated in the nutrition literature.

Ghrelin is the only known *peripheral* hormone that increases appetite and stimulates food intake¹⁰⁸⁻¹¹⁰. For example, when ghrelin is administered peripherally or centrally (especially into the arcuate nucleus) food intake increases significantly in rodents^{108, 111, 112}. When given as an infusion to healthy volunteers, ghrelin has been shown to cause an almost 30% increase in food intake¹¹³. (Of note, the dose used in this study was more than twice the physiologic normal level for these subjects.)

Ghrelin levels are highest pre-prandially and have been directly correlated with hunger scores^{110, 114}. After food consumption, ghrelin levels decline in proportion to calorie intake in normal weight people^{115, 116}. In obese individuals, the rapid and significant decrease in ghrelin is attenuated¹¹⁷.

The macronutrient content of a meal plays a role in ghrelin fluctuation. One crossover study showed that ghrelin levels decreased more after a high-carbohydrate meal than after a high-fat or high-protein meal; however, ghrelin was suppressed for a longer period of time after high-protein than after high-carbohydrate¹¹⁸. Another study showed that ghrelin decreased most after carbohydrate intake, whereas protein and fat intake led to increased ghrelin¹¹⁹. A third study also found that ghrelin was suppressed more after carbohydrate intake than after iso-caloric fat intake; however, this study did not compare either macronutrient to protein¹²⁰. Conversely, Foster-Schubert et al report that both total and acylated ghrelin decrease significantly more after protein intake than after iso-caloric amounts of carbohydrate or fat intake; they also found total and acylated ghrelin decreased more after carbohydrate than after fat¹²¹. Interestingly,

in this study, ghrelin reached the peak nadir 90 minutes after carbohydrate consumption; it then rose steadily to values above baseline over the next 4.5 hours. Ghrelin levels reached nadir 3 hours after protein intake and stayed below baseline for more than 6 hours. Al Awar et al also report that acylated ghrelin levels stayed below baseline longest, following a high-protein meal compared to high-fat or balanced meals¹²².

The effect of fiber on post-prandial ghrelin levels remains inconclusive. Lee et al reported significantly lower plasma ghrelin concentrations for 3 hours after consuming lupin kernel bread compared to an iso-caloric amount of white bread⁵⁹. Lupin kernel flour is rich in both fiber and protein, thus rendering it difficult to determine if the fiber, protein, or the combination of the two, lead to more favorable ghrelin values. Gruendel et al found the ratio of acylated to total ghrelin was significantly lower after consumption of liquid meals containing 10 or 20 g of insoluble carob fiber compared to a fiber-free control; though total ghrelin was indistinguishable between treatments¹²³. In a follow-up study by the same group, healthy subjects consumed 50 g of glucose with 0, 5, 10, and 20 g of added carob fiber; this time acylated ghrelin did not differ between treatments¹²⁴. In another study, total ghrelin was significantly *increased* two hours after a meal with 6 g of added arabinoxylan fiber compared to control¹²⁵. Arabinoxylan is often considered a soluble, viscous fiber.

The duration of time for ghrelin to return to pre-prandial levels varies. In general, ghrelin is considered short-acting and probably remains significantly suppressed for about 60 to 90 minutes after mixed-macronutrient food intake^{113, 126}. However, it clearly depends on macronutrient composition—with the possibility that

predominantly protein meals reduce ghrelin levels more slowly and with greater longevity. Some reports indicate that ghrelin may also be associated with long-term energy balance. This theory comes largely from data showing that ghrelin is chronically lower in obese individuals compared to lean^{106, 116, 117}. Of note, pre- and post-prandial ghrelin levels appear to normalize with weight loss.

The manner by which ghrelin increases hunger is unclear. However, some of its effects are mediated by the hypothalamus. At least three different pathways may contribute to ghrelin's mechanism of action: 1) ghrelin is released from the stomach into the bloodstream, where it circulates and enters the hypothalamus by crossing the blood brain barrier (BBB) 2) ghrelin is released along the GI tract where vagal afferents communicate with the hypothalamus, or 3) ghrelin is produced and acts locally in the hypothalamus^{106, 108, 127, 128}. More specifically, ghrelin signals are believed to stimulate the orexigenic neurons (neuropeptide Y (NPY) and agouti-related protein (AgRP)) in the arcuate nucleus¹⁰⁶.

Some suggest ghrelin increases GI motility (though this remains unproven)¹²⁹. If this is true, however, ghrelin could theoretically increase gastric emptying, which may reduce feelings of satiety. A review paper states ghrelin levels are inversely correlated with insulin levels; and thus suggests this as a rationale for increased hunger if ghrelin is elevated¹⁰⁹. Another theory suggests the timing of a ghrelin peak is related more to habitual meal patterns (and the anticipation of eating), than to its relationship with other hormone changes¹³⁰. Overall, it is likely that ghrelin influences appetite and food intake through both central and peripheral actions.

Glucagon-Like Peptide-1

GLP-1 is produced primarily by L cells located throughout the length of the GI tract, especially in the ileum and colon¹³¹. A smaller amount is also produced by nerve cells in the brain¹³². Two equally bio-active forms of this gut hormone exist, GLP-1₇₋₃₆ and GLP-1₇₋₃₇ (throughout the rest of this paper I will refer to them as GLP-1, collectively). Studies suggest that GLP-1 decreases appetite and food intake in rodents and humans¹³³⁻¹³⁵. And, a meta-analysis confirms that GLP-1 infusions lower appetite and food intake proportional to the amount of GLP-1 given¹³⁶. The effects are similar in lean and obese individuals¹³⁵. However, some studies indicate GLP-1 rises more significantly (and has a larger AUC) following food intake for lean individuals compared to obese. Interestingly, fasting and fed GLP-1 levels seem to increase with weight loss¹³⁷.

GLP-1 is released in proportion to calorie intake and is elevated in the blood at 5 to 120 minutes after food intake^{109, 138, 139}. Its release is regulated by nutrients in the intestinal lumen and secretion has been evaluated in response to intake of glucose, fat, protein, and certain types of dietary fiber^{36, 65, 102, 139, 140}.

One study suggests GLP-1 increases most rapidly after carbohydrate intake and peaks near 30 minutes; this rise is delayed after fat intake and may take as long as 150 minutes to reach a peak¹⁴¹. GLP-1 is also released following consumption of protein or amino acids. However, the peak is generally lower and levels return to baseline more gradually than compared to carbohydrate intake¹⁴².

Fiber may also influence GLP-1 response. One study found GLP-1 was significantly elevated after adding galactose and GG to a breakfast meal compared to a

breakfast with added water¹⁰². Similarly, plasma GLP-1 was notably increased in response to a mixed breakfast with added oligofructose compared to the same breakfast with added sucrose¹⁴³. However, a third study showed no difference in GLP-1 response when comparing low-fiber wheat bread to wheat bread with added pea fiber³⁶.

Studies of GLP-1 in rodents may offer some exciting insights when it comes to the role of gut hormones in satiety, food intake, and body weight. For example, an interesting study in rats showed significantly higher levels of GLP-1 and proglucagon mRNA after a 5-week diet with 10% oligofructose compared to a standard control diet¹⁴⁴. The oligofructose-fed rats also had lower mean food intake and weighed significantly less than control-fed rats. Thus suggesting, GLP-1 levels may vary with habitual diet.

Similarly, another study showed 6 weeks on a diet with added RS-3 led to increased plasma GLP-1, increased GLP-1 gene transcription, and lower body weight and fat compared to a control diet¹⁴⁵. Finally, a third study monitored 24-hour changes in gut hormones after rats were fed 10 days of an RS-2-enriched diet or a standard chow diet⁹⁵. Results showed significantly increased levels of GLP-1 and PYY, as well as decreased body weight and body fat when rats ate the RS diet. The authors suggest that fermentation of RS is likely responsible for the increased secretions; and, that any fiber or food that influences fermentation may also stimulate GLP-1 and PYY secretion. However, it is possible these results were influenced by the animals' body weight. Some reports suggest gut hormone levels may decrease with increasing body weight, and the rats on the control diets weighed more.

In humans, the exact mechanism explaining how GLP-1 influences satiety, has yet to be elucidated. GLP-1 receptors have been identified in the hypothalamus. It is known that vagal afferents can detect GLP-1 throughout the large intestine *and* that GLP-1 in the blood stream can reach and cross the BBB ¹¹². It is unclear whether one of these pathways plays a larger role in satiety.

Several GI and endocrine actions have also been associated with GLP-1 levels and satiety. For example, when GLP-1 is released after food intake, gastric emptying decreases and ileal brake action increases proportionally ^{109, 129, 146}. As well, GLP-1 is a strong incretin ^{109, 129, 147}. In other words, GLP-1 enhances the pancreas' ability to secrete and produce insulin in response to an elevated blood glucose level ¹⁴⁸.

Interestingly, GLP-1 analogs (or GLP-1 receptor agonists) are used in the treatment of diabetes. For instance, one study showed that 6 weeks of subcutaneous GLP-1 pump injections significantly lowered mean blood glucose, hemoglobin A1c, and body weight compared to saline ¹⁴⁹. A blood-sugar lowering medication on the market today, Byetta® is a GLP-1 analog. (GLP-1 itself is not effective for lowering blood glucose because of its rapid degradation by dipeptidyl peptidase-IV (DPP-IV). Therefore analogs, which are not as susceptible to degradation, must be used.)

Of note, glucagon-like peptide-2 (GLP-2) is simultaneously secreted with GLP-1. GLP-2, however, shows little or no effect on appetite in humans at the present time ¹⁵⁰. Some studies suggest GLP-2 may influence appetite and food intake in rodents.

Polypeptide YY

Like GLP-1, PYY is released by distal L cells, predominantly from the colon and rectum¹⁵¹. PYY₃₋₃₆ is the primary circulating form of this peptide, while PYY₁₋₃₆ makes up a smaller percentage¹⁰⁹. (PYY₁₋₃₆ is cleaved by DPP-IV to produce the active PYY₃₋₃₆. For the remainder of this section I will refer to PYY₃₋₃₆ as PYY.) Food intake, particularly protein and fat, stimulates PYY release^{112, 152}. PYY remains elevated for about 30 to 90 minutes after a meal; however, some reports have seen PYY elevated and active for as long as 6 hours post-prandially^{151, 153}. PYY, like GLP-1 and ghrelin, seems to be released in proportion to calorie intake¹⁵⁴.

The presence of PYY in the blood stream is associated with decreased food intake and feelings of satiety¹⁵⁴. The seminal study describing this relationship showed that peripheral administration of PYY reduced food intake and body weight in rats¹⁵⁵. The relationship was subsequently challenged by the Tschop lab¹⁵⁶; however, recent studies from independent laboratories have continued to support the association between PYY and food intake in rodents^{157, 158}.

Human data also support PYY's ability to reduce appetite. Batterham et al compared saline to physiologically dosed PYY infusions¹⁵⁹. Compared to saline, subjects had significant reductions in hunger (based on VAS) and in food intake at a lunch buffet. Subjects reported no adverse reactions to the infusions, yet ate 25% less after PYY than after saline. Similarly, another study by the same group, showed that a one-time infusion of PYY caused a 30% decrease in food intake at a lunch buffet (and over 24 hours) for both lean and obese subjects¹⁶⁰. Another study reported a dose-dependent decrease in food intake when PYY was given at varying amounts¹⁵⁴. In this

study, the largest PYY dose produced a 35% reduction in food intake at a buffet lunch; however, this dose was associated with nausea, fullness, and sweating in some of the subjects.

It is important to consider the fact that some infusion studies (both in humans and rodents) use pharmacologic doses of PYY—and not physiologic doses. The pharmacologic doses are interesting in the experimental setting, but can pose problems when determining application for the population at large. For example, in a study by Degen et al, two supraphysiologic doses and one physiologic dose of PYY were compared to a saline infusion¹⁵⁴. The physiologic dose had no impact on food intake compared to saline; the two supraphysiologic doses significantly reduced food intake but were associated with adverse events. Adverse events must be carefully considered when interpreting data on food intake. Nausea plays a large role in food aversion, which subsequently could reduce food intake. Incidentally, a study in mice showed conditioned taste aversion to flavored water, when the water was given along with high doses of PYY infusion¹⁶¹.

Few studies compare the differences in PYY levels when subjects consume different macronutrients. One cross-over study looked at the effects of iso-caloric high-protein, high-fat, or high-carbohydrate meals in lean and obese individuals¹⁵². The results differed slightly between the two groups. Lean individuals had significantly greater AUC_{PYY} after high-protein intake than after high-fat or high-carbohydrate. As well, AUC_{PYY} was higher after the high-fat meal than after the high-carbohydrate meal. For obese subjects, AUC_{PYY} was also greatest after high-protein intake compared to high-fat or high-carbohydrate. However, for this group, PYY levels were not different

from each other after high-fat or high-carbohydrate intake. Overall, PYY (fasting and peak levels) were consistently lower in obese subjects compared with lean. For both groups, the high-protein meal was the most satiating based on VAS questions (the VAS ratings correlated well with PYY concentration). A similarly designed study suggests PYY is elevated most after protein intake, which is followed by carbohydrate, fat, and water ¹⁶². Essah et al found that levels of PYY were 1.5 times higher after feeding obese subjects a low-carbohydrate, high-fat meal compared to an iso-caloric, high-carbohydrate, low-fat meal ¹⁶³.

The combination of these studies suggests PYY is influenced most by protein intake. However, none of these studies reported the amount of fiber included in each meal. It is possible that fiber content may also impact post-prandial PYY levels.

Weickert et al evaluated the influence of insoluble cereal fibers on post-prandial PYY and satiety ⁹⁷. They found AUC_{PYY} was significantly *lower* after 11 g of wheat-fiber bread compared to a low-fiber control bread; however, AUC_{PYY} was not different between 11 g of oat-fiber bread and the low-fiber control. Interestingly, hunger and satiety ratings were indistinguishable between the three treatments.

As mentioned previously, PYY levels are likely lower in the obese population. However, it is difficult to establish whether low PYY levels increase susceptibility to obesity, or if the obese state causes low PYY. One study showed that fasting PYY was inversely correlated with BMI ($R = -0.84$; $p < 0.001$) ¹⁶⁰. Conversely, Stock et al found no correlation between BMI and PYY when looking at normal weight and obese individuals ($R = 0.08$; NS) ¹⁶⁴. It should be noted that even though PYY levels may be

lower in the obese state, obese individuals are not resistant to the appetite-suppressing effects of PYY when it is given intravenously.

The exact mechanism by which PYY inhibits food intake is largely hypothetical and is not fully understood. It likely involves multiple systems and signals. For example, PYY action clearly involves neural reflexes—this is indicated by its secretion from the gut and its effect on appetite long before nutrients reach the distal digestive tract where PYY is released^{151, 165}. The vagal-brainstem-hypothalamic pathway clearly plays a role in the way PYY affects appetite and food intake¹⁶⁶. PYY₃₋₃₆ is known to bind with high affinity to the Y2 receptor (Y2R) in the arcuate nucleus¹⁵³. Once bound to the Y2R, PYY₃₋₃₆ likely regulates food intake by reducing NPY expression^{112, 155}. NPY expression stimulates food intake; therefore, inhibiting NPY reduces food intake. To further support this theory, PYY₃₋₃₆ injection has no effect on food intake in Y2R knockout mice. PYY may also increase POMC expression in the arcuate nucleus, but this probably is not required to reduce food intake^{155, 167}.

In addition to appetite effects, elevated PYY may decrease gastric emptying and contribute to ileal brake actions^{127, 168}. When gastric emptying is slow, nutrients are digested and absorbed over a longer period of time. This theoretically leads to greater feelings of satiety. As well, large doses of PYY given intravenously have been shown to suppress ghrelin; it is unclear whether or not this happens without pharmacologic doses^{154, 160}.

Also of interest, some data suggest that the combination of PYY₃₋₃₆ and GLP-1 may additively suppress appetite. In a crossover study, subjects were offered a buffet lunch after receiving infusions of either saline, PYY₃₋₃₆, GLP-1, or PYY₃₋₃₆ with GLP-1

¹⁶⁹. Subjects ate significantly less (27% less) from the buffet lunch after administration of PYY₃₋₃₆ with GLP-1 than after either alone. This data enhances the idea that food intake is regulated by complex hormonal interactions—rather than by just one particular hormone.

Data are sparse on the effects of chronic PYY administration in humans. However, some rodent studies suggest chronic peripheral PYY infusion or intraperitoneal PYY injections may be effective for reducing food intake and body weight ^{152, 157}. These findings would be of more interest if we could identify certain foods that continually stimulated PYY release.

Gut Hormone Summary

To summarize, food enters into the GI tract and a cascade of digestive mechanisms are set in motion. Gut hormone release is part of this. Ghrelin is actually active in the stomach before the meal begins; once it is released messages are received by the hypothalamus. Appetite is stimulated and food intake follows. Ghrelin enhances nutrient transit through the GI tract and allows for continued food intake.

GLP-1 and PYY are released soon after food intake and counteract ghrelin by slowing gastric emptying and intestinal transit (via the ileal brake). GLP-1 and PYY also counteract ghrelin by sending messages from the GI tract to the hypothalamus; these messages communicate satiety and fullness.

To further complicate matters, the origin of each of these peptides has also been identified in the brain. This means appetite is influenced by a combination of central and peripheral factors.

Overall, food intake regulation by gut hormones is complex. For example, it is generally accepted that each of these hormones are altered in proportion to calorie intake. However, it appears that macronutrient content also plays a large role. Similarly, body weight, peptide receptor status, sex, and age are just a few more factors that may affect how an individual will respond to any particular gut hormone signal.

Conclusion

Fiber intake is undoubtedly related to certain health outcomes. Current research suggests people who eat more fiber weigh less than those who do not. There is speculation that part of the mechanism for this relationship is based on greater satiety and a reduction in food intake after consuming fiber-rich foods. Gut hormones regulate appetite—specifically, GLP-1 and PYY are known to promote satiety and reduce food intake. Little is known about how gut hormone profiles are altered after consuming dietary fiber. Some studies have evaluated satiety by comparing a single type or dose of dietary fiber to a control, yet few have compared multiple types of fiber to a control, and fewer still have evaluated a dose response.

To improve understanding of how fiber influences satiety, food intake, and gut hormone response, I proposed two novel study designs. The first study compares different fiber sources to a low-fiber control, and the second study assesses the effects of different doses of mixed fibers.

Chapter Two

GREATER SATIETY RESPONSE WITH RESISTANT STARCH AND CORN BRAN IN HUMAN SUBJECTS*

*Publication Citation

Willis HJ, Eldridge AL, Beiseigel J, Thomas W, Slavin JL. Greater satiety response with resistant starch and corn bran in human subjects. *Nutr Res* 2009; 29:100–105.

Chapter Synopsis

Some studies suggest high-fiber foods are more satiating than foods with little or no fiber. However, we hypothesized that certain types of dietary fiber may enhance satiety more than others. Healthy men and women (n = 20) participated in this acute, randomized double-blind, crossover study comparing the effects of four fibers and a low-fiber treatment on satiety. On five separate visits fasting subjects consumed either a low-fiber muffin (1.6 g fiber) or one of four high-fiber muffins (8.0 - 9.6 g fiber) for breakfast. Subjects used four questions on 100 mm visual analog scales to rate satiety at baseline and at regular intervals for 180 minutes after muffin consumption. Responses were analyzed as area under the curve (AUC) and significant differences from baseline. Satiety differed among treatments. Resistant starch and corn bran had the most impact on satiety, while polydextrose had little effect and behaved similar to the low-fiber treatment. Results from this study indicate that not all fibers influence satiety equally.

Introduction

Foods high in fiber may influence satiety and ultimately body weight regulation^{12, 56, 65}. A review of studies examining the effects of fiber on body weight found that higher fiber intake was associated with increased satiety and decreased hunger¹⁷⁰. The proposed mechanisms for this relationship remain unclear, but the type of fiber may play a role.

In the gut, certain soluble fibers form a viscous gel matrix that is believed to slow gastric emptying and lead to a greater feeling of fullness^{42, 65}. As well, some viscous fibers slow absorption of glucose in the small intestine and lead to lower post-prandial glycemic and insulinemic responses^{16, 17, 171}. Both of these mechanisms are postulated to increase satiety. Some studies also report satiety improves after consumption of insoluble fiber; however, the mechanism is less clear^{24, 25}. Insoluble fiber has limited effects on gastric emptying and absorption in the small intestine, but it may be partially fermented in the large intestine. Research on RS and satiety is sparse and inconsistent^{32, 33, 94}.

Additionally, some research suggests that fiber-rich foods may influence satiety through increased mastication or changes in gut hormones (i.e. ghrelin or glucagon-like peptide-1)^{63, 145, 172, 173}.

Dietary fiber in foods is a diverse substance and is associated with many bioactive compounds. As well, when functional fibers or isolated fibers are added to foods they may be soluble, insoluble, fermentable, or viscous—all of which could impart other properties to a food. Thus, it is likely that different types of fiber will not impact satiety uniformly.

Many new types of fiber have been added to the United States food supply since the year 2000⁵⁰. The addition of fiber to many manufactured foods likely comes in response to claims that fiber may increase satiety and decrease body weight. However, few studies have compared the satiety response of different fibers in the same subject population.

We hypothesized that certain fibers would enhance satiety more than others. To test this hypothesis we evaluated and compared the satiety response from five different muffins. Four muffins contained approximately 9 g of fiber from four different sources, and one muffin contained approximately 1 g of fiber. Insight into how different types of fiber influence short-term satiety could provide useful information for designing future research, especially studies that wish to evaluate long-term appetite control, food intake, changes in appetite-regulating hormones, and body weight.

Methods and Materials

Experimental Design

This acute study was a randomized, within-subject, repeated-measures, crossover design where each subject served as his or her own control. A total of 20 subjects were enrolled. The duration of the study was five test visits, separated by at least 1 week, for a total of 100 visits. Subjects were randomly assigned to receive one treatment after an overnight fast. Subjects who dropped out were replaced.

In this study we compared a low-fiber (LF) muffin and four high-fiber muffins containing different types of fibers—Corn Bran (CB), Barley β -glucan + Oat Fiber (BG), Resistant Starch (RS), and Polydextrose. These fibers were chosen for their

diverse representation of soluble, insoluble, and resistant starch characteristics, as well as for their ability to be baked uniformly in muffins.

Subjects

The University of Minnesota Institutional Review Board Human Subjects Committee approved all aspects of this research. Subjects were recruited via flyers placed around the University of Minnesota campus. They were screened over the phone. The screen included many questions about the subject's health history. Subject eligibility was determined by meeting all of the inclusion and exclusion criteria.

Inclusion Criteria

Subjects were English speaking, healthy men and women between 18 and 65 years of age. They were nonsmoking; not taking weight loss medications; non-dieting (weight stable over last 3 months); and had a BMI less than 30.

Exclusion Criteria

Subjects were excluded if they had a history of cardiovascular disease; diabetes mellitus; any cancer in last 5 years (except basal cell carcinoma of skin); renal or hepatic disease; eating disorder; Crohns disease; ulcerative colitis; or other gastrointestinal conditions that could affect digestion and absorption. Women who were pregnant or lactating; women with irregular menstrual cycles; vegetarians; people with recent bacterial infection (< 2 months); weight change > 5 kg in prior 3 months

(intentional or unintentional); history of drug or alcohol abuse in prior 6 months; or recent participation in an intervention study (within 30 days) were also excluded.

Treatments

All five treatment muffins were similar in appearance and had nearly identical macronutrient content (see Table 2.1). They were prepared using a quick-bread muffin recipe, which varied by fiber type. All fibers were commercially available (Corn Bran, J.R. Short Milling, Kankakee, IL; Bleached Oat Fiber, SunOpta, Bedford, MA; Barley β -glucan, Cargill, Minneapolis, MN; Novelose® 330 & Hi-Maize® 260, National Starch, Bridgewater, New Jersey; Polydextrose, Danisco, Copenhagen, Denmark). After baking and cooling, the muffins were frozen at -20°C . Muffins were removed from the freezer 12 hours before each subject visit and were thawed in a sealed container at room temperature.

Visual Analog Scales (VAS)

Satiety was evaluated using questions from previously validated 100 mm VAS⁶⁸. Questions were taken directly from the original citation and can be found in Appendix A. **Hunger:** How hungry do you feel? Not hungry at all (0 mm) vs. I have never been more hungry (100 mm). **Satisfaction:** How satisfied do you feel? I am completely empty (0 mm) vs. I cannot eat another bite (100 mm). **Fullness:** How full do you feel? Not at all full (0 mm) vs. totally full (100 mm). **Prospective food intake:** How much do you think you can eat? Nothing at all (0 mm) vs. a lot (100 mm).

Five characteristics were used to assess the palatability of each muffin. Visual appeal, smell, taste, and overall pleasantness were scored as Good (0 mm) vs. Bad (100 mm). Aftertaste was scored as Much (0 mm) vs. None (100 mm).

Study Visits

Prior to study commencement each subject signed informed consent and was weighed and measured to confirm BMI. Subjects were also instructed to maintain current eating and exercise habits, and not to initiate any programs that could alter body weight.

On five separate occasions, subjects consumed either a low-fiber muffin or one of four high-fiber muffins. Subjects fasted for 12 hours the night before each visit. Visits were held between 6:30 am and 9:30 am on weekdays only. Subject visits were scheduled at least one week apart; however, women participated only during the follicular phase of their menstrual cycle so some visits were 2 to 3 weeks apart.

On the morning of each visit subjects completed baseline VAS to assess satiety. After the initial VAS were collected subjects were given their test muffin and were instructed to consume it within ten minutes. Subjects were allowed to drink 540 ml of bottled water, black decaffeinated tea, or black decaffeinated coffee during each visit. The type and amount of beverage were kept consistent at all five visits.

Additional VAS were completed at 15, 30, 45, 60, 120, and 180 minutes after baseline. Subjects also completed VAS for palatability 15 minutes after muffin consumption. During the 180-minute visits subjects were seated in a quiet room and

were allowed to read, use laptop computers, work quietly, or listen to music. Physical conditions and location of the room were consistent for all visits.

Sample Size

Sample size for this study was based on previously published research. Namely, a study by Flint et al, which found that 18 subjects was sufficient to detect a 10 mm difference on VAS when using a paired design and a study power of 0.8⁶⁸.

Randomization

A Latin Square determined subject randomization. Four 5 x 5 Latin Squares were used to assign treatments to the subjects (Appendix B). These twenty unique sequences of 5 treatments were created to minimize results based on an order effect. Each subject was assigned a row in the Latin Square and the treatment periods were assigned to the columns. Subjects were assigned to each row in order of study enrollment.

Statistical Analysis

The primary outcome was area under the curve (AUC) for responses on the VAS. AUC was calculated by the trapezoidal rule. The fiber treatments were compared by each AUC for hunger, satisfaction, fullness, and prospective food intake using a mixed effects linear model with a random subject effect (Proc Mixed). Analytic models were reduced to include only two variables, treatment and visit number (carryover, palatability characteristics, and interaction terms were tested and

eliminated). Pairwise comparisons were used to compare treatment means. Data are presented as mean AUC value \pm SEM.

Paired t-tests were used to calculate the length of time each VAS response remained significantly different from baseline; this was used to estimate duration of satiety. Data are presented as the number of minutes that each response differed significantly from the baseline response value. Significance was determined by two-sided tests with $p < 0.05$. Statistical analysis was performed with SAS version 9.1.2¹⁷⁴.

Results

Twenty subjects (7 men and 13 women) completed all five visits. Mean BMI \pm SEM was 24.5 ± 0.7 (range 21.9-26.7) and 22.9 ± 0.6 (range 19.7-26.9) for men and women, respectively. Mean age \pm SEM for the men was 28 ± 4 (range 20 – 54) and 24 ± 2 (range 18 – 50) for women. At baseline there were no statistically significant differences among scores on the VAS.

Differences Among Treatments

Figure 2-1 shows changes in satiety scores from baseline through 180 minutes. Figure 2-2 compares the mean AUC scores (for hunger, prospective food intake, satisfaction, and fullness) among treatments.

Satiety measures differed among treatments. AUC_{Hunger} suggests that subjects were less hungry after eating CB ($p=0.05$) or RS ($p=0.06$) than after eating the LF muffin. $AUC_{\text{Prospective food intake}}$ was also lower among the same treatments. CB and RS stimulated less desire for food intake than the LF treatment ($p=0.025$ and $p=0.009$,

respectively). $AUC_{\text{Satisfaction}}$ and AUC_{Fullness} were higher after CB than after either the LF or polydextrose treatments ($p < 0.049$). $AUC_{\text{Satisfaction}}$ was also marginally higher after RS than after either LF or polydextrose ($p = 0.08$).

In general, the AUC analysis suggests that CB and RS were more satiating than both the LF and polydextrose treatments.

Duration of Satiety for Individual Fibers

Each treatment influenced satiety-related feelings differently. Figure 2-3 shows how long each feeling was influenced (compared to baseline) for the individual treatments. After eating the RS muffin, mean hunger scores remained significantly lower than baseline for 120 minutes; hunger was less than baseline for only 15 minutes after polydextrose. Satisfaction and fullness levels were significantly greater than baseline for 180 minutes after the RS muffin, but for only 60 minutes after polydextrose. After CB, hunger was significantly suppressed for 60 minutes, satisfaction was maintained for 120 minutes, and fullness was maintained for 180 minutes.

Muffin Palatability

The taste of the polydextrose muffin was preferred over all other muffins, and overall pleasantness was significantly greater than the CB and RS treatments ($p < 0.05$). The LF treatment also had greater overall pleasantness than the BG treatment ($p = 0.03$). However, palatability characteristics did not explain satiety-related feelings when included in the statistical model.

Discussion

Satiation and satiety describe the feelings that lead to cessation of a meal and inhibit the desire to eat between meals. Both sensations are regulated by a multitude of environmental, central, and peripheral signals. Unfortunately, satiety cannot be assessed with one straightforward question; therefore, multi-question VAS are commonly used. For the purpose of this study we determined satiety based on differences among VAS questions about hunger, satisfaction, fullness, and prospective food intake.

Our findings suggest that not all fibers influence satiety equally. RS and CB were consistently more satiating than the LF treatment; they also influenced the duration of satiety longer than the LF, BG, and polydextrose treatments.

In this study, RS was highly satiating. Eight grams of RS kept subjects significantly less hungry than baseline for 120 minutes and more full and satisfied for the 180-minute test period. Hunger and prospective food intake were also lower after RS compared to the LF treatment. Our findings contradict two older studies, which found that RS did not improve satiety compared to other foods or fibers. de Roos et al found 30 g of two resistant starches (high-amylose corn starch or extruded and retrograded high-amylose corn starch) had little effect on appetite and satiety compared to glucose³². Raben et al reported that RS (in the form of pregelatinized potato starch) was less filling and less satiating than fully digestible potato starch³³. Interestingly, Bodinham et al reported no differences in appetite when feeding subjects 48 g of RS versus placebo; but, they did report that subjects ate significantly less at an *ad libitum* meal and in the 24-hour period following the RS treatment³¹. They suggest that the

lower 24-hour energy intakes seen with the RS supplementation could be beneficial in the management of obesity.

The mechanisms to explain why RS was more satiating in our study are not clear. However, one study found that satiety-influencing gut hormones were increased after rats were fed a high RS diet for one month ¹⁴⁵. It is possible that certain types or amounts of RS could improve satiety by increasing levels of glucagon-like peptide-1 or peptide YY. As well, another study found that RS may mediate satiety by altering colonic fermentation and gastric emptying rate ⁹⁴. In this study, colonic fermentation (as measured by breath hydrogen) was positively correlated with satiety and inversely correlated with gastric emptying.

Our research also suggests CB may impact short-term satiety, though its effects were less uniform than RS. Corn bran is an inexpensive, insoluble fiber that has not been previously evaluated for its role in satiety. However, it has been established that CB has a very low digestible starch component (less than 10% by weight in comparison to oat bran which has roughly 50%) ¹⁷⁵. Assuming an orocecal transit time of 2 to 3 hours, this may suggest that CB would behave similarly to RS in our three-hour test period. Further studies should be done with CB and other insoluble, not fermentable fibers—especially in lieu of emerging research that suggests colonic fermentation is related to longer-term satiety ⁹⁴.

Polydextrose was the least satiating. Compared to baseline, polydextrose decreased hunger only 15 minutes while all other treatments significantly decreased hunger for 60 minutes or more. Patterns in the AUC analysis suggest this fiber had little impact on satiety and behaved similar to the LF treatment. In fact, the AUC scores

for satisfaction and fullness were identical for the polydextrose and LF treatments, both were 111 and 108, respectively (refer to Figure 2-2). Our findings are similar to King et al, which reported no differences in satiety when comparing 25 g of polydextrose to a fiber-free control²⁸.

Additional studies also suggest that certain types and doses of fiber may influence satiety more than others. Samra and Anderson reported subjects had significantly lower appetite scores after eating 33 g of fiber (corn plus wheat bran) than after zero grams of fiber from white bread²⁴. Raben and colleagues found subjects felt significantly more full and had less desire to eat after consuming 25 g of pea fiber baked into wheat bread than after 9 g of fiber from plain wheat bread³⁶. And, Burley et al reported a small but significant difference in fullness when comparing how subjects felt after eating 12 g or 3 g of fiber⁹⁶.

In contrast, Weikert et al showed no difference in satiety when comparing a fiber-free bread to breads with 10 g of wheat fiber or oat fiber⁹⁷. Likewise, another study reported no difference in mean hunger ratings between 22 g of soluble fiber from psyllium and 22 g of insoluble fiber from wheat bran¹⁷⁶. Lastly, Levine et al reported no difference in hunger when comparing a fiber-free cereal to cereals with either 11 g of wheat fiber or 39 g of wheat plus corn bran fiber. However, the same subjects felt significantly less hungry after eating 18 g or 35 g of bran cereal fiber than after eating a fiber-free cereal²⁵.

Our results, as part of the bigger body of research, strengthen the notion that fiber plays a role in satiety. However, type and dose of fiber likely play an important

role. At this time it is unclear which types and doses influence satiety the most, though our study suggests RS or CB may be more effective than polydextrose.

The lack of clarity in this relationship may be partly explained by differences in study design, specifically with regard to variations in VAS, and the way fiber is administered (i.e. as a supplement mixed into liquid, baked into bread, or as part of a whole food like cooked oats).

Furthermore, palatability of a fiber-rich food may also play a role. In our study RS was highly satiating and the least palatable, while polydextrose was the inverse. Even though our muffins differed in palatability, these characteristics did not help explain satiety responses when included in the statistical model. This is not surprising, and is supported by a review paper, which reports that palatability of a food (or meal) inconsistently influences appetite and satiety⁸⁴. Highly palatable foods may increase or decrease a person's level of hunger and subsequent food intake—and the effects may not be predictable.

Our study blindly compared the impact of four different types of fiber in a macronutrient-matched breakfast. However, this study had limitations. This study did not assess *ad libitum* food intake after subjects consumed the treatment muffins. However, previous studies have used the question of, “How much do you think you can eat?” to assess prospective food intake¹⁷⁷. Additionally, two studies have found that appetite sensations, as reported on VAS, are useful predictors for spontaneous energy intake^{69, 178}. We did not control for baseline fiber intake; it is possible that subjects with chronically low fiber intake may respond differently than subjects who routinely consumed more daily fiber. It is also reasonable to hypothesize that 9 g of fiber was not

a large enough dose; perhaps a larger dose would detect more significant satiety differences. However, 9 g is a very practical dose and something the general population could reasonably consume. Higher doses would likely be less practical. Also, we must consider the impact of a low-calorie breakfast on satiety. It is possible our results were hindered by providing only 175 calories during a three-hour breakfast period. We may have seen even stronger results if we had offered foods that reached a calorie level equivalent to a typical breakfast.

The results of this study are especially important at a time when so many food products have added functional fibers. Our findings suggest added fibers will not impact satiety uniformly, and that the type of fiber must be carefully considered. Understanding ways to control appetite and food intake are critical as obesity rates rise. Thus, any dietary manipulations that could maximize satiety and decrease desire for food intake may be instrumental for helping people achieve and maintain a healthy body weight.

Future Directions

New research should focus on the fiber type when designing studies to evaluate the relationship between fiber intake and long-term appetite control or body weight regulation. Certain types of fiber may be more effective than others.

In addition, emerging research suggests some foods may simulate changes in appetite-related gut hormones, such as ghrelin, glucagon-like peptide-1, or peptide YY¹⁰⁹. Little research has been done to see how fiber may influence these hormones. Given our findings it would be interesting to know if different types of fiber evoke

different gut hormone responses.

Conclusion

In conclusion, this study suggests fiber type influences satiety response. We found that polydextrose had limited satiating capabilities and behaved similar to a LF treatment, while RS and CB enhanced short-term satiety. Further research is needed, but this study lays important groundwork for future studies. Specifically, studies should be done to determine if fiber-induced satiety is related to subsequent food intake and a healthy body weight.

Acknowledgments

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Table 2-1. Muffin Composition

Fiber Type	Total Fiber (g)^a	Insoluble Fiber (g)	Soluble Fiber (g)	Energy (kcal)	Fat (g)	Pro (g)	Sugar (g)	Weight (g)	Moisture (%)
Low Fiber (LF)	1.6	1.1	0.4	178	2.9	3.9	13.2	76	39
Resistant Starch (RS)	8.0	7.9	0.1	174	3.3	3.8	14.2	92	47
Barley β -Glucan + Oat Fiber (BG)	9.4	5.3	4.0	175	3.0	3.5	13.7	96	47
Polydextrose	9.5	1.3	8.8	177	2.9	3.5	14.2	89	38
Corn Bran (CB)	9.6	9.1	0.5	174	3.5	4.0	13.5	99	43

^a Fiber content was analyzed using standard AOAC methods. Analysis was completed at Medallion Labs; Minneapolis, MN.

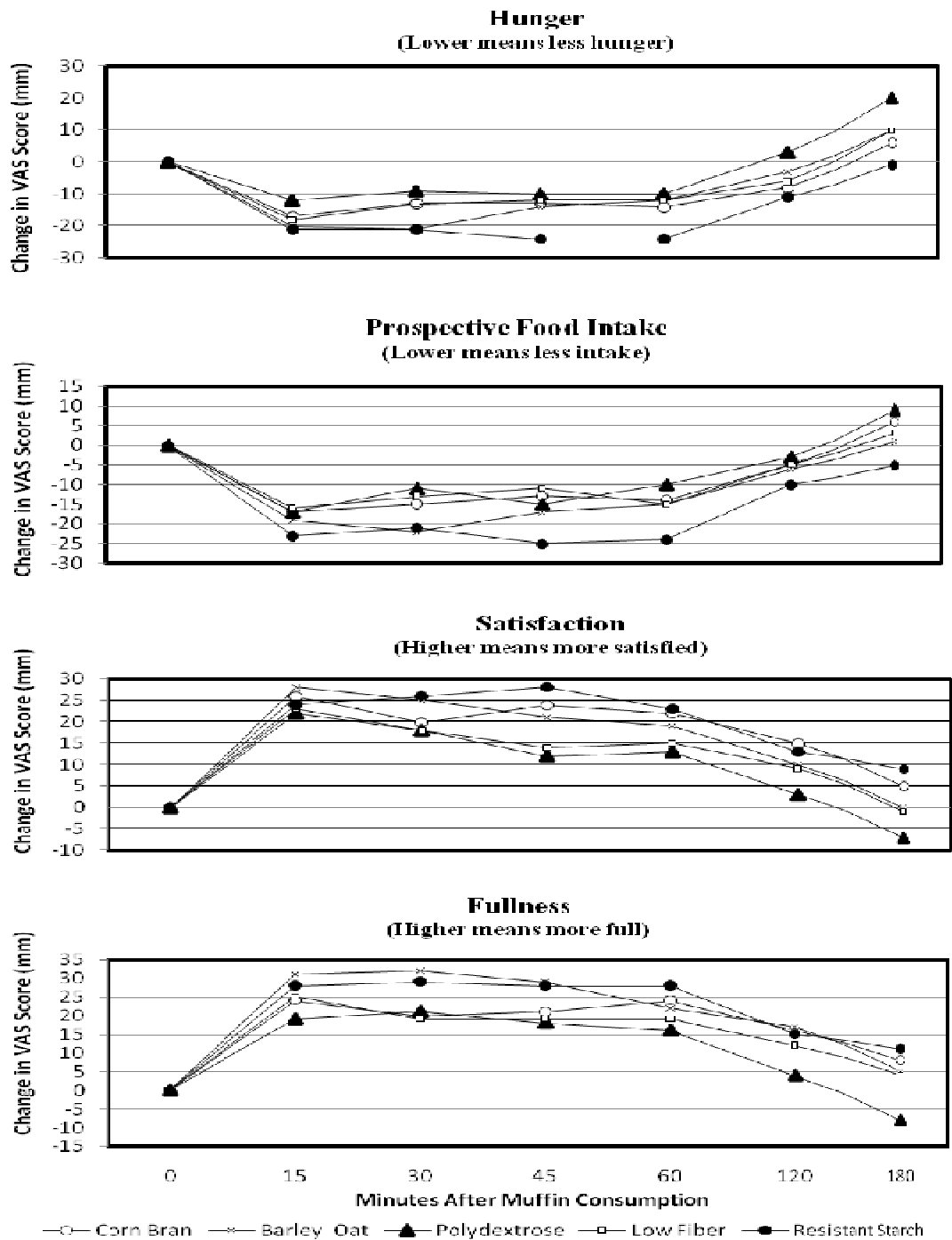


Figure 2-1. Changes in feelings of satiety based on mean VAS score, at each time point, for each question (n=20 for each line).

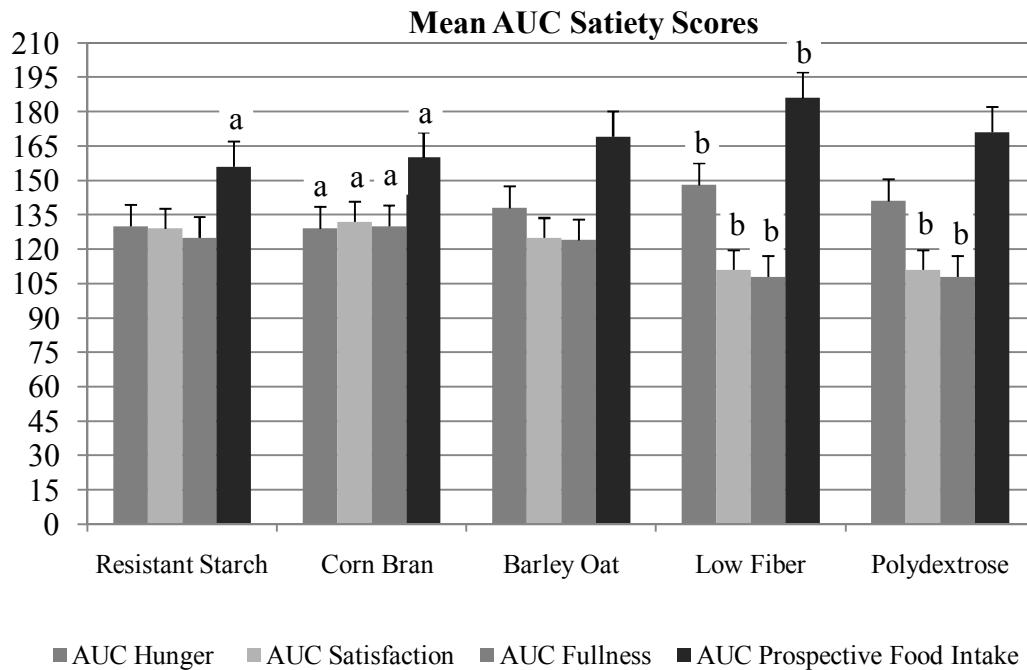


Figure 2-2. Mean AUC scores \pm SEM for each treatment and each VAS question (n=20 for each bar). AUC was calculated by the trapezoidal rule and was compared in SAS using a mixed effects linear model with a random subject effect. Within each question, treatments with different letters are significantly different at $p < 0.05$. Treatments with the same letters, or without letters, were indistinguishable from each other.

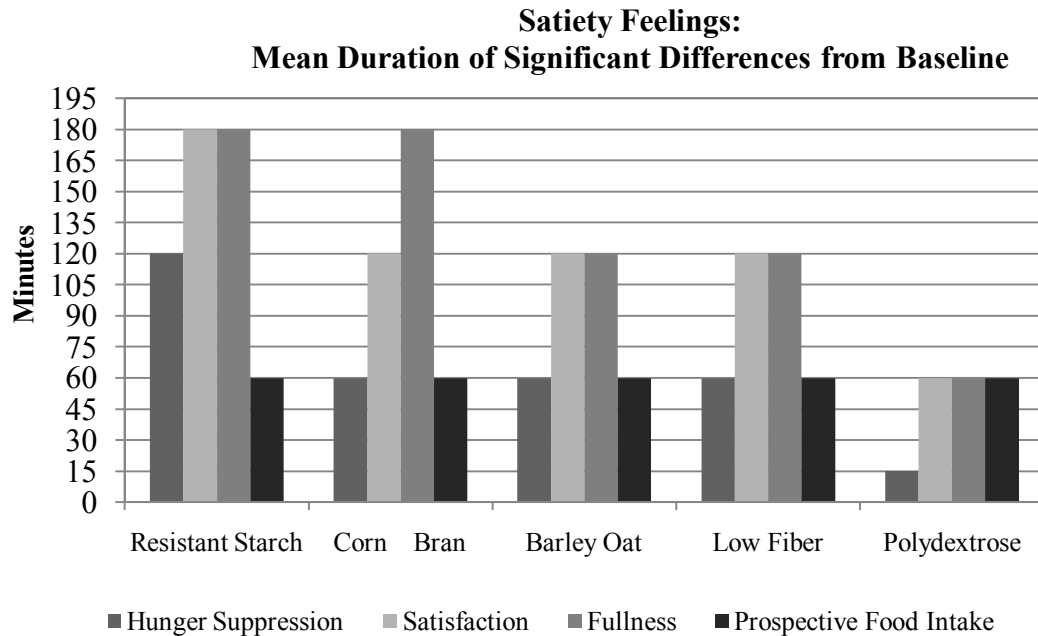


Figure 2-3. Each bar represents the mean duration of significant difference from the baseline VAS response (n=20 for each bar). For example, within polydextrose, the mean hunger level significantly differed from baseline at 15 minutes; by 30 minutes the mean hunger level was indistinguishable from baseline. The difference is shown for each question, within each fiber. Within each fiber, paired t tests were used to compare each time point to the baseline value.

Chapter Three

EFFECT OF FOUR DOSES OF MIXED FIBER ON SATIETY, FOOD INTAKE, GLUCOSE, INSULIN, AND GUT HORMONE LEVELS

Chapter Synopsis

Introduction: People who eat more fiber have a lower body weight than people who eat less fiber. Potential mechanisms include greater feelings of satiety, reduction in food intake, and changes in blood glucose, insulin, or gut hormones. We hypothesized that different doses of a mixed fiber would influence satiety response and food intake when given to subjects in muffins for breakfast.

Methods: Healthy men (n = 10) and women (n = 10) with a body mass index of 23.7 ± 0.5 (mean \pm SEM) participated in this double-blind, crossover study. On four separate visits, fasting subjects consumed a muffin with 0, 4, 8, or 12 g of mixed fibers. Muffins had 500 calories and similar macronutrient content. Visual analog scales rated hunger and satiety from 0 -180 minutes; and blood was drawn to measure glucose, insulin, and gut hormones (ghrelin, GLP-1, PYY₃₋₃₆). Food intake after muffin consumption was measured at an *ad libitum* lunch buffet and for the remainder of the day.

Results: In all satiety measures, the 12 g fiber muffin was significantly more satiating than the 0 g muffin. Muffins with 4 g and 8 g of fiber had intermediate effects. Despite significant differences in satiety measures after three hours, food intake did not differ among treatments. Glucose and insulin response did not correlate with fiber dose, but differed among some of the treatments. Ghrelin was significantly higher after 12 g of fiber than after all other doses. GLP-1 decreased consistently with fiber dose and PYY did not differ among treatments.

Conclusion: Feelings of hunger, satisfaction, fullness, and desire to eat differed after consuming 0, 4, 8, or 12 g of mixed fiber for breakfast. However, these feelings were not consistently linked to food intake or physiologic measures.

Introduction

The incidence of overweight and obese adults has increased dramatically during the past 20 years. According to recent estimates approximately 66% of adults in the United States are overweight or obese¹⁷⁹. Obesity is a complex disease, but diet and lifestyle are known to play an important role in its development.

It has been suggested that a high-fiber diet may aid in weight regulation. Observational studies have found that fiber intake is inversely associated with body weight and body fat^{21, 180, 181}. In fact, one study reported that in a 20-month period, every 1 g increase in total fiber consumed, decreased body weight by 0.25 kg and body fat by 0.25 percentage points¹⁸². Potential mechanisms include: greater feelings of satiety, reductions in food intake, and changes in blood glucose, insulin, or gut hormone levels after fiber consumption.

Increased satiety is one mechanism used to describe why fiber intake may be associated with a lower body weight. For example, fiber consumption may stimulate fullness and decrease hunger, which could lead to less food intake and a lower body weight over time. A review on the relationship between fiber, satiety, energy intake, and body weight estimated that 14 g of additional fiber per day was associated with greater satiety, a 10% decrease in energy intake, and a 2 kg weight loss over about a 4 month period⁶⁵. As well, individual studies have found that fiber-rich foods (at one meal or over multiple days) are associated with approximately 15 – 22% reductions in food intake^{24, 45, 59}.

Many theories have also been developed to explain a relationship between fiber, satiety, and glucose and insulin levels. For example, it is commonly believed that fiber

intake blunts glucose and insulin appearance in plasma, which in turn may suppress appetite. This, however, is controversial and many studies suggest the relationship is unclear and complex^{24, 100, 101}.

Alternatively, Huda et al describe hormones released from the GI tract as, “some of the most important factors controlling appetite and satiety”¹⁰⁹. These gut hormones communicate critical information about food intake to the central nervous system, which in turn influences food intake behaviors. Ghrelin, GLP-1, and PYY₃₋₃₆ are three well-studied gut hormones¹⁰⁸. However, most studies have evaluated gut hormone changes after predominantly carbohydrate, protein, or fat intake; very few studies have evaluated how these three hormones change in response to fiber intake.

Fiber research is complicated due to the fact that fiber is a highly diverse substance. It is unlikely that all fibers will behave uniformly when it comes to effects on appetite sensations, glucose, insulin, or gut hormone levels. Therefore, it is important to consider the unique characteristics and properties of individual fiber types when evaluating these endpoints.

In a large review, Slavin and Green report that certain types of fibers are more likely than placebo (or other treatments) to increase satiety¹¹. They conclude that satiating fibers may include: soy polysaccharide, boiled buckwheat groats, cellulose, native guar gum, pea fiber, pectin, barley β -glucan, and methylcellulose. They also suggest that many other types of fiber have an unclear or no established impact on satiety; that includes psyllium, hydrolyzed guar gum, resistant starch, and polydextrose.

The viscosity and fermentability characteristics of an individual fiber may help explain why some fibers influence satiety and others do not. Some studies suggest that

more viscous foods or fibers have a greater impact on satiety than less viscous foods or fibers^{11, 42, 183, 184}. However, one study found that a low-viscosity beverage was more satiating than a similar high-viscosity beverage¹⁸⁵. Interestingly, the same study reported that subjects ate significantly less for 24 hours after the high-viscosity beverage than after the low. Similarly, another study found no differences in satiety after giving subjects meals with differing viscosities; however, subjects ate significantly less after consuming the higher viscosity meals¹⁸⁶.

Alterations in gastric distention and emptying are commonly used to explain how high-viscosity foods or fibers may influence satiety. Juvonen et al found that a high viscosity oat- β -glucan beverage significantly delayed gastric emptying time compared to a similar low viscosity oat- β -glucan beverage¹⁸⁵. Conversely, another study found that gastric emptying time was indistinguishable when subjects consumed 0, 2.5, 3.5, or 4.5 g of viscous guar gum¹⁸⁷.

The fermentability of a fiber may also influence satiety. Fermentability implies that fiber in the colon is metabolized by bacteria to yield short chain fatty acids. In rodents, the fermentation process is thought to influence gut hormone release, which may suppress appetite¹⁴⁵. A human study found that a highly fermentable oligofructose treatment increased satiety and reduced hunger and prospective food intake compared to a placebo¹⁸⁸. As well, a viscous, fermentable fiber snack bar promoted greater post-prandial satiety than an iso-caloric, non-fermentable fiber snack bar¹⁸⁹. However, Howarth et al found that a non-fermentable fiber (a purified cellulose) increased satiety more than a fermentable fiber (a mixture of β -glucan and pectin)²¹.

Taken together, these findings suggest that a combination of fiber types—viscous, non-viscous, fermentable, and non-fermentable—may have an additive, positive effect on satiety.

In addition to the viscosity and fermentability characteristics, fiber dose may play a role in appetite sensations. However, to the best of our knowledge, there are few reports of dose-response studies that evaluate the relationship among multiple fiber doses and appetite.

One study randomized 200 obese women to one of the following three treatments: 8 g of mixed fiber per day, 12 g of mixed fiber per day, or placebo¹⁹⁰. Both fiber doses were more satiating than the placebo, but they were not different from each other. Another study evaluated satiety after different amounts of carrots were added to iso-caloric meals¹⁹¹. The larger carrot portions (and associated higher fiber contents) yielded significantly higher satiety scores. Even though published research on this topic is sparse, it appears that higher doses of fiber are more effective at increasing satiety than comparable low- or no-fiber foods.

Therefore, we hypothesized that a mixed fiber supplement (containing fibers generally recognized as viscous), fed at four practical doses (0, 4, 8, and 12 g) would increase satiety and decrease subsequent food intake in a dose-dependent manner. We also hypothesized that glucose, insulin, ghrelin, GLP-1, and PYY₃₋₃₆ would change proportional to the fiber dose.

Methods and Materials

This randomized, double-blind, repeated measures, crossover study included 20 healthy, normal-weight men (n=10) and women (n=10). Participants attended four study visits separated by at least 1 week, for a total of 80 study visits. Subjects who dropped out were replaced.

Subjects

The University of Minnesota Institutional Review Board Human Subjects Committee approved all aspects of this research. Subjects were recruited by flyers placed around the University of Minnesota campus. They were screened over the phone. The screen included many questions about the subject's health history. Subject eligibility was determined by meeting all inclusion and exclusion criteria.

Inclusion Criteria

Included subjects were English speaking, healthy men and women between 18 and 65 years of age. They were non-smoking; not taking medications; non-dieting (weight stable over last 3 months); and had a BMI between 18 and 27. Subjects also had to be able to give blood through an IV.

Exclusion Criteria

The following were exclusion criteria: irregular or erratic breakfast eating patterns; food allergies to ingredients commonly found in muffins or pizza; distaste for muffins or pizza; BMI less than 18 or greater than 27; weight change > 5 kg in last 3

months (intentional or unintentional); cardiovascular disease; diabetes mellitus (fasting blood sugar > 126 mg/dl); cancer in prior 5 years (except basal cell carcinoma of skin); renal or hepatic disease; Crohns disease; ulcerative colitis; any other gastrointestinal conditions that may affect digestion and absorption; recent bacterial infection (< 3 months); chronic medication use; history of drug or alcohol abuse in prior 6 months; concurrent or recent intervention study participation; vegetarians or people who ate more than approximately 15 g of fiber per day; pregnant or lactating women; and women with irregular menstrual cycles.

Treatment Muffins

In a randomized fashion, and on separate days, subjects received four treatment muffins containing: 0 g fiber, 4 g mixed fiber, 8 g mixed fiber, and 12 g mixed fiber for breakfast. The mixed fiber came from: pectin (Apple Pectin SF 50-LV, Herbstreith & Fox, Neuenbürg/Württ, Germany), barley β -glucan (Barliv, Cargill, Hammond, IN), guar gum (Guar, TIC Gums, White Marsh, MD), pea fiber (Centara Dietary Pea Fiber, Norben, Willoughby, OH), and citrus fiber (Citri-Fi 100FG, Fiberstar, Inc., Willmar, MN). These fibers were chosen based on a literature review of fiber and satiety which suggested that viscous fibers were more likely to affect appetite, and for their ability to be baked uniformly into muffins¹¹. The muffins were spice flavored and commercially made (Nestle R&D Center; Solon, OH). Attempts were made to balance macronutrient content, see Table 3-1.

After baking and cooling, the muffins were frozen at -20°C . Muffins were removed from the freezer 2 hours before each subject visit and were thawed at room temperature.

Visual Analog Scales (VAS)

Appetite sensations were measured using computerized VAS. Original VAS used a pen and paper method; however, electronic versions have also been validated⁷⁰⁻⁷².

Nine questions were taken directly from previously validated 100 mm scales⁶⁸. The following questions were asked at 0, 15, 30, 45, 60, 90, 120, and 180 minutes (Appendix A). **Hunger:** How hungry do you feel? Not hungry at all (0 mm) vs. I have never been more hungry (100 mm). **Satisfaction:** How satisfied do you feel? I am completely empty (0 mm) vs. I cannot eat another bite (100 mm). **Fullness:** How full do you feel? Not at all full (0 mm) vs. totally full (100 mm). **Prospective food intake:** How much do you think you can eat? Nothing at all (0 mm) vs. a lot (100 mm). These questions and scales have also been used in other published research^{185,192}.

At 15 minutes after muffin consumption, a set of palatability questions was also presented to each subject. Visual appeal, smell, taste, and overall pleasantness were assessed as Good (0 mm) vs. Bad (100 mm). Aftertaste was scored as Much (0 mm) vs. None (100 mm).

Computers were set so only one appetite sensation or palatability question appeared on the screen at any given time. Feelings were indicated when the subject made a tick mark (with the cursor) between 0 and 100 mm. At the end of each question

set, subjects had the opportunity to review or save each response. Once saved, responses could not be reviewed.

Screening Visit

Prior to any procedures the study coordinator obtained a signature on informed consent (Appendix C). The screening visit included height, weight, and fasting blood glucose as determined by finger stick. If the subject fulfilled the established inclusion criteria he/she was scheduled for at least one study visit. Subjects received instruction on a low-fiber, lead-in diet to be followed the day before each study visit; this included avoidance of fiber supplements such as Metamucil®, Citrucel®, Benefiber®, and others. Subjects were also instructed to maintain current body weight and activity level throughout the study period. Finally, all subjects were instructed to avoid alcohol and excessive exercise in the 24 hours prior to each visit.

Study Visits

Subjects fasted for 12 hours before each visit. Visits began between 7:00 am and 9:00 am on weekdays only and lasted approximately four and a half hours. Visits were held in a quiet room and subjects were allowed to read, use laptop computers, work quietly, or listen to music. Physical location and conditions of the room were consistent for all visits. Each visit was scheduled at least one week apart; however, women participated only during the follicular phase of their menstrual cycle so some visits were one month apart.

On the morning of each visit the subject was admitted as an outpatient to the University of Minnesota GCRC. The protocol nurse at the GCRC inserted an antecubital IV, which was left in place at least 10 minutes before drawing the baseline blood sample. This was done in attempt to reduce the possibility of elevated hormones levels after venepuncture stress ¹⁹³.

Subjects were given instructions for completing the computerized VAS and proceeded to complete their baseline appetite assessment. Fasting blood samples were drawn to evaluate glucose, insulin, ghrelin, GLP-1, and PYY ₃₋₃₆ immediately after the VAS were completed. Subjects then consumed either a low-fiber control muffin or one of three fiber-containing muffins for breakfast. The muffin and 250 ml of water were consumed within 10 minutes.

Appetite sensations were rated by VAS again at 15, 30, 45, 60, 90, 120, and 180 minutes after baseline. Blood samples for glucose and insulin were collected at the same time points. Ghrelin was sampled at 15, 30, 60, and 90 minutes, and GLP-1 and PYY₃₋₃₆ were sampled at 30 and 60 minutes. The VAS were always completed before the blood draws. The IV was removed promptly after the 180-minute blood draw.

Immediately after the IV was removed, subjects were given a lunch of pre-selected, pre-weighed pizzas and 1 liter of water (Appendix D provides detailed information on the nutrient composition and preparation of the *ad libitum* pizza lunch). Pizza has been successfully used as an *ad libitum* meal in several previous studies ^{24, 60,}
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Six personal-sized pizzas were positioned within easy reach of the subject. The subject was told to eat as much or as little as he/she wanted until comfortably satisfied;

he/she had 30 minutes to do so. After 30 minutes the remaining pizza and water were removed. The GCRC kitchen staff weighed back any remaining pizza and water; and energy intakes were calculated.

Before subjects were discharged from the GCRC they were instructed to keep a 24-hour food record, which was mailed to the study coordinator the following day. The 24-hour food records were analyzed using the dietary analysis program, Nutrition Data System for Research (NDSR)¹⁹⁵. NDSR provided detailed nutrient information, including: total energy, carbohydrate, fat, protein, and fiber intake. Subjects also completed a narrative log of their GI feelings for the remainder of the day. At each meal or snack subjects were asked to report any discomfort (i.e. bloating, cramping, gas, diarrhea) that may have been related to food intake during their study visit.

After completion of the study, subjects also completed a 51-item validated questionnaire to measure for restrained eating habits (Appendix E)¹⁹⁶. Restrained eating refers to the intention to diet to achieve or maintain a desired weight, and the eating behavior of restrained eaters is different from that of unrestrained subjects⁷⁹.

Sample Collection and Analysis

Blood samples were collected by a registered nurse at the University of Minnesota General Clinical Research Center (GCRC). Samples were collected by syringe draw at specified time points during the course of each study visit.

Plasma sample collection methods, sample handling, storage procedures, and gut hormone analysis protocols are described in detail in Appendix F.

Glucose and insulin were analyzed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center. Glucose was determined by hexokinase method (Roche Diagnostics, USA) and insulin was determined by double monoclonal antibody ELISA method (Merodia AB, Sweden). The interassay CV for glucose was <1.6%, and insulin was <6.6% across the reference range for each assay. Because, glucose and insulin were analyzed as single samples, there is no intraassay CV to report. Glucose and insulin were sampled at 0, 15, 30, 45, 60, 90, 120, and 180 minutes.

Because fiber is not digested and absorbed like carbohydrate, protein, or fat, it was difficult to choose the timing for our gut hormone analyses. Tables 3-2 through 3-4 summarize relevant gut hormone research and help explain the times we chose for analysis of each hormone. Of note, due to limited publications on this topic, the differences reported in the results column of each table were not always statistically significant. In many cases, the results were only a description of what was shown in a published graph.

Tables 3-2 through 3-4 also justify the assay kits we chose for our gut hormone analyses. Each table cites at least three recent studies that have been published using our assays. Interestingly, with respect to ghrelin, the Phoenix Pharmaceuticals assay RK-031-30 was found equivalent to the Linco assay GHRT-89HK when tested for accuracy and sensitivity¹⁹⁷. We used the GHRT-89HK assay in our research.

Ghrelin was analyzed by commercially available radioimmunoassay kits (Total Ghrelin; Cat. # GHRT-89HK; Millipore; St. Charles, MO). Components from eight radioimmunoassay kits were pooled and all samples were analyzed in a single batch on

the same days. Samples were analyzed in duplicate (Millipore Assay Services; St. Charles, MO). The CV for duplicate samples was <15% for 395 out of 399 analyzed samples. The samples with a CV >15% did not exceed 19%. In those cases, one value was chosen; the chosen value was based on the patterning of other samples from the individual. Ghrelin was sampled at 0, 15, 30, 60, and 90 minutes. One sample, out of a total of 400, was unavailable for analysis.

GLP-1 was analyzed by commercially available ELISA kits (Active Glucagon-Like Peptide-1, 96-Well Plate; Cat. # EGLP-35K; Millipore; St. Charles, MO). This non-radioactive kit quantified both GLP-1₇₋₃₆ and GLP-1₇₋₃₇ in plasma. Seven plates were used to analyze 240 samples. Each plate was balanced by gender and treatment sequence order. All of the samples for an individual were analyzed on the same plate. Samples were analyzed in duplicate at the University of Minnesota (Kurzer Lab Facilities).

Mean values were reported when duplicate values were within the reference range for the assay *and* when the CV was <15% for the pair. The CV for duplicate samples was <15% for 202 out of 240 samples analyzed. If the CV was >15%, the higher of the two values was always reported. We chose to report the higher value because the kit used was more sensitive at higher levels; therefore, making the low-end value less plausible. Fifty three samples fell below the assay detection level of 2pM. All levels less than detection were included in the statistical analysis as 2pM.

The intraassay CV for each plate was as follows: Plate 1: 12.7%; Plate 2: 10.4%; Plate 3: 11.5%; Plate 4: 14.7%; Plate 5: 8.5%; Plate 6: 4.3%; and Plate 7: 22.8%. The interassay CV was 12.1%. Of note, plate number was included in the statistical

analysis, but was not significant and was removed from the final models. GLP-1 was sampled at 0, 30, and 60 minutes.

PYY₃₋₃₆ was analyzed by commercially available radioimmunoassay kits (Human PYY₃₋₃₆; Cat. # PYY-67HK; Millipore; St. Charles, MO). This kit utilizes an antibody that only recognizes human PYY₃₋₃₆ and not PYY₁₋₃₆. Components from five radioimmunoassay kits were pooled and all samples were analyzed in a single batch on the same days. Samples were analyzed in duplicate (Millipore Assay Services; St. Charles, MO). The CV for duplicate samples was <15% for all 240 samples analyzed. Of the 240 samples, 151 fell below the assay detection level of 21.1 pg/ml. Levels less than detection were included in the statistical analysis as 21 pg/ml. PYY₃₋₃₆ was sampled at 0, 30, and 60 minutes.

Sample Size and Power Calculation

The sample size of 20 was based upon published clinical research in humans⁶⁸. Our primary endpoint was appetite sensations as determined by changes on VAS. A 10 mm difference was regarded as clinically meaningful. Since our sample size was 20 subjects, and we estimated a standard deviation of 8 –15 mm, the minimum differences we could detect with a power of 80% using a two-sided paired t-test at $\alpha = 0.05$ was as follows:

If Standard Deviation:	8 mm	10 mm	12 mm	14 mm	15 mm
Minimum Difference Detectable	5.3 mm	6.6 mm	7.9 mm	9.2 mm	9.9 mm

These power calculations were approximately based on:

$$\text{Minimum difference } D = \sigma \frac{\sqrt{(Z\alpha + Zp)^2}}{N} \quad \text{***(where } (Z\alpha + Zp)^2 = 7.9\text{)***}$$

“D” was increased to account for the t distribution that was used to compare differences among treatments. Minimum differences were calculated using the power.t.test function in R ¹⁹⁸.

Randomization

Subjects were randomized using a Williams design that was balanced for carry-over (Appendix G) ¹⁹⁹. There were 4 sequences of treatments and 5 subjects assigned to each sequence. The repeating sequences were divided evenly between a block of 10 men and 10 women. Subjects were assigned to treatment sequences in the order they enrolled in the study.

Statistical Analysis

Area under the curve (AUC) was calculated by the trapezoidal rule. AUC values, and other endpoints, were compared among treatments using a mixed effects linear model with a random subject effect (Proc Mixed). Proc Mixed was used to calculate treatment means, standard error, and statistical differences between treatment means. Statistical significance was achieved at p-values < 0.05. All treatment means were adjusted for visit. Carryover, treatment sequence, palatability and interaction terms were tested in each model; these were omitted because they were not significant at p < 0.05. All final models included treatment and visit number only. Spearman

correlation coefficient tests were performed to assess for associations between select variables. All analyses were carried out with SAS 9.1.2¹⁷⁴.

Results

Demographic Characteristics

Twenty racially diverse subjects participated in this study; 1 subject was Asian, 1 Hispanic, and 4 were African Americans. Sixteen subjects reported regular moderate exercise. Seventeen subjects reported moderate alcohol intake each week (< 2 drinks per day for men and < 1 drink per day for women).

Baseline demographic characteristics are shown in Table 3-5. There were no differences between men and women. Gender, BMI, age, and restrained eating characteristics did not differ among the treatment sequence groups (data not shown). As well, baseline responses on VAS, fasting glucose, insulin, and gut hormone levels did not differ among treatments.

Appetite Sensations

AUC hunger, prospective food intake, satisfaction, and fullness varied among treatments. The 12 g fiber dose was consistently, and significantly, more satiating than the 0 g dose. Muffins with 4 g and 8 g of fiber had intermediate effects. Data are shown in Figures 3-1 and 3-2. Appetite sensations did not change in a clear dose dependent manner.

Food Intake

Figure 3-3 shows that food intake at the lunch buffet and in the post-intervention period did not differ among treatments. In the post-intervention period, total fiber (g), total fat (g), total carbohydrate (g), total protein (g), and total food weight (g) consumed were also indistinguishable among treatments.

Glucose and Insulin

Compared to all fiber doses, AUC glucose was lowest after the 0 g fiber muffin. AUC glucose did not differ among the remaining treatment doses. Data shown in Figure 3-4. Mean glucose peaks, however, diminished as fiber doses increased. Mean glucose peaks were significantly higher after the 4 and 8 g doses, than after either the 0 or 12 g doses.

AUC insulin varied among treatments, but did not change incrementally with fiber dose. Data shown in Figure 3-4. AUC insulin was lower after the 0 g dose than after the 4 g dose; it was also lower after the 12 g dose than after the 4 g dose. Mean insulin peaks also varied.

AUC glucose and AUC insulin did not correlate with each other, or with any of the satiety endpoints (Spearman Correlation Coefficients; $p > 0.05$).

Ghrelin

Unexpectedly, AUC ghrelin was higher after the 12 g fiber dose than after all other doses. Data shown in Figure 3-5. This is inconsistent with the subjective hunger and satiety measures, which indicated that the 12 g fiber dose was most satiating.

AUC ghrelin did not correlate with hunger or with food intake at the *ad libitum* lunch buffet for any of the treatment doses (Spearman Correlation Coefficients; $p > 0.05$).

Glucagon-Like Peptide-1

Also unexpectedly, AUC GLP-1 decreased significantly with fiber dose (p for trend = 0.0017). It was lower after the 12 g dose than after all other doses. AUC GLP-1 was also lower after the 8 g dose than after the 0 g dose. Data shown in figure 3-6. AUC GLP-1 did not correlate with AUC glucose or AUC insulin across any of the treatment doses (Spearman Correlation Coefficients; $p > 0.05$).

Peptide YY₃₋₃₆

AUC PYY₃₋₃₆ did not differ among treatments. Approximately 65% of samples fell below the assay detection level of 21.1 pg/ml; therefore, graphing the mean values was not meaningful.

Palatability

The taste and overall pleasantness of the 0 g fiber muffin was preferred over all other muffins. The mean scores for taste and overall pleasantness were nearly identical for the 4, 8, and 12 g muffins. On a scale of 0 to 100 mm, the 4, 8, and 12 g doses scored about 50 mm; thus, indicating that they were of average palatability. (Data not shown.) Taste and overall pleasantness of the muffins did not correlate with any of the

satiety endpoints, or with food intake at the *ad libitum* lunch buffet (Spearman Correlation Coefficients; $p > 0.05$).

Tolerance

All treatment doses were well tolerated. Of the 80 subject visits, there were 7 complaints of *minor* GI symptoms (gas, cramps, and diarrhea). The complaints were spread evenly across the four treatments (one complaint with the 0 g dose and two complaints for the 4, 8, and 12 g doses). Symptoms were reported between 3 and 18 hours after muffin consumption.

Discussion

Satiety

The purpose of this study was to compare the effects of 3 practical doses of mixed fiber, and a placebo, on satiety, food intake, glucose, insulin, and gut hormone levels. We found that 12 g of mixed fiber, given in a muffin for breakfast, effectively improved satiety compared to a 0 g fiber control. However, there was no clear dose response when comparing muffins with 4 and 8 g of mixed fiber. Despite lack of dose response, our findings are consistent with previous reviews suggesting that fiber intake is positively associated with satiety^{11, 65}.

To the best of our knowledge, only two crossover studies have evaluated how various doses of the same fiber influence satiety. In each of these studies, the highest fiber dose was more satiating than the low- or no-fiber control. Mathern et al studied the effects of 0, 4, and 8 g of viscous fenugreek fiber on a variety of appetite sensations

²⁰⁰. They found that 8 g of fenugreek mixed into orange juice was significantly more satiating than 0 or 4 g. Similarly, Gustafsson et al found that 200 and 300 g portions of carrots, containing 5.8 and 8.7 g of fiber, were significantly more satiating than 100 g of carrots, containing 2.9 g of fiber, when incorporated into a mixed meal ¹⁹¹. This study, however, looked at fiber that was naturally present in carrots. It is possible that inherent dietary fiber influences satiety at a lower dose than an added functional fiber, such as the mixed fiber added to our muffins or the fenugreek added to the orange juice. In our study, 12 g of mixed fiber seems to be the threshold dose needed to induce satiety.

Food Intake

Despite differences in satiety, food intake did not vary among treatments. There were no differences in calories consumed during the *ad libitum* pizza lunch or in the post-intervention period. These findings are difficult to explain since they contradict the results of other published literature. A large review on dietary fiber states that the majority of studies have observed a decrease in energy intake when subjects are fed higher fiber foods compared to lower fiber foods ⁶⁵.

Many individual studies have reported that increased satiety translates into a reduction in food intake. For example, Holt et al found satiety scores after breakfast were negatively correlated with food intake at subsequent *ad libitum* meals and in the following 24 hours ⁷⁶. Similarly, Levine et al found subjects were more satiated and ate less at an *ad libitum* lunch after consuming a high-fiber breakfast than after a low-fiber breakfast ²⁵.

Our lack of relationship between appetite sensations and food intake supports the notion that food intake regulation is driven by both homeostatic and hedonic stimulus²⁰¹. In fact, a recent study suggests that hedonic hunger may override homeostatic hunger²⁰². The current food environment, and the multitude of environmental cues that stimulate food intake, are likely to blame. For example, some studies have found that habitual intake patterns, or volume of food normally consumed, may influence food intake more than appetite^{73,74}. This strongly suggests that appetite sensations explain only part of food intake behaviors.

Glucose, Insulin, and Fiber Dose

Some studies have suggested that lower post-prandial glucose and insulin levels may be partially responsible for feelings of increased satiety^{203,204}. Meanwhile, conventional wisdom suggests that consumption of fiber-rich foods will blunt glucose and insulin responses compared to low- or no-fiber foods. In particular, viscous fibers are commonly cited as highly effective for lowering post-prandial glycemic and insulinemic responses^{43,185,205}.

The majority of the fibers used in our study (pectin, barley β -glucan, guar gum, and citrus fiber) are recognized viscous fibers²⁰⁶. However, as stated in the Chapter 1 Literature Review, determining true viscosity of a fiber in a food is difficult and inconsistent.

Viscous fibers are believed to attenuate the glycemic response by: 1) slowing gastric emptying, and therefore, increasing the time it takes for glucose to come in contact with the intestinal wall, and 2) by trapping glucose within the viscous food

bolus that enters the intestinal tract. The slow glucose absorption should subsequently produce a proportionally slow insulin release. According to this theory, glucose and insulin response would decrease with increasing fiber dose. However, our results do not support this theory.

AUC glucose and insulin levels did vary among our treatments, but not in a dose-dependent manner, and not as we expected. AUC glucose was indistinguishable among the 4, 8, and 12 g fiber doses, and was significantly lower after the 0 g fiber control. Part of these findings may be explained by small differences in carbohydrate and fat content among our treatments. The challenge of disguising fiber in our muffins made it difficult to balance macronutrient content across treatments. Because of this, carbohydrate content increased from 74 g in the 0 g fiber muffin, up to 93 g in the 12 g fiber muffin. With a nearly 20 g carbohydrate difference, it is somewhat reasonable to expect AUC glucose to differ between the 0 g and 12 g fiber doses. The 0 g fiber treatment also contained significantly more fat than the other treatments (19.5 g versus 10 – 13 g, respectively); this also may have contributed to the lower mean glucose level after the 0 g fiber dose.

Despite slight macronutrient differences in the 0 g fiber dose, the remaining muffins were nearly identical and varied only by fiber content. Therefore, it is not clear why AUC glucose was indistinguishable among the remaining doses. It is possible the differences in fiber were not large enough in the context of a 500 calorie breakfast. Perhaps glucose would have been significantly affected if we had larger increments between our fiber doses, or fewer carbohydrate calories in each of the muffins. However, other studies have also found AUC glucose does not vary across iso-caloric

meals with varying fiber doses^{24,98}. Additionally, Kim et al found no differences in AUC glucose after feeding subjects 5 doses of wheat and barley fiber (8.8 g to 20.1 g of fiber)²⁰⁷. These findings conflict with a number of other studies, which have suggested that fibers are beneficial for lowering post-prandial glucose^{18, 20, 171, 205}.

Interestingly, Casiraghi et al showed differences in glycemic and insulinemic responses when similar doses of barley and wheat were baked into either cookies or crackers¹⁷. This implies that processing (i.e. baking time, temperature, or moisture content) may impact the glycemic or insulinemic response to a particular food. Therefore, it is possible that the variation in processing techniques may help explain the discrepancy between studies that find fibers effective for attenuating the glucose response and those that do not.

We did, however, find significant differences in the mean glucose peaks among fiber doses. Despite the 20 g difference in carbohydrate value between the 0 g and 12 g fiber treatments, the mean glucose peaks were not significantly different. Mean glucose peaks were significantly higher after the 4 and 8 g fiber doses. Thus, suggesting that higher fiber content may indeed slow the appearance of glucose, but not necessarily change the overall amount of glucose released during a period of time. This finding supports the notion that increasing fiber may be useful for maintaining a steady blood glucose level in normoglycemic people. It is also possible that glucose levels would behave differently in people with glucose intolerance than in young, healthy people, like those in our study population.

AUC insulin was highest after our 4 g fiber dose; it was significantly higher than after the 0 g and 12 g fiber doses. The AUC insulin difference between the 0 g and 4 g

fiber doses was expected since there was also a lower AUC glucose response after the 0 g control. The lower AUC insulin response after the 12 g fiber dose, however, is difficult to explain since AUC glucose was similar after the 4 and 12 g fiber treatments. Nonetheless, our 12 g fiber treatment did blunt AUC insulin response. Thus, our findings are consistent with current beliefs that higher fiber foods may be beneficial for maintaining lower insulin levels.

Contrary to our findings, Kim et al reported a true dose response when evaluating the relationship between 5 doses of viscous β -glucan fiber and insulin levels²⁰⁷. As the β -glucan content increased, AUC insulin levels decreased proportionally. The most significant difference was between their highest and lowest β -glucan doses. Interestingly, like our data, Kim et al did not find an obvious correlation between AUC glucose and AUC insulin. They suggest that the discrepancy between glucose and insulin levels was most likely due to differences in gut hormone release; however, gut hormone levels were not tested in their study.

Glucose, Insulin, and Satiety

Our findings, as well as those of others, suggest that glucose and insulin levels do not play a significant (or consistent) role in satiety. For example, Mathern et al found differences in satiety after 3 doses of fenugreek fiber, but mean glucose levels were indistinguishable among the treatments²⁰⁰. Holt et al reported no correlations between satiety and glucose or insulin levels after feeding subjects 38 test foods¹⁰¹. Similarly, a recent meta-analysis concluded that glucose levels were not significantly associated with hunger, satiety, or energy intake, but that higher insulin levels may be

related to greater satiety, less hunger, and less energy intake in some normal weight subjects¹⁰⁰. The latter contradicts our findings. Our 4 g fiber treatment produced a significantly higher mean insulin response, but was less satiating than the 8 and 12 g treatments. Interestingly, two studies suggest that insulin does not influence appetite if glucose levels remain stable^{103, 104}. In our study, glucose levels were similar across treatments; thus, suggesting that the alterations in insulin would not affect appetite sensations. In the context of these findings, we conclude that postprandial glucose and insulin levels are not key drivers for satiety after fiber intake.

Gut Hormones

On the contrary, many reviews have suggested that gut hormones—like ghrelin, GLP-1, and PYY₃₋₃₆—play highly influential roles in appetite^{108, 109, 129, 208, 209}.

Ghrelin

Ghrelin is the only peripheral hormone known to stimulate appetite. It is generally accepted that ghrelin levels rise pre-prandially and fall post-prandially. Elevated ghrelin is typically correlated with hunger, while ghrelin suppression is correlated with satiety¹²⁹. In our study, total ghrelin levels changed significantly after muffin consumption. However, AUC ghrelin was higher after the 12 g fiber dose than after all other doses. This finding is unexpected, since the 12 g fiber muffin was rated the most satiating on VAS. Interestingly, several other studies have also reported that fiber intake may inhibit ghrelin suppression.

Karhunen et al found that ghrelin responses varied significantly after subjects consumed five test meals with varying amounts of fiber²¹⁰. Two psyllium fiber meals, with 26 and 27 g of fiber, inhibited ghrelin suppression during a 2-hour period compared to three low-fiber meals, with 3 to 7 g fiber. Mohlig et al also found total ghrelin was significantly higher 2 hours after consumption of a soluble, viscous fiber than after a no-fiber control¹²⁵. Similarly, Weickert et al showed ghrelin was higher 2 hours after subjects consumed 10 g of wheat fiber compared to a low-fiber control⁹⁷. (Of note, in the same study, ghrelin levels were indistinguishable from the low-fiber control after subjects ate 10 g of oat fiber). Finally, another study reported ghrelin levels remained unchanged from baseline after subjects consumed 21 g of guar gum in water²¹¹. These findings suggest that certain types of fiber may inhibit ghrelin suppression.

However, additional studies designed to evaluate the relationship between ghrelin and fiber intake reported mixed, or conflicting, results. Gruendel et al reported total ghrelin was decreased after a 10 g dose of insoluble fiber, but not after a 5 or 20 g dose of the same fiber¹²⁴. Another study found psyllium fiber, given for breakfast, significantly suppressed ghrelin levels in healthy adults²¹².

Macronutrient content plays a known role in ghrelin changes. Many reviews suggest that digestible carbohydrates are the most effective for suppressing post-prandial ghrelin, followed by protein, then fat^{129, 151, 213}. However, the effect of consuming a predominantly fiber food, or consuming fiber as part of a mixed meal, is not clearly understood.

It is possible that the viscous nature of our fibers increased gastric distention and slowed gastric emptying; thus, leading to fullness and less hunger by means of mechanical changes in digestion or absorption, and not by way of ghrelin suppression^{42, 185}. It is plausible that a viscous food could prohibit sufficient nutrient contact with intestinal sensors, such that ghrelin would not be suppressed. Our 0, 4, and 8 g fiber muffins had less carbohydrate than the 12 g fiber muffin, yet they still suppressed ghrelin more.

Based on our findings, we believe that fiber content clearly influences appetite sensations, but the mechanisms are independent of ghrelin suppression. Further research is needed to better understand why ghrelin (and other gut hormone secretions) did not correlate with appetite after fiber intake in the same way it does after digestible carbohydrate intake.

Ghrelin and Food Intake

AUC ghrelin did not correlate with hunger or subsequent food intake in our study. We found no association between post-prandial ghrelin levels and hunger or food intake 3 hours later. Additional studies have also reported no correlation between post-prandial ghrelin, hunger, or food intake^{119, 214}. However, this is contrary to the findings of others.

Typically, in studies where ghrelin has been administered intravenously (sometimes at doses well beyond physiologic levels), ghrelin shows a consistent positive correlation with hunger and food intake^{110, 113}. Studies evaluating ghrelin

levels after mixed macronutrient meals (without fiber) also confirm these relationships
115, 116.

Glucagon-Like Peptide-1

Glucagon-like peptide-1 is typically very low in the fasting state, but rises quickly after food intake. The rise of GLP-1 has been correlated with increased satiety and less hunger^{135, 136}. However, our results indicate that the fiber content of a meal may blunt GLP-1 release without decreasing satiety. In our study, AUC GLP-1 decreased as fiber dose increased, and the dose response was significant. Similar to our findings with ghrelin, this is contrary to what we would expect. The 12 g fiber dose produced the greatest feeling of satiety, but the lowest levels of GLP-1.

Carbohydrates are known to be a strong stimulus for GLP-1 release; though, the type and structural form of carbohydrate likely matters. In our study, GLP-1 response was lowest after our 12 g fiber treatment, yet it had more carbohydrate than the 0, 4, and 8 g fiber treatments. This suggests that fiber may modify the impact of carbohydrate on GLP-1 release. Additional studies also support this idea.

One study reported differences in GLP-1 response after feeding subjects 50 g of digestible carbohydrate from a variety of wheat, barley, and rye products²¹⁵. In this study, whole-kernel rye bread and whole-meal pasta yielded significantly lower GLP-1 levels than did white-wheat bread (fiber content: 12.8 g, 5.6 g, vs. 3.1 g, respectively). As well, another crossover study fed subjects 75 g of carbohydrate from glucose, brown rice, or barley; GLP-1 was significantly higher after the glucose treatment than after the other two treatments¹⁴¹. In this study the authors suggest that the substantial GLP-1

increase after the glucose treatment was due to the incretin action of GLP-1. However, this is not consistent with our findings, since our 0 g fiber muffin mounted the smallest glucose and insulin response, and the largest GLP-1 response.

Contrary to the results of others, GLP-1 was not correlated with either glucose or insulin response for any of our treatments. Interestingly, Najjar et al has also reported no correlation between GLP-1, glucose, or insulin when comparing responses after consumption of four different types of bread with varying amounts of fiber ²¹⁶. The relationship between GLP-1, glucose, and insulin response after fiber intake clearly warrants further study.

We believe GLP-1 suppression may be attributed to mechanical differences in digestion after consuming increasing fiber doses. We again hypothesize that gastric emptying time and overall nutrient absorption may have been slower after our higher fiber treatments; thus, fewer stimuli (nutrients) were available to promote GLP-1 release. It is conceivable that in the low and no fiber treatments, nutrients interfaced with intestinal cells and nerve fibers more rapidly, which subsequently produced a greater GLP-1 response. This theory is somewhat supported by Juvonen et al ¹⁸⁵. They compared high and low-viscosity beverages with equivalent fiber content and found that the high-viscosity beverage significantly slowed gastric emptying and suppressed GLP-1 release compared to the equivalent low-viscosity beverage. This relationship is also supported by the work of Miholic et al; they found that gastric emptying time was positively correlated with GLP-1 levels ²¹⁷. Specifically, they state that faster gastric emptying time was related to higher GLP-1 concentrations. This is contrary to the findings of others, who have reported that GLP-1 concentrations are inversely

associated with gastric emptying time^{135, 146, 218}. However, these studies looked at gastric emptying after GLP-1 infusions, or GLP-1 stimulated by fiber-free meals.

The addition of mixed fiber to a meal inevitably influences digestion and absorption. But, it is difficult to make concrete inferences about the relationship between GLP-1 release and gastric emptying after fiber intake, since we did not evaluate this endpoint in our study. Our study certainly suggests that mixed fiber intake blunts GLP-1 response; though, whether or not gastric emptying time plays a significant role warrants further investigation.

It is also possible that 60 minutes was not enough time to assess the full picture of GLP-1 release. One review suggests that GLP-1 may have a biphasic secretion profile—with an initial peak about 30 minutes after food ingestion and another peak 60 to 120 minutes later¹³². If this is true, it is possible that the AUC GLP-1 response would have been different at 120 or 180 minutes after intake. Though, this is somewhat unlikely.

Peptide YY₃₋₃₆

Similar to GLP-1, PYY₃₋₃₆ is generally low in the fasted state and rises with food intake. Several studies have indicated that satiety increases in proportion to plasma levels of PYY₃₋₃₆; however, this is most often seen after exogenous administration and not by way of endogenous production after food intake^{154, 160, 219}.

PYY₃₋₃₆ did not rise substantially after any of our muffins were consumed. The majority of our subjects' blood samples remained below the assay detection range for PYY₃₋₃₆. Therefore, quantified levels were not reported. We have no reason to believe

this was an assay error, since preparation and analysis techniques were the same as those previously described in the literature^{220, 221}. PYY₃₋₃₆ was consistently detectable in 7 of our 20 subjects; though, there was significant variability in the baseline values between subjects and between test days (22 pg/ml to 161 pg/ml). This suggests that basal levels of PYY₃₋₃₆ are highly variable within, and between, individuals.

PYY₃₋₃₆ concentrations are expected to increase within 15 to 30 minutes after food intake and reach a peak within 1 to 2 hours²²². The change in PYY₃₋₃₆ concentration is believed to reflect calorie content and the macronutrient (carbohydrate, protein, or fat) composition of a meal. However, there are no published human studies evaluating changes in PYY₃₋₃₆ after fiber is consumed as part of a mixed macronutrient meal. Weickert et al evaluated change in total PYY (PYY₁₋₃₆ and PYY₃₋₃₆) after 10 g of either oat or wheat fiber, and found that total PYY was lower after wheat fiber than after a low-fiber control⁹⁷. In the 3 hours after the wheat bread was consumed, total PYY remained at levels similar to baseline (or dipped below baseline) whereas the control bread caused total PYY to increase to approximately 150% of baseline values. This is relevant because PYY₃₋₃₆ represents the majority of total PYY, implying that if PYY₃₋₃₆ were tested this would have been lower after the wheat bread as well.

Interestingly, PYY₃₋₃₆ was detectable in 10 of our 20 subjects after the 0 g fiber muffin was consumed. Conversely, PYY₃₋₃₆ levels were detectable in only 6 or 7 subjects after the 8 g and 12 g fiber doses, respectively. Statistically, we cannot make conclusions with so few subjects; however, these findings are interesting and suggest that increasing fiber doses may suppress the PYY₃₋₃₆ response.

Neary and Batterham confirm that very little is currently understood about the mechanisms underlying PYY release; they also emphasize the need for a better understanding of how habitual diet and other lifestyle factors may influence release of PYY²²². Thus, altering the fiber content of test meals should certainly be evaluated in future PYY₃₋₃₆ studies.

Conclusions

This study was the first of its kind and had many strengths. For example, we used a crossover design, which allowed each subject to serve as his or her own control; we included a balanced, homogenous group of men and women; and we used practical doses of fiber that could reasonably be consumed during one meal. We also were the first group to simultaneously evaluate satiety and gut hormone responses after increasing doses of fiber. On the other hand, our work also included some potential limitations. It is possible our fiber doses were too similar; perhaps if the discrepancy between doses had been slightly larger we would have seen clearer dose responses. Ideally, our muffins would have had identical macronutrient profiles; however, this is difficult when trying to conceal fiber in baked goods.

This research offers many new insights into the relationship between increasing doses of mixed fiber, appetite sensations, and commonly associated physiologic markers. We found that feelings of hunger, satisfaction, fullness, and desire to eat differed after subjects consumed 0, 4, 8, or 12 g of mixed fiber for breakfast. There was no clear dose response. However, in all satiety measures the 12 g fiber treatment was

consistently more satiating than the 0 g treatment, while the 4 and 8 g treatments had intermediate effects.

Satiety was not inversely correlated to food intake as we had expected. This may reflect the ability of hedonic hunger to override the physiologic need for food intake.

Subjective appetite sensations were also inconsistent with our physiologic markers for satiety. Fiber intake clearly influenced ghrelin and GLP-1 levels; however, the effects did not relate to appetite in the manner we expected. The 12 g fiber dose left subjects feeling the most satiated, despite inhibiting ghrelin suppression and blunting GLP-1 response. It is difficult to explain these findings, though it seems that after fiber intake, satiety may be influenced more by differences in digestion and absorption, than by gut hormone release. It is possible that our higher fiber muffins may have prevented interaction between nutrients and the GI mucosa, such that enteroendocrine cells were not stimulated enough for gut hormone release or suppression. Though, this was not evaluated in our study.

Our findings are consistent with other studies, which have also indicated that subjective appetite does not always correlate with food intake behavior, or with the post-prandial responses of glucose, insulin, or gut hormones. This emphasizes the complexity of appetite and food intake regulation, and suggests that appetitive sensations and behaviors are likely driven by a combination of many factors including, the fiber content of a meal, typical eating habits, environment, and/or physiologic changes.

In conclusion, understanding factors that control appetite and food intake are critical as obesity, and obesity-related morbidity, continue to rise. We believe dietary manipulations that maximize satiety are important and may be part of the reason why people who consume high-fiber diets tend to weigh less than people who consume low-fiber diets. In this study, we did not see a dose response in satiety when giving subjects increasing amounts of mixed fiber. However, 12 g of mixed fiber was significantly more satiating than 0 g of fiber. Interestingly, the commonly accepted physiologic markers for satiety were not consistent with subjective satiety ratings after mixed fiber intake in our subjects. Further research is needed to explain these inconsistencies.

Future Directions

Information generated from this study will be useful when designing future research. The following paragraphs describe a few of my original ideas for upcoming studies.

Subjective Satiety and Food Intake Studies

Even though the 12 g fiber dose was significantly more satiating, food intake was indistinguishable among the 4 fiber doses. These results suggest that many people are out of touch with the appetitive signals sent during and shortly after eating a meal. I envision research that incorporates a counseling component into a similar fiber dose study. Counseling would encourage subjects to recognize and respond to hunger and fullness while eating an *ad libitum* meal; this may alter food intake behaviors. I would then compare food intake after feeding the various fiber doses. Results from this type

of study could be potentially useful when designing future weight loss studies. It is possible that the people who weigh less after consuming high-fiber diets are more aware of appetite signals.

This study indicated clear differences in satiety after a 3-hour period. Future research should determine whether differences in satiety would persist with chronic fiber intake—and whether these differences would influence long-term food intake. Perhaps food intake would differ if the subjects were acclimated to the feelings of satiety after chronic high fiber consumption.

Satiety Mechanisms

Satiety differed among fiber doses, but glucose, insulin, and gut hormones levels could not explain these differences. Ideally, I would like to repeat the study while simultaneously evaluating gastric emptying time, as well as characteristics of the food bolus during intestinal transit.

Gut Hormones

At this time it is difficult to interpret the importance of gut hormone changes before and after fiber intake. We evaluated three hormones that are known to play a significant role in appetite after digestible macronutrients are consumed. However, our results showed that subjective appetite was inconsistently linked to these hormones when fiber was consumed as part of the meal. Additional research should be done to confirm our findings, and to explain the mechanisms of this relationship.

Our research suggests gut hormones vary significantly within a relatively homogenous population. Future studies should be designed to determine whether or not “normal” gut hormone ranges exist. For example, gut hormones may be different in people who habitually eat high-fiber diets. Animal data suggests that chronic fiber consumption may alter gut hormones levels and release; this could subsequently alter appetite, food intake, fat metabolism, and/or fat storage.

Future research should also evaluate gut hormone levels at time points later in the digestive process. We evaluated these hormones for a relatively short period of time and may have missed important changes. Specifically, it would be interesting to see if gut hormone levels would change once our fiber mixture reached the colon and began the fermentation process. We chose time points for gut hormone evaluation based on research from digestible macronutrients; however, we know that fiber behaves differently.

Finally, it is clear that gut hormones are not released in isolation; and thus it might not make sense to look at a handful of hormones until we have a better understanding of the interconnectedness of many hormones. Studies that evaluate the orchestration of many gut hormones would be beneficial and may be more important than understanding how two or three gut hormones change in the acute setting.

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Table 3-1. Composition of Treatment Muffins¹

Mixed Fiber Dose	Total Fiber (g)	Soluble Fiber (g)	Insoluble Fiber (g)	Kcals	Total fat (g)	Total Carb (g)	Protein (g)	Moisture Content (g)	Ash (g)	Serving Size (g)
0 g	<1	n/a	n/a	502	19.5	74	11	24	1	144
4 g	5.7	2.5	3.2	488	13	81	12	68	3	176
8 g	8.9	4.0	4.9	493	10	89	12	62	3	175
12 g	12.8	6.1	6.7	544	13	93	13	81	3	204

¹Content listed per serving. Fiber, fat, protein, moisture, and ash analysis were determined by appropriate AOAC Methodology. Carbohydrate and calorie content were estimated by United States Department of Agriculture calculations. This analysis was completed at Covance Labs; Madison, WI.

Table 3-2. Ghrelin Summary

Study Summary	Analytic Method*	Results
Ghrelin levels after 500 kcal chocolate breakfast drink ²²³	“Established” RIA	Ghrelin decreased steadily from baseline through 120 minutes.
Ghrelin levels after white bread, bread with added wheat fiber, or bread with added oat fiber ⁹⁷	RIA; RK-031-30 Phoenix Pharmaceuticals	Ghrelin decreased most after 30 and 60 minutes, but was still low at 90 minutes. Upward trend obvious by 120 and 180 minutes.
Ghrelin levels after 0, 5, 10, and 20 g doses of Carob Pulp (an insoluble fiber) ¹²³	RIA; GHRT-89HK Linco Research	Ghrelin decreased substantially through 50 minutes, but only after the 5 and 20 g fiber doses. It changed sporadically through 100 minutes after 0 and 10 g doses.
Ghrelin levels after consumption of hydrolyzed gelatin protein ²²¹	RIA; GHRT-89HK Linco Research	Ghrelin was lower than baseline through 90 minutes after consumption of gelatin. Levels trended upward between 90 and 120 minutes.
Ghrelin response after carbohydrate-enriched breakfast, low-carbohydrate breakfast, or water ²²⁴	RIA; Phoenix Pharmaceuticals	Ghrelin decreased through 90 minutes after both meals; it did not change after water. Levels trended towards baseline between 90 and 120 minutes.
Ghrelin response after various protein or glucose loads ¹⁴²	RIA; RK-031-30 Phoenix Pharmaceuticals	Ghrelin decreased continuously from 30 to 90 minutes after all meals. Levels started to trend upward between 120 and 180 minutes.
Ghrelin responses after 280 calorie snack vs. no snack before lunch. Subjects were old, young, under, and normal nourished ²²⁵	RIA; Phoenix Pharmaceuticals	Ghrelin appeared to have a gradual, yet substantial, decrease at 30, 60, and 90 minutes after snack consumption.
Relationship among ghrelin and other gut hormones in lactating women ²²⁶	RIA; GHRT-89HK Linco Research	Mean ghrelin levels in women were between 831 ± 184 and $1,329 \pm 204$ pg/ml for women during different stages of lactation.
Ghrelin Decision: Collect Plasma Sample at: 0, 15, 30, 60, and 90 minutes after muffin consumption		

* Linco®, Chemicon®, and Upstate® merged in 2008; they now operate under the name Millipore®. In Chapter 3, we report use of Millipore® assay kits for our gut hormone analysis; these are the same as the Linco kits reported in these charts.

Table 3-3. Glucagon-Like Peptide-1 Summary

Study Summary	Analytic Method*	Results
GLP-1 levels in response to galactose with guar gum + std breakfast or water + std breakfast ¹⁰²	ELISA; EGLP-35k Linco Research	GLP-1 increased from baseline at 30 and 60 minutes.
GLP-1 levels after subjects ate bread, milk, and egg breakfast ²²⁷	ELISA; EGLP-35k Linco Research	GLP-1 had the most pronounced increase at 30 and 60 minutes after food intake.
GLP-1 changes during normal and high protein diets ²²⁸	ELISA; EGLP-35K Linco Research	GLP-1 increased from 15 to 60 minutes after meal intake. It remained elevated at 90 minutes, but peaked around 60 minutes.
GLP-1 levels after 500 kcal chocolate breakfast drink ²²³	“Established” RIA	GLP-1 appeared to increase from about 30 to 90 minutes after food intake.
GLP-1 response after various protein or glucose loads ¹⁴²	Fluorescence Immunoassay; Linco Research	Peak GLP-1 values appeared about 30 to 90 minutes after treatments.
GLP-1 responses after 280 calorie snack vs. no snack before lunch. Subjects were old, young, under, and normal nourished ²²⁵	“Established” RIA	GLP-1 peaked at 30 minutes, but was still slightly higher than baseline 60 minutes after intake.
GLP-1 Decision: Collect Plasma Sample at: 0, 30, and 60 minutes after muffin consumption		

* Linco®, Chemicon®, and Upstate® merged in 2008; they now operate under the name Millipore®. In Chapter 3, we report use of Millipore® assay kits for our gut hormone analysis; these are the same as the Linco kits reported in these charts.

Table 3-4. PYY Summary

Study Summary	Analytic Method*	Results
Changes in PYY 3-36 levels after a high-calorie meal, when subjects exercised before, after, or not at all ²²⁹	RIA for PYY 3-36; PYY- 67HK Linco Research	PYY 3–36 increased significantly and reached a peak 60 minutes after the meal in all conditions.
PYY 3-36 response after different macronutrient meals in obese females ²²⁰	RIA for PYY 3-36; PYY-67HK Linco Research	PYY 3–36 increased after all meals, but was highest after the high carbohydrate meal. PYY 3-36 peaked about 60 minutes after food intake.
PYY 3-36 levels after consumption of hydrolyzed gelatin protein ²²¹	RIA for PYY 3-36; PYY-67HK Linco Research	PYY 3-36 did not change much from baseline level after protein intake. Levels actually decreased significantly at 150 minutes after intake.
PYY levels after 500 kcal chocolate breakfast drink ²²³	“Established” RIA for total PYY (1-36 and 3-36)	PYY appears to increase from 30 – 150 minutes after intake. It trends toward baseline by 180 minutes.
PYY levels after white bread, bread with added wheat fiber, or bread with added oat fiber ⁹⁷	“Established” RIA for total PYY (1-36 and 3-36)	Oat fiber and white bread control caused PYY to rise 30 and 60 minutes after intake. Wheat fiber caused PYY to <i>decrease</i> at 30 and 60 minutes with a peak 120 minutes after intake.
PYY levels after high-carbohydrate/low-fat meals and after high-fat/low carbohydrate meals ¹⁶⁵	RIA for total PYY (1-36 and 3-36); PYYT-66HK Linco Research	PYY peaked by 90 minutes after a high-carbohydrate meal. PYY peak was at 30 minutes after a high-fat meal.
PYY 3-36 CONCLUSIONS: Sample Collection at 0, 30, 60 minutes after muffin consumption		

* Linco®, Chemicon®, and Upstate® merged in 2008; they now operate under the name Millipore®. In Chapter 3, we report use of Millipore® assay kits for our gut hormone analysis; these are the same as the Linco kits reported in these charts.

Table 3-5: Baseline Demographic Characteristics

	N	BMI	Age	Restrained Eaters
Men	10	25 \pm 2	29 \pm 2	4
Women	10	23 \pm 2	22 \pm 12	3
Pooled	20	24 \pm 2	26 \pm 7	7

Mean \pm SE.

Changes in Hunger and Prospective Food Intake

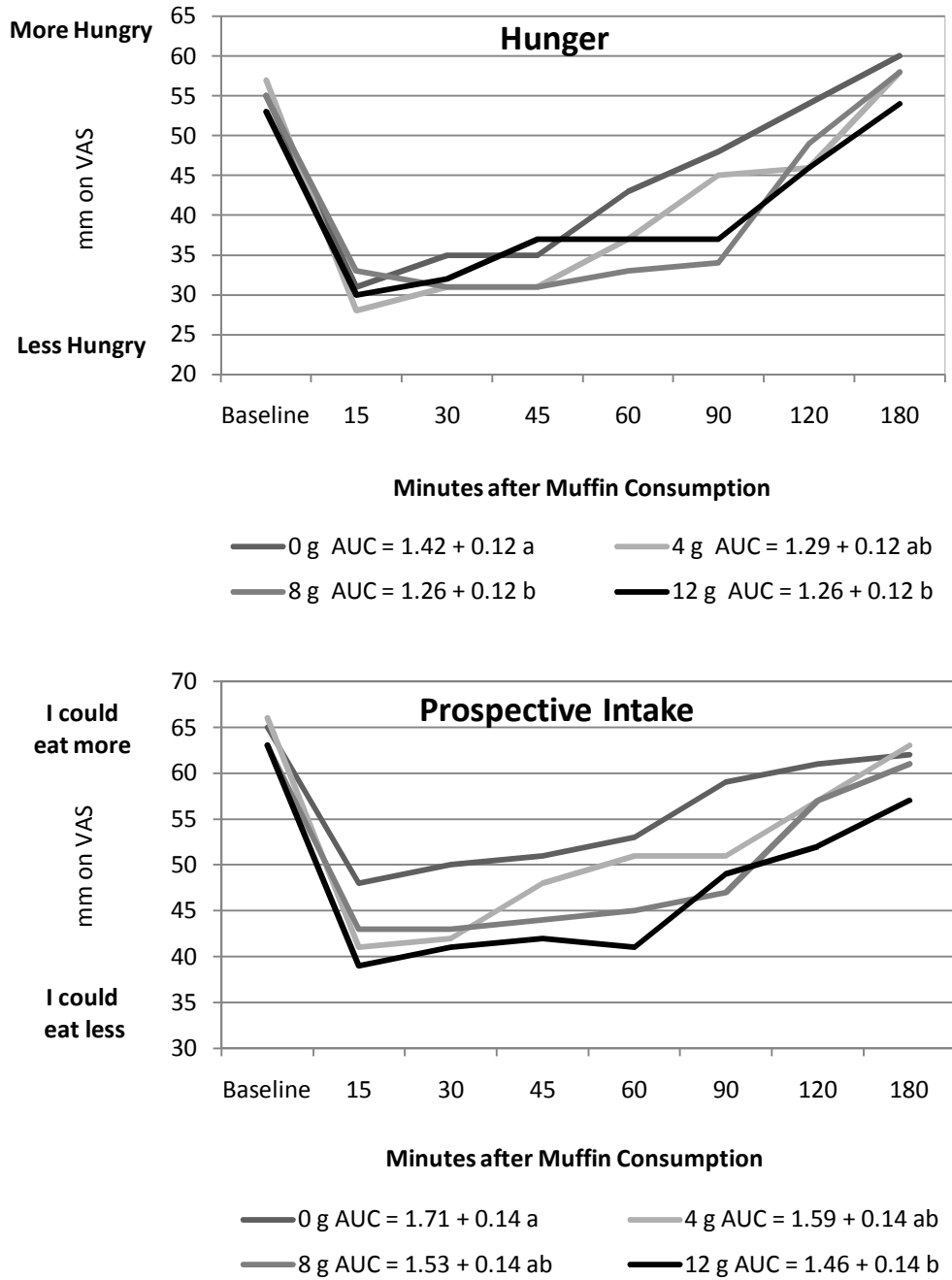


Figure 3-1. Millimeters on VAS for hunger and prospective food intake. In the legend, the numbers after each treatment dose represent the AUC score ± SEM. The treatments with different letters have statistically different AUC. N=20 for each line.

Changes in Satisfaction and Fullness

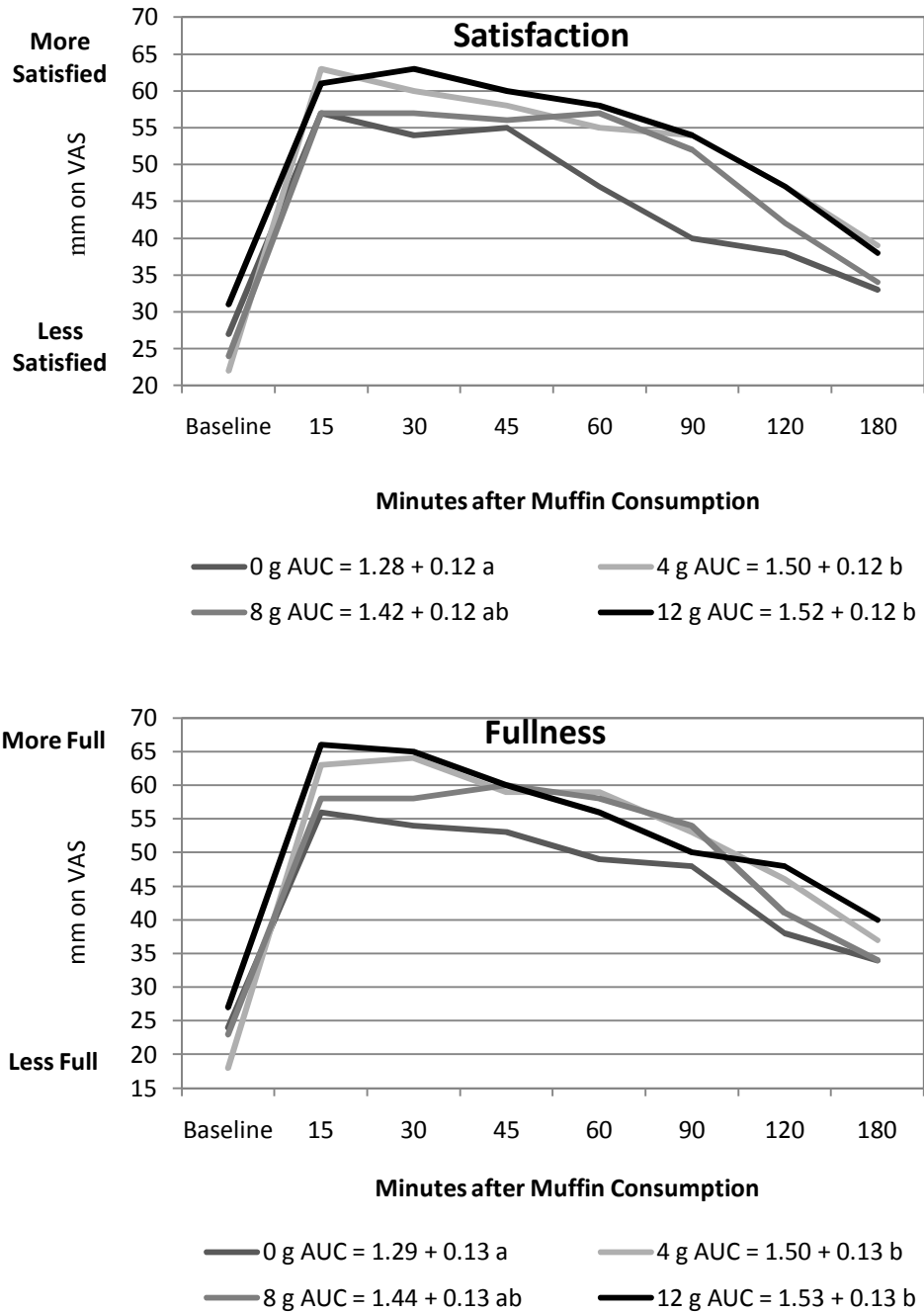


Figure 3-2. Millimeters on VAS for satisfaction and fullness. In the legend, the numbers after each treatment dose represent the AUC score \pm SEM. The treatments with different letters have statistically different AUC. N=20 for each line.

Mean Calories Consumed

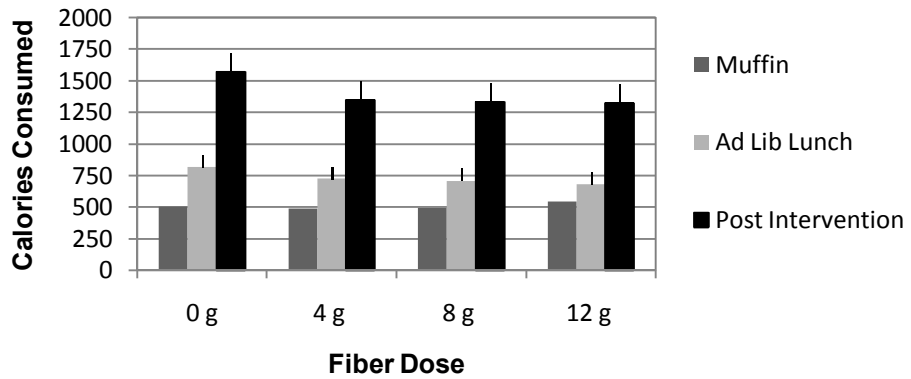


Figure 3-3. Mean (\pm SEM) calorie intake during 24-hour intervention day. There were no differences among treatments for intake at the lunch buffet or during the post-intervention period.

Mean AUC Glucose and AUC Insulin

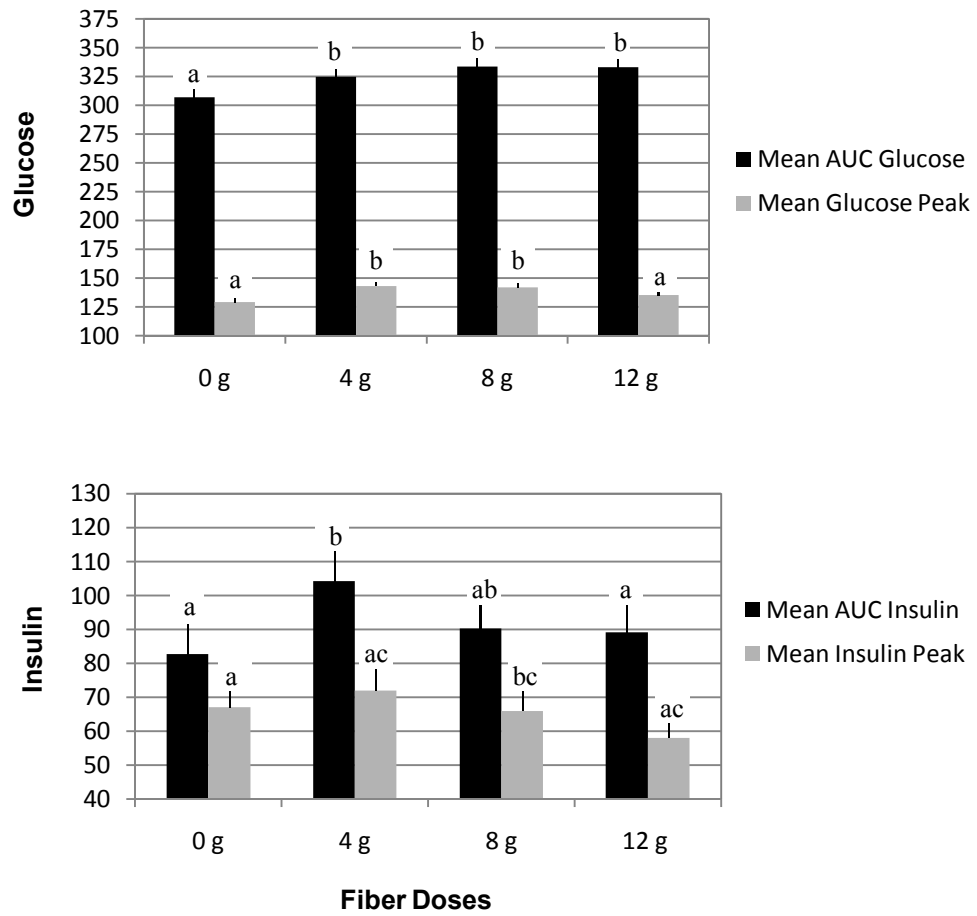


Figure 3-4. Means \pm SEM are presented for AUC, and peak, glucose and insulin values.

Units for AUC glucose and insulin are $\text{mg}\cdot\text{dl}^{-1}\cdot\text{min}$ and $\text{mU}\cdot\text{L}^{-1}\cdot\text{min}$, respectively.

Units for the peak glucose and insulin values are mg/dL and mU/L , respectively.

Within each series, treatments with different letters are statistically different.

Changes in Ghrelin

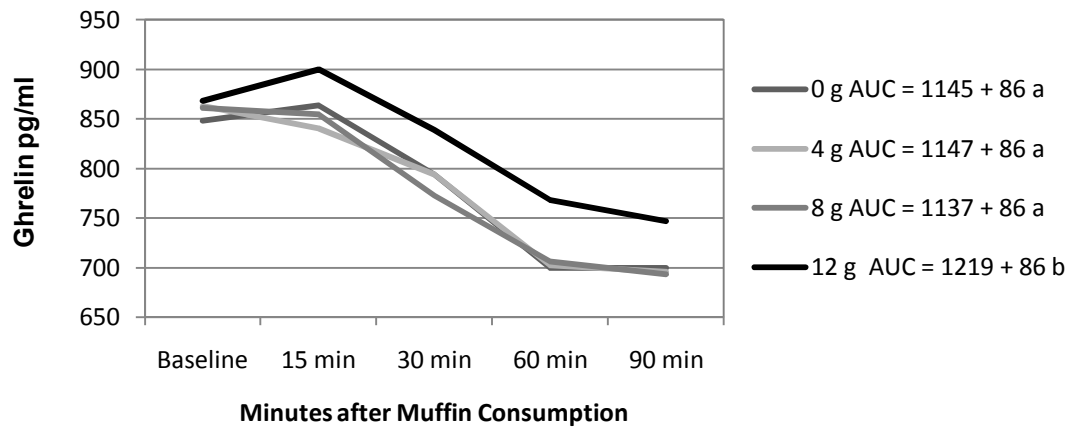


Figure 3-5. Change in ghrelin after muffin consumption. In the legend, the numbers after each treatment dose represent the AUC score \pm SEM. The treatments with different letters have statistically different AUC. N=20 for each line.

Changes in Glucagon-Like Peptide-1

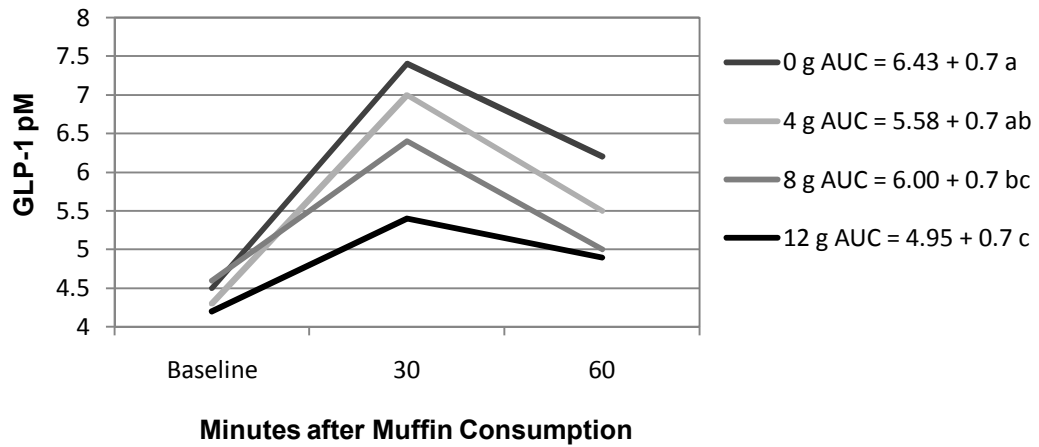


Figure 3-6. Change in GLP-1 after muffin consumption. In the legend, the numbers after each treatment dose represent the AUC score \pm SEM. The treatments with different letters have statistically different AUC. N=19 for each line. One subject was removed from the analysis due to significantly elevated GLP-1 levels; values for this subject ranged from 21 pM to 37 pM.

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Appendix A. 100 mm Visual Analog Scales

Satiety Questions

How hungry do you feel?

I am not _____ I have never
hungry at all _____ been more hungry

How satisfied do you feel?

I am _____ I cannot eat
completely _____ another bite
empty

How full do you feel?

_____ Totally full
Not at all full

How much do you think you can eat?

_____ A lot
Nothing at all

Palatability Questions

Visual appeal

Good _____ Bad

Smell

Good _____ Bad

Taste

Good _____ Bad

Aftertaste

Much _____ None

Overall Pleasantness

Good _____ Bad

Appendix B. Latin Square Randomization

Sbj # Treatment Order

1	B C A E D
2	E D B C A
3	A E C D B
4	D B E A C
5	C A D B E
6	E C D B A
7	B E C A D
8	D A B E C
9	C B A D E
10	A D E C B
11	C A E B D
12	B E A D C
13	E C D A B
14	A D B C E
15	D B C E A
16	A C D B E
17	C B A E D
18	E A C D B
19	B D E A C
20	D E B C A

Appendix C. Mixed Fiber Muffin Study Informed Consent

SATIETY RESPONSE AFTER MIXED FIBER SUPPLEMENT

CONSENT FORM

Please read this document and ask any questions you may have before agreeing to be in the study.

Joanne Slavin, PhD, RD and Holly Willis, MS, RD in the Department of Food Science and Nutrition are conducting this study. The Department of Food Science and Nutrition at the University of Minnesota is in the College of Food, Agricultural and Natural Resource Sciences. The study is sponsored by Nestle.

Description and Purpose of the Study

You are being asked to participate in a study of a mixed dietary fiber and its effect on hunger and certain markers in your blood. The fibers you will consume are already used in food products and are safe to eat. Approximately 20 subjects will participate in this study. This study will require you to attend 5 visits. The first screening visit will last approximately 30 minutes. The next four visits will each last about four hours. All visits will be conducted at the General Clinical Research Center at the University of Minnesota East Bank Campus. All visits are necessary to complete the study itself.

You were selected for this study because you are a man or woman in good health.

At visit 1, you will be weighed and measured, and you will have a finger prick to test the amount of sugar in your blood. At visits 2-5, you will consume a muffin for breakfast. The muffin will contain different amounts of fiber each week.

Study Procedures

At visits 2-5, you will be given a muffin that contains a total of 0, 4, 8, or 12 grams of fiber. You will also be asked to complete a survey about your level of hunger at baseline and for 4 hours after the fiber. You will be given a pizza lunch to consume 3 hours after the fiber treatment. An I.V. will be placed in your arm when you arrive and blood samples will be drawn from this line at baseline, 15, 30, 45, 60, 90, 120, and 180 minutes after the fiber treatments. Information from these visits will be recorded in your Fairview Medical Center medical chart.

Risks Associated with the Study

The fibers used in this study are provided in amounts commonly found in foods. There are no known side effects of the fibers in the amounts used in this study.

The finger prick will draw a small amount of blood from your finger. The risks associated with this may be a small amount of pain or a small bruise. Blood samples will be drawn from a vein in your arm. The risks associated with drawing blood through an I.V. are pain, bruising, lightheadedness, and rarely infection.

Benefits Associated with the Study

There is no guarantee that you will receive any benefit by participating in this study.

Compensation for Health Problems Related to the Study

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment, and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. The sponsor of the study has some funds available to pay for care for injuries resulting directly from being this study. If you think you have suffered a research-related injury and that you may be eligible for reimbursement of some medical care costs, let the study physicians know right away.

Compensation for Participation

Study related visits, procedures, tests, and the fiber-containing muffins for the study will be provided at no cost to you. You will also receive:

\$150.00 for each completed scheduled visit (for visits 2-5), if you do not complete the whole study.

\$200.00 additional at the end of the study if you complete all four food records

\$800.00 total if you complete the whole study

Confidentiality and Document Review

The results of this research study may be presented at meetings or in publications, so absolute confidentiality cannot be guaranteed. However, your identity will not be disclosed in these presentations. Data will be kept for 3 years after collection for completing the analysis of data and reporting of the information in the scientific literature.

Alternative Treatment

The alternative is to not participate in this study. You may consume fiber without participating in this study.

Voluntary Nature of Participation

Your decision whether or not to be in this study will not affect your current or future relations with the University of Minnesota. If you decide to be in this study, you are free to withdraw your consent and to stop participation at any time. Withdrawing your consent and stopping participation will not affect your relationship with the University of Minnesota.

New Information

If, during the course of this research study, there are significant new findings discovered that might influence your willingness to continue, the researchers will inform you of those findings.

Contacts and Questions

You may ask any questions you have now.

You may contact the investigators of the study, Dr. Joanne Slavin, in the Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108; telephone (612) 624-1290. Or Holly Willis, in the Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108; telephone (612) 625-5264.

If you have any other questions or concerns regarding the study and would like to talk to someone other than the researchers, you are encouraged to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this department in writing or in person at Fairview University Medical Center – Riverside Campus, #815 Professional Building, 2450 Riverside Avenue, Minneapolis, MN 55454.

You will be given a copy of this form to keep for your records.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Signature _____ Date _____

Signature of Investigator or Person Obtaining Consent

Signature _____ Date _____

Appendix D. Nutrient Composition and Preparation Method for Pizza Lunch

Nutrition Facts

Lean Cuisine Casual Eating Four Cheese Pizza

Serving Size: 1 Package (6 ounces)

Servings Per Package: 1

Amount Per Serving

Calories 360

Calories From Fat 70

Total Fat (g) 8

Saturated Fat (g) 3.5

Trans Fat 0

Polyunsaturated Fat (g) 2

Monounsaturated Fat (g) 1.5

Cholesterol (mg) 10

Sodium (mg) 690

Potassium (mg) 120

Total Carbohydrates (g) 55

Dietary Fiber (g) 2

Sugars (g) 6

Protein (g) 17

%Daily Value*

12%

18%

3%

29%

3%

18%

8%

<

Nutrition Facts

Lean Cuisine Casual Eating Pepperoni Pizza

Serving Size: 1 Package (6 ounces)

Servings Per Package: 1

Amount Per Serving

Calories 370

Calories From Fat 80

Total Fat (g) 9

Saturated Fat (g) 3

Trans Fat 0

Polyunsaturated Fat (g) 1.5

Monounsaturated Fat (g) 3

Cholesterol (mg) 30

Sodium (mg) 690

Potassium (mg) 440

Total Carbohydrates (g) 53

Dietary Fiber (g) 4

Sugars (g) 9

Protein (g) 20

%Daily Value*

14%

15%

10%

29%

13%

18%

14%

<

6 cheese pizzas provided:

2,160 calories

42 grams of fat

330 grams of carbohydrate

102 grams of protein

6 pepperoni pizzas provided:

2,220 calories

54 grams of fat

318 grams of carbohydrate

120 grams of protein

Pizza Preparation

1. Preheat oven to 400°F
2. Remove 6 Lean Cuisine pizzas from box (cheese or pepperoni specified on subject menu)
3. Remove plastic film
4. Place pizzas on aluminum foil covered cookie sheet
5. Cook 15 minutes
6. Tare plate on scale
7. Weigh each pizza individually and record each pizza weight
8. Add up total weight of pizzas
9. Cut each pizza into pieces
10. Place each pizza on separate plate and cover to keep warm
11. Await return of all uneaten pizzas to the kitchen (approximately 30 minutes later)
12. Tare plate on scale and weigh back all remaining pieces of pizza
13. Determine weight of pizza eaten by subtracting weight of remaining pizza from total pizza weight

Appendix E. Original Three Factor Eating Questionnaire from Stunkard & Messick¹

Part I: Please answer the following questions by circling True or False.

If part of the sentence is false, consider the whole sentence false and choose F.

- | | | |
|---|---|---|
| 1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal. | T | F |
| 2. I usually eat too much at social occasions, like parties and picnics. | T | F |
| 3. I am usually so hungry that I eat more than three times a day. | T | F |
| 4. When I have eaten my quota of calories, I am usually good about not eating anymore. | T | F |
| 5. Dieting is so hard for me because I just get too hungry. | T | F |
| 6. I deliberately take small helpings as a means of controlling my weight. | T | F |
| 7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry. | T | F |
| 8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat. | T | F |
| 9. When I feel anxious, I find myself eating. | T | F |
| 10. Life is too short to worry about dieting. | T | F |
| 11. Since my weight goes up and down, I have gone on reducing diets more than once. | T | F |
| 12. I often feel so hungry that I just have to eat something. | T | F |
| 13. When I am with someone who is overeating, I usually overeat too. | T | F |
| 14. I have a pretty good idea of the number of calories in common food. | T | F |
| 15. Sometimes when I start eating, I just can't seem to stop. | T | F |
| 16. It is not difficult for me to leave something on my plate. | T | F |
| 17. At certain times of the day, I get hungry because I have gotten used to eating then. | T | F |
| 18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. | T | F |
| 19. Being with someone who is eating often makes me hungry enough to eat also. | T | F |
| 20. When I feel blue, I often overeat. | T | F |
| 21. I enjoy eating too much to spoil it by counting calories or watching my weight. | T | F |
| 22. When I see a real delicacy, I often get so hungry that I have to eat right away. | T | F |
| 23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat. | T | F |
| 24. I get so hungry that my stomach often seems like a bottomless pit. | T | F |

- | | | |
|--|---|---|
| 25. My weight has hardly changed at all in the last ten years. | T | F |
| 26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate. | T | F |
| 27. When I feel lonely, I console myself by eating. | T | F |
| 28. I consciously hold back at meals in order not to gain weight. | T | F |
| 29. I sometimes get very hungry late in the evening or at night. | T | F |
| 30. I eat anything I want, any time I want. | T | F |
| 31. Without even thinking about it, I take a long time to eat. | T | F |
| 32. I count calories as a conscious means of controlling my weight. | T | F |
| 33. I do not eat some foods because they make me fat. | T | F |
| 34. I am always hungry enough to eat at any time. | T | F |
| 35. I pay a great deal of attention to changes in my figure. | T | F |
| 36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods. | T | F |

Part II: Please circle the number and response that is appropriate to you for each question.

37. How often are you dieting in a conscious effort to control your weight?
- | | | | |
|--------|-----------|---------|--------|
| 1 | 2 | 3 | 4 |
| rarely | Sometimes | usually | always |
38. Would a weight fluctuation of 5 lbs affect the way you live your life?
- | | | | |
|------------|----------|------------|-----------|
| 1 | 2 | 3 | 4 |
| not at all | Slightly | moderately | very much |
39. How often do you feel hungry?
- | | | | |
|-------------------|-------------------------|---------------------|---------------|
| 1 | 2 | 3 | 4 |
| only at mealtimes | Sometimes between meals | often between meals | almost always |
40. Do your feelings of guilt about overeating help you to control your food intake?
- | | | | |
|-------|--------|-------|--------|
| 1 | 2 | 3 | 4 |
| never | Rarely | often | always |
41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?
- | | | | |
|------|--------------------|----------------------|----------------|
| 1 | 2 | 3 | 4 |
| easy | Slightly difficult | moderately difficult | very difficult |
42. How conscious are you of what you are eating?
- | | | | |
|------------|----------|------------|-----------|
| 1 | 2 | 3 | 4 |
| not at all | slightly | moderately | extremely |

43. How frequently do you avoid 'stocking up' on tempting foods?
- | | | | |
|--------------|--------|---------|---------------|
| 1 | 2 | 3 | 4 |
| almost never | seldom | usually | almost always |
44. How likely are you to shop for low calorie foods?
- | | | | |
|----------|-------------------|-------------------|-------------|
| 1 | 2 | 3 | 4 |
| unlikely | slightly unlikely | moderately likely | very likely |
45. Do you eat sensibly in front of others and splurge alone?
- | | | | |
|-------|--------|-------|--------|
| 1 | 2 | 3 | 4 |
| never | rarely | often | always |
46. How likely are you to consciously eat slowly in order to cut down on how much you eat?
- | | | | |
|----------|-----------------|-------------------|-------------|
| 1 | 2 | 3 | 4 |
| unlikely | slightly likely | moderately likely | very likely |
47. How frequently do you skip dessert because you are no longer hungry?
- | | | | |
|--------------|--------|----------------------|------------------|
| 1 | 2 | 3 | 4 |
| almost never | seldom | at least once a week | almost every day |
48. How likely are you to consciously eat less than you want?
- | | | | |
|----------|-----------------|-------------------|-------------|
| 1 | 2 | 3 | 4 |
| unlikely | slightly likely | moderately likely | very likely |
49. Do you go on eating binges though you are not hungry?
- | | | | |
|-------|--------|-----------|----------------------|
| 1 | 2 | 3 | 4 |
| never | rarely | sometimes | at least once a week |
50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?
- | |
|---|
| 0 |
| eat whatever you want, whenever you want it |
| 1 |
| usually eat whatever you want, whenever you want it |
| 2 |
| often eat whatever you want, whenever you want it |
| 3 |
| often limit food intake, but often 'give in' |
| 4 |
| usually limit food intake, rarely 'give in' |
| 5 |
| constantly limiting food intake, never 'giving in' |

51. To what extent does this statement describe your eating behavior? “I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.”

1	2	3	4
not like me	little not like me	pretty good description of me	describes me perfectly

1. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res.* 1985;29:71-83.

Appendix F. Plasma Sample Collection Methods and Gut Hormone Analysis Protocols

Plasma Sample Collection Methods

Insulin and Glucose

- 5 mL whole blood collected in Red Top Tube with gel separator
- Tubes inverted several times immediately after collection
- Samples stood at room temperature 30 minutes
- Centrifuged 10 minutes at 3200 rpm
- Aliquoted ~2ml plasma into GLUCOSE and INSULIN labeled screw-cap vial
- Frozen at -20°C

Ghrelin

- 3 mL whole blood collected in EDTA Purple Top Plasma Tube
- Tube inverted several times immediately after collection
- Tube placed in ice bucket and remained there until centrifuged
- Spun in refrigerated centrifuge at 3200 rpm for 10 minutes
- Aspirated **at least** 300 mcl of plasma into 3mL screw-cap vial labeled for Ghrelin
- Sample placed on dry ice and transferred to -70 freezer

GLP-1

- 20 mcl DPP-IV inhibitor added to EDTA Purple Top Plasma Tube
- Collection tubes, with additives, were refrigerated up to 24 hours before collection
- 2 mL whole blood syringe drawn and immediately transferred to chilled vacutainer tube
- Tube inverted several times immediately after collection
- Tube placed in ice bucket and remained there until centrifuged
- Spun in refrigerated centrifuge at 3200 rpm for 10 minutes
- Aspirated **at least** 300 mcl of plasma into 3mL screw-cap vial labeled for GLP-1
- Sample placed on dry ice and transferred to -70 freezer

PYY₃₋₃₆

- 77 mcl aprotinin and 20 mcl DPP-IV inhibitor added to EDTA Purple Top Plasma Tube
- Collection tubes, with additives, were refrigerated up to 24 hours before collection
- 2 mL whole blood syringe drawn and immediately transferred to chilled vacutainer tube
- Tube inverted several times immediately after collection
- Tube placed in ice bucket and remained there until centrifuged
- Spun in refrigerated centrifuge at 3200 rpm for 10 minutes
- Aspirated **at least** 300 mcl of plasma into 3mL screw-cap vial labeled for PYY
- Sample placed on dry ice and transferred to -70 freezer after each visit

Gut Hormone Analysis Protocols

Glucagon-Like Peptide-1 (Using Millipore Kit; EGLP-35K)

Day One

___ Dilute Wash Buffer 1:10 with deionized water (___ ml DI water and ___ ml wash buffer)

___ Add 300 mcl of diluted Wash Buffer to each well in Plate #1

___ Incubate 5 minutes

Time Incubation Started Plate #1 _____

Time Incubation Ended Plate #1 _____

___ Decant and tap out excess Wash Buffer on Towels

___ Add 200 mcl Assay Buffer to wells a1, a2 (NSB wells)

___ Add 100 mcl Assay Buffer to wells a3 thru h12

___ Add 100 mcl 2pm GLP-1 to wells a3, a4

___ Add 100 mcl 5pm GLP-1 to wells a5, a6

___ Add 100 mcl 10pm GLP-1 to wells a7, a8

___ Add 100 mcl 20pm GLP-1 to wells a9, a10

___ Add 100 mcl 50pm GLP-1 to wells a11, a12

___ Add 100 mcl 100pm GLP-1 to wells b1, b2

___ Add 100 mcl QC1 to wells b3, b4

___ Add 100 mcl QC2 to wells b5, b6

___ Add 100 mcl Internal Control sample b7, b8

___ **Wells b9, b10, b11, b12 are BLANK!**

___ Add 100 mcl sample IN DUPLICATE to wells c1 through h12

Plate # X → Row C & D = _____

Row E & F = _____

Row G & H = _____

___ Shake plate gently to mix

___ Cover plate with sealer

___ Incubate at 4° C for 22 hours

Time Incubation Starts Plate #1 _____ Date _____

Time Incubation Expected to end (22 hours after start time) _____

Time Incubation actually ends Plate #1 _____ Date _____

Day Two

___ Decant liquid from plate #1 and tap out excess fluid on absorbent towels

___ Wash Plate #1 with 300 mcl Wash Buffer per well x 3 _____

___ Wash Plate #1 with 300 mcl Wash Buffer per well (this is the start of the 4th wash)

___ Incubate 5 minutes in plate washer

___ Wash Plate #1 with 300 mcl Wash Buffer per well (this is the 5th wash)

___ Tap out excess Buffer on absorbent towel

___ IMMEDIATELY add 200 mcl Detection Conjugate to each well of plate #1

__ Incubate at room temperature for 2 hours
Time Incubation Starts Plate #1 _____
Time Incubation Expected to end Plate #1 _____
Time Incubation Actually ends Plate #1 _____

__ Decant wells from plate #1 after 2 hour incubation
__ Wash plate #1 with 300 mcl diluted Wash Buffer x 3 ____ ____ ____
__ Tap out excess buffer on absorbent towels

Prepare Substrate for Plate #1:

__ Hydrate substrate with 1ml deionized water *right before use*.

Prepare 1:200 dilution:

__ Add 100 mcl hydrated substrate into 20 ml diluent
__ Add 200 mcl diluted substrate into each well of plates #1
__ Incubate minimum 20 minutes in the dark (upstairs in room with plate reader)
Time Incubation Starts Plate #1 _____
Time Incubation Expected to end Plate #1 _____

__ Monitor plate #1 for signal noise with the lowest point on std. curve
__ Incubate longer if needed – determine when sufficient fluorochrome generated
Time Incubation Actually ends Plate #1 _____

__ Add 50 mcl Stop Solution to each well in order that the substrate was added
__ Incubate Plate #1 for 5 minutes in the dark to arrest phosphatase activity
Time Incubation Starts Plate #1 _____
Time Incubation Actually ends Plate #1 _____

__ Read on plate reader with excitation/emission wavelength of 355nm/460nm

Ghrelin (Using Millipore Kit; GHRT-89HK)

Day One

1. Pipette 300 µl of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 200 µl of Assay Buffer in the Reference (Bo) tubes (5-6). Pipette 100 µl of Assay Buffer to tubes seven through the end of the assay.
2. Pipette 100 µl of Standards and Quality Controls in duplicate.
3. Pipette 100 µl of each sample in duplicate.
4. Pipette 100 µl of Ghrelin Antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
5. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.

Day Two

6. Hydrate the 125I-Ghrelin tracer with 13.5 ml of Label Hydrating Buffer. Gently mix. Pipette 100 µl of 125I-Ghrelin to all tubes.
7. Vortex, cover and incubate overnight (22-24 hours) at 4°C.

Day Three

8. Add 1.0 ml of cold (4°C) Precipitating Reagent to all tubes except Total Count tubes (1-2).
9. Vortex and incubate 20 minutes at 4°C.
10. Centrifuge, at 4°C, for 20 minutes at 2,000-3,000 x g.
11. Immediately decant supernatant from all centrifuged tubes except Total Count tubes (1-2). Drain tubes for 15-60 seconds (be consistent between racks), blot excess liquid from lip of tubes and count pellet using the gamma counter according to the manufacturer's instructions.

PYY 3-36 (Using Millipore Kit; PYY-67HK)

Day One

1. Pipette 200 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 100 µL of Assay Buffer in the Reference (Bo) tubes (5-6) and sample tubes 25 through the end of the assay. Do not add buffer to standard and QC tubes.
2. Pipette 100 µL of Matrix Solution to the Non-Specific Binding (NSB) tubes (3-4), Reference (Bo) tubes (5-6), Standard tubes (7-20), and Quality Control tubes (21-24).
3. Pipette 100 µL of each Standard (tubes 7-20) and Quality Controls (tubes 21-24).
4. Pipette 100 µL of each sample in duplicate.
5. Pipette 100 µL of PYY (3-36) Antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.

Day Two

7. Hydrate the 125I-PYY tracer with 13.5 mL of Assay Buffer and gently mix. Pipette 100 µl of 125I-PYY to all tubes.
8. Vortex, cover and incubate overnight (22-24 hours) at 4°C.

Day Three

9. Add 10µL of Guinea Pig Carrier to all tubes except Total Count tubes (1-2).
10. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes except Total Count tubes (1-2).
11. Vortex and incubate 20 minutes at 4°C.
12. Centrifuge, at 4°C, for 20 minutes at 2,000-3,000 x g.
13. Immediately decant supernatant from all centrifuged tubes except Total Count tubes (1-2). Drain tubes for 15-60 seconds (be consistent between racks), blot excess liquid from lip of tubes and count pellet using the gamma counter according to the manufacturer's instructions.

Appendix G. Williams Design Randomization

Sbj #	Study Visit			
	2	3	4	5
1	B 628	A 245	D 413	C 769
2	D 413	B 628	C 769	A 245
3	A 245	C 769	B 628	D 413
4	C 769	D 413	A 245	B 628
5	B 628	A 245	D 413	C 769
6	C 769	D 413	A 245	B 628
7	A 245	C 769	B 628	D 413
8	D 413	B 628	C 769	A 245
9	D 413	B 628	C 769	A 245
10	C 769	D 413	A 245	B 628
} Women				
11	B 628	A 245	D 413	C 769
12	A 245	C 769	B 628	D 413
13	A 245	C 769	B 628	D 413
14	C 769	D 413	A 245	B 628
15	B 628	A 245	D 413	C 769
16	D 413	B 628	C 769	A 245
17	C 769	D 413	A 245	B 628
18	D 413	B 628	C 769	A 245
19	A 245	C 769	B 628	D 413
20	B 628	A 245	D 413	C 769
} Men				

Treatment Codes:

413 = 0 g control

769 = 4 g mixed fiber

628 = 8 g mixed fiber

245 = 12 g mixed fiber