SATIETY, GLYCEMIC, AND GASTROINTESTINAL EFFECTS OF NOVEL FIBERS

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

ABBY SUSAN KLOSTERBUER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

JOANNE L. SLAVIN, PHD, RD, ADVISER

JUNE 2012

Acknowledgements

This dissertation would not have been possible without the guidance and support of my family, friends, and academic community. Without them, I would never have made it to this point, and I owe them the deepest gratitude.

I'd first like to thank my adviser, Dr. Joanne Slavin, for providing me with countless opportunities and the flexibility to pursue my interests. Her knowledge, advice, and ability to put things in perspective were invaluable throughout this process. Joanne has been an absolute pleasure to work with and I feel very lucky to have been a part of her lab.

I'd also like to thank my committee members – Dr. Will Thomas, Dr. Catherine Kotz, and Dr. Kim Robien – for their time, insightful feedback, and their ability to get me to think more critically about my research.

I thank my research subjects for enduring my constant emails and reminders, as well as the wonderful nurses and staff at the clinical research center. This research truly would not have been possible without their participation.

I also thank my wonderful lab mates, past and present. A special thanks to Holly for all her advice and for paving the way for a satiety study and saving me countless hours of work; Derek, for going through this whole process with me from the start and always being a source of support; Jackie, for her willingness to help and for keeping me optimistic; and Katie, for her help in the lab, without which I may never have finished!

Lastly, I thank my family and friends for their constant support and encouragement. I thank my Dad for his frequent emails and good vibes; my Mom for her phone calls and making sure I celebrated my successes along the way; my sister Sarah for her hilarious emails that provided a much needed distraction; Joe, for his patience and for motivating me to finish writing so we could start working on accomplishing a long list of fun things (and house projects); and my grandma, for instilling in me the importance of higher education and the pursuit of knowledge.

Abstract

Epidemiological studies have shown that dietary fiber consumption is inversely associated with body weight, and some research suggests that foods high in fiber increase satiety and reduce energy intake. The mechanism for this relationship is unknown, but may be related to changes in glucose, insulin, or gut hormone concentrations. Fiber may also benefit health by improving laxation, altering the gut microbiota, and increasing production of short chain fatty acids (SCFA). The following work describes two review articles, as well as an intervention study designed to help examine these effects.

The first review focuses on the benefits of dietary fiber in clinical nutrition. This allowed for evaluation of the physiological effects of different types and combinations of fiber in subjects on a controlled diet. In general, blends of fibers with varying physicochemical properties provided greater benefits and were better tolerated than single fiber sources.

Next, a systematic review of the effects of fiber intake on gut hormone concentrations examined the evidence for this relationship. Considerable variation was found in study design, population, fiber type and dose, which made comparisons difficult. Few studies reported a significant effect of fiber on gut hormone levels, and data suggest caloric load may have a more significant influence.

Lastly, a randomized, double-blind, crossover study examined the effects of three novel fibers with varying physicochemical properties on satiety, stool characteristics, and the role of gut hormones, glucose, and insulin in appetite regulation. On Day 1 of the study, healthy men and women consumed either a low-fiber control breakfast or 1 of 4

breakfasts containing 25 g fiber from soluble corn fiber (SCF) or resistant starch (RS), alone or in combination with pullulan (SCF+P and RS+P). Subjects rated satiety using visual analog scales (VAS), and blood samples were collected at various time points for 3 hours following breakfast. The fiber treatments did not influence satiety or energy intake compared to control. The RS+P treatment significantly reduced glucose, insulin, and GLP-1 concentrations.

To examine the effects of chronic fiber intake, subjects consumed the fiber treatments at home for 6 additional days, with a 3 week washout between periods. Stool samples were collected on Day 7 and tolerance was assessed following fiber intake on Day 1 and Day 6. Fiber did not alter stool weight or stool consistency. SCF reduced pH and increased total SCFA production compared to control, while RS+P increased the percentage of butyrate. Overall, fiber was well tolerated, although treatments containing pullulan tended to cause minor increases in symptoms. Both SCF treatments resulted in a significant shift in the microbial community.

Results from these studies confirm that different fibers vary in their physiological effects, and consuming fiber from a variety of sources may be most beneficial. Although increased satiety and improved bowel function are commonly reported benefits of fiber intake, it is clear that not all fibers exert these effects. In addition, the relationship between fiber and potential biomarkers of satiety remains unclear. Thus, it is important to evaluate the effects of different fibers in human studies to better guide recommendations for their use.

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Chapter One

LITERATURE REVIEW

Defining Fiber

The definition of fiber is a topic of considerable debate and differs among various organizations and regulatory agencies. The term "dietary fiber" was first coined in 1953, and referred to the non-digestible constituents that make up the plant cell wall.[1] Over the years, other definitions were developed based primarily on analytical methods or physiological effects. In 2002, the Institute of Medicine (IOM) proposed a definition for fiber which has been widely used in the United States. The IOM definition separated fiber into the following categories:[2]

- *Dietary fiber* is the non-digestible carbohydrates and lignin occurring intrinsically and intact in plants.
- *Functional fiber* encompasses isolated, non-digestible carbohydrates which exert beneficial physiological effects in humans.
- *Total fiber* describes the sum of dietary fiber and functional fiber.

More recently, the Codex Alimentarius Commission of the FAO/WHO Food Standards Program developed a new definition of fiber. The Codex definition states that "dietary fiber means carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans." Polymers with 3 to 9 monomeric units may also be included as fiber, depending on regulations at the national level. Similar to the IOM definition, fiber is divided into three categories:

- Edible carbohydrate polymers naturally occurring in the food as consumed.
- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a

physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

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

In both the IOM and the Codex definitions, synthetic or extracted/isolated fibers must demonstrate beneficial physiological effects, while fiber contained naturally in food does not need to meet this requirement.

Physicochemical Properties of Fiber

It is well recognized that different fibers exert different physiological effects. Traditionally, fiber has been classified as soluble or insoluble according to analytical methods agreed upon by the Association of Official Analytical Chemists (AOAC).[2] While these methods are useful, they have several limitations. Many methods rely on alcohol precipitation, yet certain carbohydrates, such as oligosaccharides and fructans, are soluble in ethanol yet indigestible by humans.[2] Thus, these constituents should be classified as fiber, but may go undetected by traditional methods. Resistant starch (RS) is another fiber component that may be classified incorrectly.[2]

In addition, there is increasing recognition that solubility is not the most physiologically relevant basis for classification. More recently, the physicochemical properties of viscosity and fermentability are being viewed as more meaningful characteristics.[2] Soluble fibers are typically described as being viscous and fermentable,

and having cholesterol-lowering properties. However, not all soluble fibers have a high viscosity (e.g. partially hydrolyzed guar gum and inulin), and low-viscosity, soluble fibers typically do not reduce cholesterol levels.[3] Likewise, insoluble fibers are often reported to be resistant to fermentation and have fecal bulking properties. However, some insoluble fibers are readily fermentable (e.g. soy polysaccharide and RS) and scientific evidence for increased stool weight with insoluble fiber is inconsistent.[3] Thus, it is not accurate to assume physiological benefits based on fiber solubility.

While viscosity and fermentability may be more predictive of the physiological effects of a fiber, determining these characteristics can also be problematic. Viscosity refers to the extent to which a fiber thickens or forms a gel when mixed with fluids. [4] While viscosity is fairly simple to measure in a solution, evaluating viscosity in the gastrointestinal (GI) tract is far more complex. Experimental data suggest viscosity is influenced by food processing, pH, and gut motility, and is likely to vary along regions of the GI tract.[4] Thus, it is difficult to determine how a viscous fiber might act in the body once consumed.

Fermentability refers to the extent to which fiber is metabolized by colonic bacteria, yielding products such as short chain fatty acids (SCFA), which may have beneficial effects on health. Unfortunately, current methodologies for evaluating fermentation patterns of dietary fiber have a number of limitations. *In vitro* methods may not provide a representative model of dynamic changes and metabolite usage in the human colon, while *in vivo* methods are limited to measurements of byproducts such as gas or SCFA concentrations in blood or feces.[5] The relationship between these

measurements and actual fermentation events in the colon is not well understood.[5] Furthermore, differences in host microbiota influence the extent to which a particular type of fiber will be fermented in an individual.

Due to the great variation in physical, chemical, and physiological properties of different fibers, classification of fiber into meaningful categories is difficult. This is further complicated by methodological limitations in assessing the physicochemical characteristics of different types of fiber. Thus, assigning health benefits to broad categories of fiber may not be accurate. This highlights the importance of evaluating the physiological effects of different types of fiber in human studies.

Specific Types of Fiber

Three different fibers were used in my research (Chapters 4 and 5). All are glucose polymers that are resistant to digestion, but differ in a number of properties. These fibers will be described in brief below.

Resistant Starch (RS)

The term "resistant starch" refers to starch and products of starch digestion that are not absorbed in the small intestines of healthy people and pass to the colon.[6] The molecular structure of RS is similar to that of digestible starch, consisting of D-glucose units connected via α-4 and α-6 glucosidic bonds.[7] Thus, RS is theoretically capable of digestion by pancreatic α-amylase, but resists breakdown due to a number of physical and chemical properties.[8] RS is typically classified into four different subtypes:[6]

- *RS1* is starch that is physically inaccessible and is common in whole or partially milled grains and seeds
- *RS2* is resistant due to a certain granular form that limits access by digestive enzymes. This occurs in starch granules that are ingested raw (uncooked), in which the starch is tightly compacted in a radial pattern. The most common sources of RS2 are green (unripe) bananas and raw potato starch.
- *RS3* includes retrograded starches. Retrogradation occurs when a starch is cooked and then cooled, allowing the formation of crystals that resist digestion. Starches with a high amylose (vs. amylopectin) content are more resistant to digestion and are also more likely to undergo retrogradation upon cooking and cooling.
- *RS4* is starch that has been chemically modified to increase functionality in processed foods. These starches include chemical bonds other than the α-1,4 and α-1,6 typically present in starch.

The RS used in this study is non-granular and not chemically modified. It is formed from heat-moisture treated high amylose maize starch and is classified as RS3.

Interest in the health effects of RS increased following epidemiological studies reporting a protective effect of starch on risk for colorectal and intestinal cancer.[9, 10] Since RS passes to the colon, it can be used as a substrate for bacterial fermentation, leading to the production of SCFA.[11] Fermentation of RS typically results in increased butyrate and decreased acetate compared to other fibers.[11] RS has been studied as a prebiotic and can promote the growth of bifidobacteria.[12] Consumption of RS can lower the postprandial glucose and insulin response to a meal and may be of benefit to

individuals with type 2 diabetes.[13, 14] Animal studies, as well as some human studies, have also shown a cholesterol-lowering effect for RS.[15, 16] However, there is currently little evidence that RS has a significant impact on satiety.[13, 17]

Soluble Corn Fiber (SCF)

The soluble corn fiber used in this research is produced from nutritive sugars obtained by hydrolysis of corn starch.[18] It is mainly comprised of α-1,6 linkages, but some α-1,4 bonds are also present.[18] Addition of 25 g SCF to a lemonade drink has been shown to reduce the glycemic and insulinemic response compared to a glucose control.[18] In another study, subjects consumed 12 g/d SCF or placebo in a crossover design for 14 days. Treatment had no effect on fasting glucose, insulin, or ghrelin levels.[19] Few studies have measured the effects of SCF on satiety. Consumption of two beverage preloads containing 11.8 g SCF each did not alter appetite ratings or energy intake compared to control, suggesting little satiating effect when provided as a beverage. [20]

Pullulan

Pullulan is a linear glucose homopolysaccharide composed of α-1,6 linked maltotriose subunits synthesized in large quantities via fermentation of starch by the fungus *Aureobasidium pullulans*.[21, 22] It is water-soluble and forms a viscous, colorless, clear, adhesive solution when dissolved.[21] Resistance to degradation by human digestive enzymes is due to the presence of α-1,6 bonds as well as to steric hindrance which limits access to α-1,4 linkages.[18] Some pullulanases are produced by bacteria present in the

human gut, and pullulan has been shown to promote the growth of several strains of bifidobacteria.[23] *In vitro*, pullulan fermentation by human fecal inocula increases gas production, decreases fecal pH, and increases production of butyrate and total SCFA when compared to a readily digestible maltodextrin control.[22]

Few controlled feeding trials have examined the effects of pullulan in humans. A study by Wolf, et al. found that 50 g pullulan mixed with a beverage attenuated the postprandial glucose response compared to a maltodextrin control. Peak glucose concentrations were 54% lower and positive incremental area under the curve (AUC) was 50% lower in subjects consuming pullulan.[24] Flatulence was increased, but all other GI symptoms were comparable to control.[24] A similar suppression in the glycemic response has also been observed with a dose of 25 g pullulan.[18, 23] Few studies have evaluated the effect of pullulan on satiety. However, acute ingestion of beverage containing 15 g pullulan reduced appetite compared to a maltodextrin beverage, although treatments were not matched for available carbohydrates. [25]

Recommendations for Fiber Intake

The Dietary Reference Intake (DRI) for fiber is 14 g/1000 kcal, based on the level of intake observed to reduce risk of coronary heart disease. This translates to approximately 25 g/d for women and 38 g/d for men. However, actual intake is much lower, with Americans consuming on average only 15 g/d.[26] The main sources of fiber in the American diet are white flour and potatoes; while these foods are not high in fiber, they are major contributors due to their widespread consumption.[3] Legumes and whole

grains provide a more concentrated source of fiber, but are consumed to a lesser extent. Addition of functional fiber to processed foods is becoming increasingly common in everything from cereal bars to sugar packets. However, research suggests that whole foods are more protective against chronic disease than isolated food components (e.g. dietary fiber or antioxidants).[3] The 2010 Dietary Guidelines for Americans highlight fiber as a nutrient of concern and recommend increased intake of whole foods that contain dietary fiber (e.g. whole grains, fruits, and vegetables).

Fiber and Health

In recent years, dietary fiber has been increasingly recognized as a health promoting agent. High fiber intake is associated with reduced risk for a number of chronic conditions, including cardiovascular disease, diabetes, and GI disorders.[27] In addition, observational studies suggest that dietary fiber may play a role in weight management.[28] Fiber consumption is inversely associated with body weight, body fat and BMI in cross-sectional studies, and fiber supplementation has been shown to improve weight loss in intervention trials.[29-33] However, it is unknown precisely how fiber exerts these effects.

Several mechanisms have been proposed as to how fiber may aid in weight regulation. Foods high in fiber have a lower energy density than foods rich in fat or refined carbohydrates and may displace calories in the diet.[34] Some fibers form viscous gels in the gut, which may delay gastric emptying, prolong small intestinal transit time, and improve the postprandial glycemic response, all of which may increase satiety.[35]

Fiber may also act through effects on gut hormone secretion. The digestive tract releases a large number of peptides in response to the nutritional state, and these act both peripherally and centrally to regulate energy balance.[36] Although it is known diet composition plays an important role, the effects of fiber on gut hormone release are not well understood.

Fiber consumption may also have additional health benefits beyond effects on weight management. Fiber tends to have a normalizing effect on bowel function, and the colon is inhabited by a large number of bacteria which metabolize fiber and produce SCFA. Certain fibers stimulate the growth of favorable bacteria which can increase the production of specific SCFA that promote a healthy colonic environment and may reduce the risk for conditions such as colon cancer.[37]

Satiety and Energy Intake

The term satiation refers to the satisfaction of appetite over the course of a meal and ultimately causes termination of eating. Satiety occurs as a result of eating and is defined as a state in which further eating is inhibited.[38] Appetite and satiety can be assessed either subjectively or by objective measurements such as food intake.

Subjective measures often take the form of visual analog scales (VAS) in which subjects rate hunger, desire to eat, and other appetite parameters. The scales consist of a horizontal line of varying length (usually 100-150 mm) anchored by statements indicating the extremes of the sensation of interest (for example, 'I am not hungry at all'/ 'I have never been more hungry').[39, 40] Subjects mark a line along the continuum that

corresponds to their current feeling, and the distance from the left end of the scale to the mark is measured for analysis.[41] Although the anchor statements can vary, the most commonly used terminology was developed by Rogers and Blundell, and includes 4 statements relating to hunger, desire to eat, prospective consumption, and fullness.[42] Numerous studies have found these ratings to be correlated with subsequent food intake.[39, 43, 44] VAS results have been found to be reproducible, but these measures are best used for within-subject comparisons due to variable response patterns among subjects.[41] (See Appendix A for an example of the VAS used in our research).

An alternate method for assessing appetite is to measure food intake, either short-term or long-term. There is also interest in identifying biomarkers (objective physiological measures) that indicate one's level of hunger or satiety. These include gastric distention and changes in hormone levels and neuronal activity.[43, 45] Unfortunately, while simple assays can be used to determine hormone levels, no such test exists to obtain a truly objective measure of satiety.

Many factors are thought to affect satiety and energy intake. Some studies have reported that certain macronutrients are more satiating than others. In particular, fat is often reported to be less satiating than carbohydrates,[46] although not all studies support this.[47] Alternatively, others suggest energy density and food volume are more important factors.[48] Related to this, the concept of "expected satiety" suggests that intake during a meal will depend on previous experiences with foods and beliefs about how much one needs to eat to be satisfied.[49] This effect persists despite manipulation of the energy density of the meal.[50] The palatability of a food may also be important, and studies

suggest higher palatability leads to greater food intake within a meal.[51] However, the influence of palatability on appetite ratings in the period following a meal is not consistent.[52] Sensory-specific satiety may also play a role. A food becomes less pleasant compared to other foods as it is consumed, and this contributes to the cessation of eating.[53] Increasing attention has also been given to the role of food form in satiety, and studies suggest that liquids are less satiating than solid foods.[54, 55]

It is also important to remember that people eat for a variety of reasons that have nothing to do with physiological feelings of hunger or appetite. Availability, cost, boredom, holiday traditions, stress, social circumstances and other factors all affect food intake.[35, 56] It is clear that environmental and psychological influences play an important role in appetite. While it is impossible to remove all these influences, clinical trials assessing satiety can help control for these factors by ensuring subjects are fasted prior to the meal and using a randomized, crossover design.

Fiber and Satiety: Mechanisms of Action

Several review articles have summarized the ability of dietary fiber to increase satiety and reduce energy intake.[35, 38, 57] However, variability in the literature on this topic makes generalizations difficult. The characteristics of the fiber (solubility, fermentability, viscosity, etc), dose, duration of intake, and how the fiber is consumed may all impact the level of satiety achieved. Fiber has diverse effects on the body, and a number of different mechanisms have been proposed as to how fiber may induce satiety and reduce energy intake.[57]

Foods rich in fiber have a lower energy density and greater volume compared to foods high in fat or sugar. Rolls proposed that humans may eat a constant weight or volume of food, regardless of caloric value.[58] Therefore, high-fiber foods may displace other calories in the diet and result in an overall reduction in energy intake. Additionally, foods rich in fiber often require increased time and effort to chew, which results in a slower rate of ingestion and subsequently enhanced satiety.[35, 59] Increased mastication also stimulates secretion of saliva and gastric juices and causes stomach expansion. Gastric distention has been shown to increase feelings of fullness and reduce energy intake, but these effects are short-lived.[60]

In addition to causing gastric expansion, fiber has other effects on the GI tract. In particular, viscous fibers delay gastric emptying, which may lengthen the duration of fullness.[61, 62] These fibers can also form a gel in the small intestine, which acts to delay nutrient absorption and slow the delivery of glucose into the bloodstream.[63, 64] As a result, the insulin response is decreased, and postprandial glucose and insulin levels remain more stable compared to a meal without fiber. This slow, sustained glucose response may lead to greater satiety, as discussed later.

Related to the rate of gastric emptying is the influence fiber has on the "ileal brake," a feedback mechanism that inhibits GI motility and secretions and thereby controls GI transit to optimize digestion and absorption.[65] Activation of this mechanism by ileal nutrient infusion has been shown to delay gastric emptying, increase small intestinal transit time, reduce energy intake, and increase feelings of satiety.[66-68] Ileal brake activation is affected by the caloric load and nutrient composition of the meal. Fat

has been studied most extensively and is considered the most potent activator of the ileal brake, though carbohydrates, protein, and fiber have also been shown to have an effect.[69-71] Gut hormones, such as polypeptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which are released in response to food intake, also stimulate the ileal brake and impact satiety.[72] In addition, fermentable fibers are metabolized by colonic bacteria to yield SCFA, and these may play a role in satiety in addition to having other health benefits.[37]

Intervention Studies

In recent years, an increasing number of human intervention studies have examined the relationship between fiber and satiety. Samra et al. found that a high fiber cereal (33 g insoluble fiber) significantly reduced the appetite AUC compared to an isocaloric low fiber cereal (1 g fiber).[73] In subjects consuming meals that differed only in fiber content (11 g vs. 3 g), researchers found that the high fiber meal induced late satiety (4-4.5 hours after the meal) compared to control.[74] The same group also reported greater fullness following a high fiber meal (12 g vs. 3 g), but no difference in hunger or desire to eat.[75]

Other studies have compared the effects of individual types of fiber on satiety. Addition of 5 g pectin to orange juice increased satiety for up to 4 hours after ingestion in US Army employees.[76] Similarly, consumption of 25 g pea fiber incorporated into wheat bread led to significantly greater fullness and reduced prospective consumption compared to 9 g fiber from control wheat bread. However, there were no differences in ratings of hunger and satiety.[77] Biscuits supplemented with 12.6 g fiber from barley reduced desire

to eat and increased satiety compared to a low-fiber biscuit matched for energy and macronutrients.[78]

Studies comparing different types of fiber provide evidence that not all fibers are equally satiating. Preloads containing 22-24 g fiber as soluble fiber dextrin, SCF, polydextrose, or RS were compared to a low-fiber control. Only soluble fiber dextrin suppressed appetite.[20] In another study, muffins containing 8-9.6 g fiber as corn bran, barley β-glucan plus oat fiber, RS, or polydextrose were compared to a low-fiber muffin. Corn bran and RS were most satiating, while polydextrose acted similar to control and had little effect on satiety.[79]

Several studies have examined different doses of the same fiber. Consumption of 8 g fenugreek fiber at breakfast increased satiety and fullness and reduced hunger and prospective food intake compared to control, while no effect was seen with a 4 g dose.[80] In a study by Willis et al, subjects consumed muffins with 0, 4, 8, or 12 g mixed fibers. Appetite ratings did not changes in a dose-dependent manner; fullness and satisfaction were greater following the 4 g fiber muffin compared to the 0 g fiber muffin, but no other differences among treatments were observed.[81]

Viscosity of the fiber may also be important. Several studies have found that more viscous fibers induce greater satiety than non-viscous fibers.[82, 83] In contrast, others have reported no effect[84] or the opposite effect of viscosity on satiety.[61] Still, others have reported no effect of fiber on satiety using a variety of types and doses of fiber.[84-87]

A number of studies support the theory that fiber can reduce energy intake. Burley et al. found that 29 g of sugar beet fiber reduced energy intake at lunch by 14%

compared to a low fiber control meal.[74] Pasman et al. reported that consumption of 40 g/d partially hydrolyzed guar gum (PHGG) for one week reduced energy intake by 19% compared to control.[88] Consumption of a high fiber cereal (33 g insoluble fiber) significantly reduced ad libitum intake at a subsequent meal compared to a low-fiber treatment.[73] Similarly, a cereal preload containing 41 g insoluble fiber also reduced energy intake at an ad libitum meal 1 hour later compared to a low fiber cereal (1 g fiber) matched for calories, macronutrients, weight, and volume. However, appetite ratings did not differ between meals.[89] Likewise, a low-calorie beverage supplemented with 8 g pectin reduced energy intake at lunch compared to an equicaloric control, although ratings of appetite did not differ.[90] Despite these findings, many others have reported no effect of fiber on energy intake.[78, 81, 84-86]

The available literature on the effects of fiber on satiety and/or energy intake shows mixed results. The studies vary greatly in experimental design, type/dose of fiber used, length of intervention, and choice of control, which makes generalization difficult. Overall, it is clear that that not all fibers are equally satiating, and the effective dose likely varies by fiber type. Furthermore, even within a certain type of fiber, differences in the source/supplier, method of processing, and the food matrix it is supplied in may influence the effect on satiety. This highlights the importance of testing the effects of different fibers on satiety to better guide their use in food products.

Glucose, Insulin, and Satiety

Mayer first proposed the glucostatic theory in 1953, and in it suggested that glucoreceptors in the hypothalamus and periphery sense the concentration of glucose in the blood and regulate short term energy intake accordingly.[91] A drop in blood glucose stimulates hunger, whereas an increase signals satiety. In support of this theory, many studies have observed declines in blood glucose prior to meal initiation, and that these declines are correlated with increased hunger and reduced satiety.[92] Similar theories have been proposed for insulin, whereby higher insulin concentrations are associated with increased satiety and reduced ad libitum energy intake.

A meta-analysis of 7 randomized feeding trials involving 136 subjects investigated the relationship between glucose and insulin concentrations and feelings of hunger and satiety, as well as subsequent ad libitum intake.[93] In normal weight individuals, higher postprandial insulin levels were associated with increased satiety and decreased hunger and energy intake. No associations were seen in obese subjects. In contrast, glucose levels were not significantly associated with any of the study outcomes.[93] Holt et al. studied the glycemic and insulinemic response to isoenergetic portions of 38 common foods and found that a higher insulin concentration at 120 min was associated with decreased energy intake.[94] However, infusion studies do not support a direct satiating effect for insulin in the absence of elevated blood glucose.[95, 96]

An alternative theory is that foods that produce a slower, sustained glucose response are associated with increased satiety as well beneficial effects on risk factors for chronic disease.[97, 98] This attenuated response typically describes foods with a low glycemic

index, and thus a *lower* incremental AUC for postprandial glucose. While low glycemic index foods are not necessarily high in fiber, increased fiber content is typically associated with lower glycemic index values.[99] In particular, viscous soluble fibers have been shown to lower the glycemic response to a meal.[100]

Granfeldt et al. found that 4 barley products (15-18% dietary fiber) elicited lower blood glucose responses and also resulted in significantly higher satiety ratings than a white bread control.[101] Similarly, Benini et al. fed subjects a high fiber meal (20 g/1000 kcal) or a low fiber meal (4 g/1000 kcal) in a crossover design and found that glucose AUC was significantly lower for the high fiber meal.[76] Although there were no differences in satiety scores, there was a quicker return of hunger in the low-fiber group.[102] Rigaud et al. gave subjects a preload with 7.4 g of added psyllium or placebo prior to a test lunch. Glucose AUC was significantly lower for the psyllium treatment, and this was accompanied with a significant reduction in hunger scores and subsequent energy intake.[33] These effects have also been reported with low doses of fiber. The addition of 5 g guar gum to a glucose drink significantly lowered the glucose and insulin response and increased satiety ratings in healthy adults.[103]

However, not all research supports this relationship. Keogh et al. fed subjects a meal including bread and muffins made with high-fiber barley flour (14.5 g fiber) or a low fiber white flour control. Despite a significantly lower glucose and insulin response with the barley products, there were no differences in any appetite ratings, nor were satiety scores correlated with plasma measures.[86] Subsequent ad libitum energy intake was also significantly greater for the barley meal.[86] However, the meals were not matched for

available carbohydrates, so this may have altered the glucose response. Similarly, Holt et al. examined the glycemic response to 7 isoenergetic breads with varying fiber content (1.8-33.5 g). There were no correlations between glucose levels and any measures of satiety. However, the breads differed in portion size and macronutrient content, which may have influenced satiety through other mechanisms.[104]

Therefore, it seems that while glucose and insulin are likely important in overall appetite regulation, they do not appear to be primary indicators of satiety. It is more likely they contribute to appetite control through complex interactions with other physiological and environmental factors.

Gut Hormones

A variety of peptides are released from the GI tract in response to the nutritional state. Traditionally, these were thought to act fairly locally to control gut function and facilitate digestion.[105] However, it is now well known that these hormones interact with brain centers to influence the regulation of appetite and energy expenditure in a pathway referred to as the gut-brain axis.[106] Although this pathway is widely recognized, the interactions between the gut and brain circuits are highly complex and the mechanisms by which gut hormones modify feeding behavior are under continuing investigation. While many more peptides and hormones are produced by the GI tract and may impact satiety, only two – ghrelin and GLP-1 – were chosen for investigation in my research. The following sections present what is known about the relationship between these hormones, food intake, and satiety. The complex mechanisms and neural pathways

regulating the effects of gut hormones on appetite are beyond the scope of this research, and will be discussed in brief. Further discussion of the effects of fiber consumption on gut hormone concentrations is presented in Chapter 3.

Ghrelin

Ghrelin is a 28 amino-acid peptide hormone identified as the endogenous ligand for the growth hormone secretagogue receptor.[107] The stomach is the primary site of ghrelin production, and accounts for approximately 75% of circulating ghrelin.[108] Some ghrelin is also formed in the small intestine, with much smaller amounts originating in other organs such as the lungs, kidney, and brain.[109] Ghrelin receptors are distributed widely throughout the body, including the brain, stomach, intestines, pancreas, gonads, thymus, and heart.[107] Circulating ghrelin exists in either an acylated or des-acylated form, but only the acylated form is considered active and able to bind the ghrelin receptor. However, since the majority of acylated ghrelin circulates bound to larger molecules and may be undetected in assays, total ghrelin is most commonly reported in the literature.[110]

Ghrelin exerts a number of biological actions, but most important is its role in appetite regulation.[107, 111] Unlike the more abundant satiety hormones, ghrelin is the only peripheral hormone known to be a powerful stimulant of appetite and food intake.[111] Numerous animal studies have reported significant increases in food intake following central or peripheral administration of ghrelin.[112-114] In humans, peripheral infusion of ghrelin increased energy intake by 28% and significantly increased hunger, although the

dose used was outside of the physiological range.[113] Plasma levels of ghrelin increase markedly prior to a meal and return to baseline within an hour of eating, suggesting a role in meal initiation.[115] Ghrelin may also play a role in long-term weight regulation as levels increase with weight loss and decrease with weight gain.[116]

In general, nutrient intake suppresses plasma ghrelin levels, although the caloric load and macronutrient content of the meal are important factors. When all other variables are equal, meals with higher energy content suppress ghrelin to a greater extent and for a longer period of time.[117] In a crossover design, healthy subjects were given three isocaloric meals with different macronutrient composition. All suppressed ghrelin compared to baseline, but the high carbohydrate meal produced a significantly greater decrease than the high fat and high protein meals.[118] However, the high protein meal suppressed ghrelin levels for the longest amount of time.[118] Al Awar et al. found that ghrelin decreased significantly following a high-protein or balanced meal, but no suppression was observed after an isocaloric fat meal. The high-protein meal again led to longer duration of suppression.[119] Monteleone et al. also reported significantly greater ghrelin suppression following a high carbohydrate meal compared to an isocaloric high fat meal.[120] Conversely, another study found that ghrelin decreased significantly after a carbohydrate-rich meal, but *increased* following a fat-rich or protein-rich meal containing similar amounts of calories.[121] Others have reported no difference in postprandial ghrelin levels among meals rich in each macronutrient.[122] Overall, it appears that carbohydrates cause the most pronounced decrease in ghrelin levels, but protein sustains this

suppression for a longer time. Studies measuring ghrelin in response to fiber consumption have yielded mixed results, and are described in Chapter 3.

The mechanisms by which ghrelin affects appetite are not fully understood. Ghrelin is thought to interact with neuropeptide Y (NPY) and agouti-related peptide (AgRP)-expressing neurons of the arcuate nucleus of the hypothalamus.[36] NPY and AgRP are orexigenic peptides and promote food intake.[36] The rate at which circulating ghrelin passes the blood brain barrier is low, which suggests that central activation is not the primary mechanism. Instead, peripheral ghrelin is thought to stimulate these brain regions via indirect pathways.[123]

Animal studies provide some insight into the mechanism of ghrelin action. Ghrelin receptors have been identified on vagal afferent neurons in rats, so ghrelin released in the stomach can send signals to the brain via the vagus nerve.[124-126] Vagotomy has been shown to reduce the ability of ghrelin to stimulate food intake, highlighting the importance of this pathway.[124] Ghrelin administration increases activity of AMP-activated protein kinase (AMPK) in the hypothalamus, which has been linked to increased food intake in mice.[127, 128] Uncoupling protein 2 (UCP2) appears to be required for the appetite-stimulating effects of ghrelin, since ghrelin administration has no effect on NPY levels or AMPK in UCP2-deficient mice.[123]

Despite increasing research in this area, the mechanism by which ghrelin is secreted from the stomach is not known. Secretion increases during fasting, but it is not clear what neural or hormonal factors are involved. The vagus nerve also appears

important for ghrelin synthesis, since blockage of the nerve prevents the rise in ghrelin observed during fasting.[129]

Hormones may influence the expression and secretion of ghrelin. In rats, administration of insulin inhibits ghrelin release from stomach tissue and reduces serum ghrelin levels.[130, 131] Similarly, in humans undergoing a euglycemic clamp study, insulin administration induced a significant decrease in plasma ghrelin.[132, 133] However, the relationship between insulin and ghrelin is less clear under physiological conditions with fluctuating glucose levels.[134] Other hormones such as glucagon, somatostatin, growth hormone, leptin, and estrogen have also been suggested to influence ghrelin secretion, but results of experimental studies are mixed.[135]

Some studies have shown that ghrelin increases secretion of gastric acid and gastric motility.[136, 137] Theoretically, the increased rate of gastric emptying could remove satiety signals from gastric distention and lead to intake of additional food. Overall, it appears that multiple mechanisms are involved in mediating the effects of ghrelin on appetite and food intake.

Glucagon-like Peptide-1

GLP-1 is produced primarily in the L-cells of the distal small intestine and is also expressed in the brain. GLP-1 is formed from the cleavage of proglucagon, which produces either a 36- or 37-amino acid molecule. Further N-terminal truncation is required to produce biologically active forms of the molecule.[105] The most abundant bioactive form in human plasma is GLP-1(7-36), although GLP-1(7-37) is equipotent and

will be referred to collectively as GLP-1. Both forms are rapidly cleaved and inactivated by dipeptidyl peptidase-IV (DPP-IV) such that only about 10-15% of secreted GLP-1 reaches the systemic circulation intact.[138] GLP-1 receptors are widely distributed throughout the body, located in pancreatic islet cells, the brain, heart, kidney, and GI tract.

GLP-1 has been implicated in the regulation of appetite and food intake. Infusions of GLP-1 have been shown to significantly increase satiety and decrease food intake in healthy normal weight and obese subjects, as well as in those with type 2 diabetes.[139-142] A meta-analysis by Verdich et al. concluded that infusion of physiological amounts of GLP-1 results in a 12% reduction in food intake.[142] These effects are similar in both lean and obese subjects, with reductions of 13.2% and 9.3%, respectively. Of interest, differences in plasma GLP-1 concentrations were correlated with differences in fullness, prospective food consumption, and hunger, but not with *ad libitum* energy intake.[142]

Due to these effects, GLP-1 mimetics and DPP-IV inhibitors have been studied as potential anti-obesity agents. Injection of GLP-1 mimetics is associated with significant weight loss, as well as improved blood glucose control.[143] Liraglutide, a GLP-1 analogue, has been shown to reduce energy intake, shorten meal duration, slow gastric emptying, and reduce body weight when injected daily for 4 weeks.[144] Importantly, these effects were not associated with increased nausea or adverse effects compared to placebo.

Upon food intake, GLP-1 levels increase in the plasma within minutes. In general, plasma levels rise within 10-15 minutes and peak by 40 minutes.[145] The

magnitude of the response is dependent on meal size and composition.[146] Carbohydrates are a strong stimulus of GLP-1 release, yet not all carbohydrates elicit the same response. After 75 g doses of oral glucose or fructose, GLP-1 showed the same time pattern of release, but the response was significantly greater after glucose.[147] An early study found that GLP-1 peaked most quickly (around 30 minutes) after a carbohydrate test meal, while maximum GLP-1 levels were not observed until up to 150 minutes after an equicaloric fat load.[148] Protein also stimulates GLP-1 release. In response to meals enriched in fat, carbohydrates, protein, or alcohol, the protein meal produced the highest response and the greatest AUC, followed closely by carbohydrates, fats, and finally alcohol.[149] Other studies have shown higher peaks with glucose compared to protein, but GLP-1 remains elevated longer following protein consumption.[150] Studies on GLP-1 response to fiber ingestion have been mixed, and are reviewed in Chapter 3.

The mechanism by which GLP-1 may influence satiety is not fully understood, but it is known that an intact GLP-1 receptor is required for an effect.[151] This receptor is expressed in the gut, brainstem, and hypothalamus, as well as on vagal afferent nerves. GLP-1 is also able to cross the blood-brain barrier, but it is unknown whether this pathway significantly contributes to appetite regulation.[114] Since most GLP-1 is rapidly degraded by DPP-IV, it is suspected that paracrine-like signaling via vagal afferents is a more significant pathway.

Since GLP-1 levels increase prior to nutrients reaching the site of GLP-1 secretion in the intestine, some researchers have suggested there is an interaction between the stomach and small intestine in appetite control and secretion of gut peptides. Steinert et

al. gave subjects an intra-gastric load or an intra-duodenal infusion of glucose or a mixed liquid meal.[152] The intra-gastric load resulted in significantly higher GLP-1 levels, increased fullness, and decreased hunger compared to the intra-duodenal infusion. This suggests a neural link between the stomach and small intestine that influences hormone release. Furthermore, stomach distention alone does not increase gut hormone levels,[153] so it appears the presence of nutrients is important in addition to mechanical signals.

Colonic fermentation may also influence GLP-1 levels. SCFA have been shown to trigger secretion of GLP-1 from colon cells *in vitro*, and mice lacking a SCFA receptor have impaired GLP-1 secretion.[154] In one study, rats were given resistant starch (a fermentable fiber) for 10 days, after which gut hormone levels were measured over a 24 hour period.[155] There was a significant increase in GLP-1 levels that was not due to meal effects. The authors concluded that fermentation of RS occurred throughout the day and was responsible for the increase in GLP-1. A similar effect was reported in humans. Hyperinsulinemic subjects consumed a high-fiber (20 g) cereal or placebo daily for one year.[156] GLP-1 and plasma SCFA concentrations were increased in the high-fiber group, but not until 9 months on the diet. This suggests that colonic bacteria adapted to the fiber over time, resulting in increased fermentation and changes in gut hormone levels.

Additionally, GLP-1 exerts several physiological effects that may influence appetite. GLP-1 is an incretin hormone, and amplifies the insulin response to glucose intake and is important for normal glucose tolerance in humans.[36, 111, 151] In the GI tract, GLP-1 inhibits gastric and pancreatic exocrine secretion as well as gastric emptying.[36, 111, 157] This is part of the "ileal brake effect" which may extend feelings of satiety.

Summary

The arrival of food to the GI tract causes the release of a number of peptides and hormones that act to optimize the digestive process and regulate appetite and energy expenditure. Ghrelin rises prior to a meal and acts via vagal afferents to stimulate appetite and energy intake. It also enhances gastric motility, which may act to promote additional food consumption. GLP-1 rises soon after a meal and has actions that oppose those of ghrelin. It acts via the vagus nerve to relay satiety signals and suppress appetite and also activates the ileal brake, thereby slowing gastric emptying and extending feelings of fullness. However, the precise role of gut hormones in the regulation of eating behavior is not clear. Levels of these hormones are influenced by meal composition, caloric load, body weight, gender, and other factors that complicate investigation of the relationship between these hormones and appetite.

Fiber and Laxation

Laxation refers to a number of GI effects, including increased stool weight and water content, decreased transit time, and symptoms such as loose stools, bloating, flatus, and abdominal discomfort.[158] A commonly reported benefit of dietary fiber intake is improved bowel function. This mainly refers to increasing fecal bulk, normalizing the number of bowel movements per day, and improving the ease with which a stool is passed.[26]

In general, stool weight increases as dietary fiber increases.[159] The increased weight is due to the physical presence of the fiber, water held by the fiber, and increased

bacterial mass from fermentation of the fiber.[160] A meta-analysis by Cummings et al. found that stool weight was highly correlated with fiber intake, and low stool weight was associated with increased risk for colon cancer.[161] Several studies have suggested that consuming enough fiber to achieve a stool weight of >150 g/d may improve transit time and reduce risk of colon cancer.[161-163] However, different types of fiber vary in their bulking capacity. In general, insoluble fibers exhibit greater bulking properties than soluble fibers. Wheat bran is considered the "gold standard" and increases stool weight by around 5 g/g wheat bran consumed. Cellulose, another insoluble fiber, has been shown to increase stool weight by 3 g/g consumed.[164] In contrast, other fibers have minimal effects on stool weight. For example, consumption of 20 g/d inulin for 3 weeks had no effect on stool weight in healthy male volunteers.[165]

In addition to increasing fecal bulk, fiber may improve other aspects of bowel function. "Normal" bowel function encompasses a wide range of stool frequency, and is often defined as bowel movements between three times per day and three times per week.[166, 167] Constipation can be defined as three or fewer bowel movements per week, and is characterized by hard, difficult to evacuate feces.[168] This occurs as a result of increased water absorption as the feces remains in the colon for longer periods of time. Alternatively, diarrhea is characterized by watery stools and greater than three bowel movements per day.[169] Fiber tends to normalize bowel frequency to one bowel movement per day and transit time to 2 to 4 days.[3]

Tolerance is an important consideration, since side effects such as flatulence and bloating may discourage people from consuming fiber. Tolerance is typically assessed

via questionnaires, where subjects report subjective ratings of a wide variety of symptoms. However, there is little standardization in questionnaires among studies, which can make comparisons difficult (Appendix B has a sample of the questionnaire used in our research). Certain fibers tend to cause intolerance at fairly low levels. For example, bloating was significantly more intense during consumption of 2.5 or 5 g/d short-chain fructooligosaccharides (FOS) compared to placebo, although frequency of symptoms did not differ.[170] In contrast, other fibers such as polydextrose and RS may be tolerated at very high levels (over 40 g/d).[171] Tolerance appears to depend on characteristics of the fiber (chain length, molecular structure, fermentability) as well as how it is provided (liquid or solid meal; as a sole source of fiber or as part of a blend). In addition, individual differences, including microbiota composition, also play a role.

Gut Microbiota

The human gut is inhabited by a large and diverse population of microorganisms. It is estimated that the colon contains roughly 10^{14} bacterial cells, and these organisms make up 40-55% of solid stool matter.[6] The microbiota is involved a number of processes, including immunity, fermentation of non-digestible dietary components to SCFA, protein metabolism, biotransformation of bile acids, and vitamin synthesis.[172] Over 50 bacterial phyla have been identified, but the human gut is dominated primarily by Bacteroidetes and Firmicutes.[173] Proteobacteria, Actinobacteria (which contains Bifidobacteria spp.) and others make up a minor proportion of the total.[173] While the major groups that dominate the microbiota are consistent among individuals,

considerable interindividual variation occurs when the microbiota is examined at the genera or species level. The number of species reported varies in different studies, but is estimated to be between 500 and 1,000.[174]

Due to certain physiological effects, some bacteria are thought to be more beneficial or detrimental to human health. Although not the most abundant colonic species, *Bifidobacterium* and *Lactobacillus* have received the most attention as health promoting due to their ability to produce lactic acid, which can lower colonic pH and help prevent the growth of pathogenic bacteria such as *Clostridium*.[170] While this classification of "good" and "bad" bacteria may be overly simplistic, it does provide some basis for identifying foods that may modulate the microbiota to influence health.

Prebiotics are defined as "non-digestible substances that when consumed provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria."[175] Currently, only a few types of fiber fulfill the criteria for classification as prebiotics. However, others have been shown to have some prebiotic effects.

Several studies have demonstrated the prebiotic ability of different dietary fibers. Gibson et al. fed subjects 15 g of inulin or oligofructose (OF) for 15 days and collected stool samples for the last 5 days on the diet.[176] Compared to a sucrose control, treatment significantly increased Bifidobacteria counts but had no effect on total bacteria, indicating selective stimulation.[176] Clostridia counts were reduced after OF feeding, but not after inulin.[176] Similar results were achieved using the same fibers, but half the dose (7.5 g/d).[177] In a crossover study, subjects consumed biscuits containing 6.6 g FOS and

3.4 g PHGG per day or placebo biscuits for 21 days.[178] Numbers of *Bifidobacterium* spp. in feces were significantly increased following the experimental biscuits, but no changes in *Bacteroides* spp., *Clostridium* spp., or *Lactobacillus* spp. were observed.[178]

Bouhnik et al. studied the effects of various non-digestible carbohydrates on gut microbiota composition in humans. Consumption of 10 g/d RS3 (debranched retrograded tapioca maltodextrin) for 7 days was bifidogenic compared to placebo. Counts of total anaerobes, *Lactobacillus, Bacteroides,* and enterobacteria were unaffected.[170] This study provides evidence that microbiota can be altered after a relatively short (7 day) feeding period. However, no changes in bacterial counts were detected after 3 week treatment with a lower (2.5 g/day) dose of FOS, suggesting that higher doses are necessary for an effect.[179]

Despite the high interest in prebiotics, there is now increasing recognition of functional redundancy in the gut microbiota, and it appears that many different species of bacteria perform similar metabolic functions. Thus, although certain groups may be indicative of a healthier microbiota, it is likely that different microbial profiles correlate with similar health effects. More recently, interest has shifted away from measuring specific bacteria in isolation (e.g. bifidobacteria and lactobacilli) and more towards looking at the entire microbial community as well as its metabolic activity.

Recent evidence suggests that the gut microbiota may play a role in obesity. Several studies have found that the microbiota of obese individuals differs from that of lean subjects, with obese subjects generally having fewer *Bacteroidetes* and greater *Firmicutes*.[180-182] Ley et al. placed 12 obese individuals on low-calorie diets (low-

carbohydrate or low-fat) and gut microbiota was measured over 1 year. Shifts in the bacterial population towards the lean profile were strongly correlated with weight loss.[183] Similarly, in obese adolescents following a weight loss diet, individuals who lost the most weight had more total bacteria and a distinct microbial profile at baseline compared to individuals who had only minor weight loss.[184] This suggests the gut microbiota may play a role in weight loss success, although the mechanisms for this effect were not examined.

Animal studies provide some insight into the mechanism by which the microbiota may influence energy balance. Turnbaugh et al. transplanted microbiota from lean and obese mice into wild-type mice. After two weeks, the mice with the obese mouse microbiota had increased fat and extracted more energy from food as compared to the mice with the lean microbiota.[185] In another study, germ-free mice were colonized with one or two bacterial strains. Colonization with two species led to greater energy harvest from food and a greater increase in body fat compared to mice colonized with only one strain.[186] This highlights the role of cross-feeding among bacteria and the importance of studying the entire microbial community.

Researchers have also studied changes in the composition and metabolic activity of the gut microbiota in animal models of diet-induced obesity, which is thought to more closely resemble development of obesity in humans. Mice fed a high-fat, low fiber diet became obese and exhibited a reduction in saccharolytic bacteria.[172] This change in microbiota was also accompanied by increased intestinal permeability, systemic inflammation, and the development of insulin resistance and type 2 diabetes.

Interestingly, these effects were reversed when the mice were fed oligofructose (a fermentable fiber).[187] In addition, mice fed a high-fat diet experienced an increase in *Firmicutes* and a reduction in *Bacteroidetes*,[188] which has also been reported in genetic models of obesity.

The effect of diet interventions on microbiota seems to be short-lived. Following cessation of dietary treatment, bacteria levels tend to return to baseline within one or two weeks. In addition, baseline bacterial counts are an important determinant of the magnitude of the effect that can be achieved by dietary intervention. In the case of prebiotics, individuals with low initial bifidobacteria counts typically have the most significant changes in bifidobacteria, while little effect is seen in subjects with normal or high levels.

In summary, the gut microbiota clearly plays an important role in human health and energy balance, although much remains unknown about the complexities of this relationship. Recent research has found that the gut microbiota is altered in obesity, and that the microbial population can be altered by diet. Dietary fiber is fermented by colonic bacteria, and may have beneficial effects on the microbial community. However, additional research is needed to define a healthy microbiota and determine dietary interventions to improve host health.

Short Chain Fatty Acids

SCFA are produced in the colon from bacterial fermentation of non-digestible carbohydrates. In addition to SCFA, this process also yields gases (CO_2, CH_4, and H_2), and heat.[37, 189] SCFA are organic molecules containing 1 to 6 carbon atoms, the most abundant of which are acetate, propionate, and butyrate.[189] SCFA are produced in a relatively constant ratio (60:20:20 acetate:propionate:butyrate) in both the proximal and distal colon, but this distribution can be altered by dietary and other changes.[190]

SCFA are important for colonic health, and increased concentrations are associated with reduced risk for diseases such as irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease, and cancer.[191] Patients with inflammatory bowel disorders such as ulcerative colitis have been shown to have decreased concentrations of colonic SCFA when compared to normal subjects.[192] SCFA enemas and consumption of various fermentable fibers have been shown to improve symptoms of these conditions.[193, 194]

Increased amounts of SCFA reduce pH, which inhibits growth of potential pathogens such as clostridia.[195] SCFAs also decrease solubility of bile acids and aid in the absorption of minerals such as calcium and magnesium.[196-198] The major SCFAs each exert some unique physiological effects. Acetate, the most abundant SCFA, is rapidly absorbed and metabolized by the liver, muscle, and other tissues for energy.[195] It serves as a substrate for fatty acid synthesis, and has been shown to increase colonic blood flow and enhance ileal motility.[199] Acetate may also be converted to butyrate by gut bacteria.[200] Propionate is suggested to inhibit cholesterol synthesis and have a

hypolipidemic effect, but the results of human trials have been inconsistent.[37, 198, 201] Butyrate is considered the most important SCFA for colonic health. It is the preferred fuel for colonic epithelial cells, which metabolize 70-90% of the butyrate produced.[189] Butyrate is also thought to have a role in preventing colon cancer. While butyrate stimulates proliferation of normal colonocytes, it suppresses that of cancerous cells by promoting apoptosis and inducing differentiation of neoplastic colonocytes.[202]

SCFA production varies according to the number and type of gut microbiota, as well as substrate availability. The highest concentration of bacteria, and hence the greatest amount of fermentation, occurs in the proximal colon. This is also the site of greatest substrate availability. The concentration of total SCFAs is estimated to be 70-140 mM in the proximal colon, which drops to only 20-70 mM in the distal colon.[189] Low concentrations of SCFA in the distal colon have been implicated in a number of GI disorders and cancer.[191] Thus, it is of interest to identify methods to increase total SCFA production as well as delivery of SCFA to the distal colon.

Since diet provides the fermentation substrates, dietary factors can have a significant influence on SCFA production. Certain fibers are fermented more quickly or slowly than others, and there is increased interest in identifying slowly fermented fibers which may provide health benefits in the distal colon.[203] Soluble fibers, such as pectins, hemicelluloses, and gums, are typically fermented to a greater extent than insoluble fibers such as cellulose or wheat bran.[37, 204] Although insoluble fiber is more resistant to fermentation, it may indirectly increase SCFA production by increasing fecal bulk and carrying fermentable sugars and starches to the colon.[205]

The assessment of colonic fermentation and SCFA production in controlled human feeding studies is generally limited to measurement of fecal SCFA. However, this is complicated due to the fact that greater than 95% of SCFA are absorbed in the colon.[189] While fecal measurement of SCFA is useful in detecting changes in excretion, it is limited in its ability to assess changes in production.[189]

Several human intervention studies have measured fecal SCFA in response to dietary interventions using RS, and most have found increases in butyrate production. Jenkins et al. fed subjects 21.5 g RS2 or 27.9 g RS3 incorporated into cereal and muffins for two weeks and reported an increase in butyrate concentration and the butyrate:total SCFA ratio versus a low fiber control.[205] Similarly, Phillips et al. fed either a low or high RS diet from a variety of sources, dosed based on energy needs, for 3 weeks.[11] The high-RS diet resulted in a significant increase in fecal butyrate concentrations and significantly greater daily excretion of butyrate, acetate, and total SCFA as compared to the low-RS diet.[11] In a recent study by Fastinger et al., healthy adults were fed 7.5 or 15 g of maltodextrin or resistant maltodextrin (RM) for three weeks.[206] After RM treatment, the proportion of acetate was significantly decreased and the proportion of butyrate increased as compared to maltodextrin. This effect remained throughout the washout period, possibly due to an increase in *Bifidobacterium* observed in the RM group. Bird et al. fed subjects a diet containing a novel high-amylose, high-RS barley (45 g fiber/day), whole-wheat cereals (32 g fiber/day) or refined cereals (21 g fiber/day) for 4 weeks in a crossover design.[207] Consumption of the novel barley resulted in significant increases in fecal concentration and excretion of butyrate compared to the other two treatments.

The identification of foods that increase production of SCFAs, namely butyrate, may be beneficial for colonic health. While many sources of fiber have been found to raise SCFA levels *in vitro* and in animal models, little data is available on the effects in humans.

Conclusion

Intake of dietary fiber clearly has implications for human health. Evidence suggests fiber may aid in weight regulation, possibly by inducing satiety and reducing energy intake. This effect may be mediated by gut hormones such as ghrelin and GLP-1, which are released in response to the nutritional state and interact with appetite centers in the central nervous system to regulate energy balance. However, little is known about the effects of fiber on gut hormone release. Fiber consumption can also alter the gut microbiota and produce beneficial changes in the colon, although the effects of different fibers on these parameters in humans are not well studied.

To better understand the effects of fiber on these outcomes, several approaches were taken. First, the benefits of fiber in clinical nutrition were reviewed. This allowed for evaluation of the physiological effects of different types and combinations of fiber under controlled settings. Next, a systematic review of the effects of fiber intake on gut hormone concentrations was completed to examine the evidence for this relationship. Lastly, a human study which examined the effects of three novel fibers with varying physicochemical properties on satiety, stool characteristics, and the role of gut hormones, glucose, and insulin in appetite regulation is presented. Chapter 4 describes the effects on

satiety and blood parameters following acute intake of the fibers, while chapter 5 discusses the GI effects following chronic fiber intake.

Chapter Two

BENEFITS OF DIETARY FIBER IN CLINICAL NUTRITION*[1]

*Publication Citation

Klosterbuer A, Roughead ZF, Slavin JL. Benefits of Dietary Fiber in Clinical Nutrition. Nutr Clin Pract. 2011;26(5):625-635.

[1] This review was supported by Nestlé Nutrition.

Chapter Synopsis

Dietary fiber is widely recognized as an important part of a healthy diet and is a common addition to enteral nutrition (EN) formulas. Fiber sources differ in characteristics such as solubility, fermentability, and viscosity, and it is now well known that different types of fiber exert varying physiological effects in the body. Clinical studies suggest fiber can exert a wide range of benefits in areas such as bowel function, gut health, immunity, blood glucose control, and serum lipid levels. Although early clinical nutrition products contained fiber from a single source, it is now thought that blends of fiber from multiple sources more closely resemble a regular diet and may provide a greater range of benefits for the patient. Current recommendations support the use of dietary fiber in clinical nutrition when no contraindications exist, but little information exists about which types and combinations of fibers provide the relevant benefit in certain patient populations. This article summarizes the different types of fiber commonly added to EN products and reviews the current literature on the use of fiber blends in clinical nutrition.

Introduction

Dietary fiber is widely recognized as beneficial for human health, and increased intake of certain fibers has been shown to improve serum lipid concentrations, promote regularity of bowel movements, improve blood glucose control, aid in weight maintenance, and improve immune function.[3] Providing adequate and appropriate nutrition is especially important in the clinical setting, and fiber supplementation during this time may benefit patient health outcomes as well as quality of life. It is well known that the term *fiber* encompasses compounds with a wide range of physicochemical properties and physiological effects. Therefore, it may be possible to select specific fibers or combinations of fibers that may be most beneficial for certain patient populations. The use of fiber in clinical nutrition products should be evidence based, both in fiber source and dose provided. This review provides a description of the most commonly supplemented fibers, as well as a summary of the current research on the use of fiber blends in clinical nutrition.

The importance of nutrition in the clinical setting has received increasing attention in the past several decades. Patients unable to consume an oral diet or patients unable to meet their nutrition needs with food alone benefit from the use of enteral nutrition (EN). The provision of EN is associated with improved clinical outcomes and prevention of adverse changes in gut integrity.[208] Polymeric standard formulas, which are designed for patients with normal gastrointestinal (GI) function, are the most commonly used source of nutrition for patients requiring EN. In the past, enteral formulas were often designed to be fiber free because of concerns with tube occlusion, as well as

the belief that bowel rest may be beneficial for the patient. Although fiber-free formulas are appropriate for patients requiring a low-residue diet for diagnostic procedures or surgery, fiber supplementation is useful for preventing some negative side effects associated with EN. Specifically, alterations in bowel function are commonly associated with the use of fiber-free enteral formulas. In the acute and hospital setting, diarrhea is the most common complaint among patients on fiber-free formulas, occurring in up to 68% of intensive care unit (ICU) patients.[209] In contrast, constipation and need for laxatives are more common side effects of long-term and home enteral feeding.[210]

Fiber has the potential to exert a number of health benefits for the tube-fed patient. Fiber increases the water content and bulk of alimentary contents, normalizing the progression of stool through the intestine.[211] The increased bulk is due to the presence of fiber itself, the water held by the fiber, and increased bacterial mass from fiber fermentation.[3] In this manner, dietary fiber contributes to improving the regularity of bowel movements, facilitating the generation of soft-formed stools, and improving ease and control of stool evacuation. In the distal bowel, certain fibers are fermented by bacteria to yield short chain fatty acids (SCFAs), including butyrate, acetate, and propionate. Butyrate is the major energy source for colonic epithelial cells and is important for normal cell proliferation and differentiation.[191] SCFAs also help regulate absorption of water and electrolytes and can help reverse fluid secretion in the ascending colon associated with enteral feeding and may thus be useful in the control of diarrhea in this population.[212]

In addition to the effects on stool frequency and consistency, fiber exerts further benefits in the colon. Approximately 10^{14} microorganisms representing >500 bacterial species are present in the typical adult colon.[213] The balance between beneficial and pathogenic bacteria is important to maintaining normal intestinal physiology, as this balance has direct effects on immune function and nutrient digestion and absorption. A healthy and diverse microbiota acts as a barrier to potentially pathogenic microorganisms (PPMs). However, this balance can be upset during illness, when the microbiota may be altered by stress, diet changes, medications, or pathology. By definition, a prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microbiota that confers benefits upon host well-being and health."[214] Most studies have focused on an increase in bifidobacteria and/or lactobacilli, but there is also interest in assessing changes in the entire bacterial population.[215]

Prebiotic fibers may exert a number of health benefits, related to both the increase in health-promoting bacteria, as well as the increase in SCFAs as a result of bacterial fermentation of fiber. Intake of prebiotic fibers can stimulate mucin production, possibly due to a drop in pH caused by SCFA production.[216] Increased mucin improves mucosal barrier function and helps to reduce translocation of bacteria across the epithelial wall to the bloodstream.[217] This reduction in pH can also decrease the solubility of bile acids, help increase mineral absorption, and inhibit the growth of bacteria such as *Clostridium difficile.*[218] In addition, attachment of beneficial bacteria, such as lactobacilli or bifidobacteria, to the epithelial wall can prevent adherence of PPM (e.g., *Escherichia coli*, *Salmonella typhimurium*), as well as cause competition for nutrients.[219]

Bifidobacteria may also interact with immune cells and antigens to improve host immune response.[220] SCFAs, especially butyrate, have also been shown to exhibit anti-inflammatory properties.[221]

Soluble, viscous fibers have been found to have a number of metabolic benefits. The presence of these fibers increases the viscosity of intestinal contents and can interfere with absorption of bile acids in the ileum, causing an increase in fecal bile acid loss. As a result, low-density lipoprotein (LDL) cholesterol is removed from the blood by the liver and converted into bile acids, causing a reduction in serum cholesterol.[222] Viscous fibers may also attenuate the glucose and insulin response to nutrient ingestion.[223] These fibers can increase the viscosity of stomach contents, thus delaying gastric emptying. In addition, the increased viscosity of the chyme slows the rate of intestinal glucose absorption and reduces the need for insulin.[223]

A growing number of clinical trials have evaluated the effects of adding fiber to enteral formulas. Most studies have focused primarily on bowel function, whereas other outcomes, such as changes in microbiota, glucose and insulin response, and SCFA, have been examined to a lesser extent. In general, the effects of fiber on GI function in clinical trials have not been consistent, in part because of differences in population (healthy volunteers vs. patients), length of intervention, fiber source, fiber dose, and lack of a universal definition for diarrhea and constipation. However, a recent meta-analysis including 51 studies on fiber-supplemented enteral formulas found that fiber significantly reduced the incidence of diarrhea in the acute setting, especially in populations with a high baseline incidence of diarrhea.[224] In addition, in both healthy subjects and patients,

fiber significantly reduced stool frequency when high and increased frequency when low, which is supportive of a moderating effect of fiber on bowel function.[224]

Summary of Current Recommendations

The Dietary Reference Intakes (DRIs) current recommendation for adults is to consume 14 g dietary fiber per 1000 kcal ingested, which translates into a daily intake of about 25 g/d for women and 38 g/d for men. This value is based on protection from heart disease from fiber consumption observed in epidemiological and clinical data.[3] However, average fiber intake in the United States is about half the recommended value, and Americans typically consume only 15 g per day. A number of organizations that promote research and organize consensus statements regarding clinical nutrition have issued guidelines for the use of fiber in clinical nutrition. The Fiber Consensus Panel, which met in 2004, recommended the inclusion of fiber in the diets of all patients if no contraindication exists, based on benefits on diarrhea, constipation, and feeding tolerance.[225] In 2006, the European Society for Clinical Nutrition and Metabolism (ESPEN) recommended the use of fiber in EN and a mixture of bulking and fermentable fibers for all non-ICU patients.[226] The American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) and the Society for Critical Care Medicine (SCCM) also recognize the benefits of fiber for laxation, improvement in blood lipid concentrations, and reduction of the glycemic response[227] and published nutrition support therapy guidelines for adult critically ill patients in 2009.[228] These recommendations are summarized in **Table 2-1**.

Fiber Sources Contained in Enteral Formulas

Enteral nutrition formulas are supplemented with fibers from a variety of sources, either alone or as part of a mixture. As mentioned previously, fibers from different sources may vary in a number of physical and chemical characteristics. Although fiber was traditionally classified according to solubility, additional properties, such as viscosity and fermentability, are now being recognized as more important in terms of specific physiological benefits (see **Table 2-2**).[2] In general, soluble fibers are more completely fermented and have a higher viscosity than insoluble fibers. However, not all soluble fibers are viscous (e.g., acacia gum, partially hydrolyzed guar gum), and some insoluble fibers may be well fermented (e.g., finely ground soy polysaccharides). A description of the most common fiber sources used in clinical nutrition is included below.

Soy Polysaccharides

Soy polysaccharides are a fiber source obtained from soy cotyledon and consist of a number of fiber components, including cellulose, hemicelluloses, lignin, and pectin-like molecules. Although soy polysaccharides are typically 75%–85% insoluble, they have been shown to be highly fermentable in humans, likely because of their small particle size.[229] Fermentation of soy fiber results in high proportions of propionate and butyrate compared with other fibers.[230]

Soy fiber was a popular addition to early enteral formulas, as it was nonviscous and easy to incorporate into products without altering quality or causing tube clogging. Acute supplementation with soy fiber has been shown to reduce duration of diarrhea in

infants and toddlers compared with a fiber-free formula.[231, 232] Long-term use of a soy polysaccharide–supplemented enteral formula in constipated children increased daily stool frequency and moisture, suggesting some benefits on constipation.[233]

However, the effects of soy fiber supplementation are less clear in adults. In healthy subjects, consumption of a liquid diet with added soy fiber has been found to have inconsistent effects on transit time, stool weight, and stool frequency, but is generally less effective than a self-selected diet.[234-236] Clinical trials also show minimal effectiveness when soy fiber is used as the sole fiber source. In a crossover study among head-injured patients (n = 9) receiving tube feeding with a fiber-free formula or one supplemented with soy polysaccharide, fiber had no impact on stool weight, consistency, or incidence of diarrhea.[237] Similarly, compared with a fiber-free feed, use of a formula supplemented with 21 g/L soy polysaccharide had no effect on frequency of diarrhea in ICU patients (n = 91).[238] Furthermore, in acutely ill patients receiving EN (n = 100), use of a soy polysaccharide–supplemented formula (14.4 g/L) did not significantly lower the incidence of diarrhea.[239]

In contrast, use of an enteral formula supplemented with 10 g soy polysaccharide/500 mL for 5 days resulted in a significantly lower diarrhea score compared with a fiber-free control in postoperative patients (n = 60) who had undergone antibiotic treatment.[240] Similarly, in elderly tube-fed patients (n = 148), use of a formula with 13.2 g soy fiber/L decreased the rate EN-associated diarrhea compared with fiber-free control, independent of antibiotic use.[241]

These studies suggest that the effects of soy fiber supplementation may vary according to patient population and medication use. It is possible that because of its high fermentability, soy polysaccharide may be less effective at altering laxation compared with other insoluble fibers. Although enteral formulas with soy fiber as the single fiber source are generally no longer used, soy polysaccharide is commonly added as a source of insoluble fiber in mixed fiber blends.

Partially Hydrolyzed Guar Gum

Partially hydrolyzed guar gum (PHGG) is a soluble fiber produced by controlled partial enzymatic hydrolysis of guar gum, a highly viscous fiber. The structure consists of a mannose backbone with galactose side units. PHGG is a soluble fiber with only marginal effects on viscosity, yet it seems to retain the ability of native guar gum to lower glucose and insulin levels. Several studies have shown that PHGG is beneficial in glycemic control and can attenuate the postprandial increase in blood glucose in healthy individuals as well as in those with non-insulin-dependent diabetes.[242-246] PHGG has also been shown to produce high levels of SCFA while favoring production of butyrate and propionate.[247] In addition, individuals given 21 g/d PHGG (in 3 divided doses) for 2 weeks experienced a significant increase in the growth of bifidobacteria in stool, suggesting benefits on gut microbiota.[248]

PHGG is most notable for its laxation benefits, and a number of trials using PHGG as the single fiber source have shown benefits on bowel function. In healthy individuals, PHGG supplementation increases fecal bulk,[249] prolongs transit time

compared with a fiber-free formula,[235, 250] and increases defecation frequency in constipated individuals.[251] Similar benefits are observed in clinical trials. In ICU patients with persistent diarrhea (n = 20), enrichment of EN with 2% PHGG (20 g/L) for 4 days resulted in significantly fewer diarrheal episodes and better GI tolerance than a fiber-free feed.[245] Similarly, among surgical or medical patients (n = 100), addition of 20 g/L PHGG to total or supplemental EN resulted in significantly fewer patients with diarrhea and fewer days with diarrhea vs. control.[252] Supplementation with 22 g/d PHGG for at least 6 days resulted in a significant reduction in the frequency of diarrhea and mean diarrhea score in patients (n = 25) with sepsis or shock.[253] In elderly tube-fed patients experiencing diarrhea (n = 20), increasing doses (7–28 g/d) of PHGG for 4 weeks significantly reduced stool frequency and increased SCFA production.[254] Supplementation with PHGG has also been shown to reduce enema requirements and laxative use among nursing home residents, suggesting benefits on constipation as well as diarrhea.[255, 256]

Acacia Gum

Acacia gum (AG) is a nonviscous, soluble fiber that has received increasing attention in recent years because of its prebiotic effects and exceptional tolerance. It is obtained as an exudate from the branches and stems of *Acacia senegal* and *Acacia seyal* and is a highly branched, high molecular weight molecule consisting of galactose, arabinose, rhamnose, and glucuronic acid units. AG is slowly fermented compared with other soluble fibers and increases production of SCFA and therefore may benefit the

distal colon.[257] Low doses of AG (3 g/d), when combined with 3 g/d fructo-oligosaccharides, have been shown to be prebiotic and support the growth of bifidobacteria in humans.[258] A dose of 10 g/d AG for 4 weeks was found to cause a greater increase in fecal bifidobacteria than an equal dose of inulin,[259] but the study was not crossover in design, and results may have been affected by interindividual variation. Animal studies suggest an ability of AG to improve symptoms of diarrhea,[260] and human trials have shown effects on normalizing bowel function.[257, 261] In addition, 5 g AG added to a meal has been shown to lower the glycemic response, and chronic consumption of 25 g/d has a lipid-lowering effect.[262] Consumption of AG is well tolerated up to high doses (50 g/d) and produces fewer GI symptoms than other fermentable fibers.[257]

Inulin, Oligofructose, and Fructo-Oligosaccharides

Inulin, oligofructose (OF), and fructo-oligosaccharides (FOS) belong to a larger class called inulin-type fructans, which refers to all linear fructans that contain β-2,1 fructosyl-fructose glycosidic bonds.[263] These molecules differ in chain length and method of extraction or synthesis, yet nomenclature is inconsistent in the literature. In general, inulin refers to molecules with an average degree of polymerization ≥10, whereas FOS and OF refer to shorter chain molecules.[263] FOS, OF, and inulin are nonviscous, soluble fibers obtained from a number of foods (primarily chicory root) or produced synthetically by adding fructose units to a sucrose molecule via β-1,2 linkages (FOS only).[263]

These compounds are some of the most well-studied prebiotic fibers and have been shown to increase fecal bifidobacteria in a number of populations, including infants, adults, and elderly, typically at doses of 5 g/d or more.[264, 265] As shown in vitro, fermentation of these fibers leads to high levels of SCFA and an increased molar ratio of butyrate to total SCFA compared with other fibers.[266] In addition, these fibers have been shown to enhance immune response in children[267] and elderly patients[268] and reduce inflammation in patients with ulcerative colitis.[269] These fibers have some bulking properties, and addition of FOS to an enteral formula has been shown to reduce constipation.[176, 270-272] Consumption of FOS and inulin has been found to enhance mineral absorption, especially that of calcium, and therefore may have implications for bone health.[273-276]

In some studies, the rapid fermentation of FOS has been associated with excess gas and GI discomfort.[277] Similarly, when used as the single source of fiber in EN, 30–35 g/d inulin caused a significant increase in flatulence.[278] However, low doses (<15 g/d) are generally well tolerated and can easily be incorporated into foods and beverages, making them useful sources of added fiber.

Resistant Starch

Resistant starch (RS) refers to starch and products of starch digestion that are not absorbed in the small intestines of healthy people and pass to the colon.[6] RS can be classified according to the characteristics that make it resistant to digestion (physically inaccessible, granular form, retrograded, or chemically modified). Fermentation of RS

typically results in increased butyrate and decreased acetate production compared with other fibers,[11] and RS can promote the growth of bifidobacteria.[12] Consumption of RS has been shown to lower postprandial glucose and insulin response[13, 14] and may also benefit cholesterol levels.[15] Although RS has not been used as a sole source of fiber in EN, it is commonly used as a component in mixed fiber blends.

Cellulose

Cellulose is an insoluble fiber consisting of glucose polymers with β-1,4 linkages, present in plant cell walls[279]. Cellulose is effective at increasing stool weight[164] and has been shown to suppress osmotic diarrhea.[280] Cellulose is poorly fermented and has little effects on glycemia or cholesterol levels.[279, 281] Similar to RS, cellulose commonly appears as a source of insoluble fiber in mixed fiber blends.

Outer Pea Fiber

Outer pea fiber is an insoluble fiber obtained from the hulls of the field pea and is composed of hemicelluloses, cellulose, and pectic substances.[282] Pea fiber is primarily used to enhance the fiber content of products, without modifying functional or technical properties, and increases stool weight in healthy individuals.[283] Intake of low doses of pea fiber has been shown to increase stool frequency in individuals with infrequent bowel movements, suggesting a normalizing effect on bowel function. This effect has been observed in both elderly and pediatric populations.[284, 285]

Rationale for Use of Fiber Blends

Early enteral formulas were primarily supplemented with a single fiber source, which was largely driven by technical concerns related to tube clogging and product quality. However, new processing techniques allow for the incorporation of a wide range of fibers into enteral formulas. Just as nutrition professionals recommend obtaining nutrients from a variety of different foods, it also seems reasonable to consume fiber from a variety of different sources to achieve a balanced intake of fiber. Likewise, current recommendations support the use of fiber blends. The Fiber Consensus Panel recommends that in patients requiring long-term EN, both nonfermentable, bulking fiber and fermentable fibers are appropriate for supplementation.[225] ESPEN guidelines also recommend a mixture of bulking and fermentable fibers (see Table 2-1).[226].[286]

Because it is well recognized that different fibers exert different physiological effects in the body, the use of fiber blends in clinical nutrition has become increasingly common. Blends more closely resemble a normal mixed diet, which contains small amounts of fiber from multiple sources, rather than a larger dose from a single source. Fermentable, prebiotic fibers can be added to promote growth of healthy gut microbiota, whereas less fermentable fibers can enhance stool consistency and mass.[3] Although intake of specific fiber components in the general population is difficult to estimate, studies suggest Americans consume 12–17 g/d nonstarch polysaccharides[9] and average 4.9 g/d RS,[286] 2.6 g/d inulin, and 2.5 g/d oligofructose.[287] There are currently no official recommendations for the ratio of soluble to insoluble fiber; however, a typical mixed diet consists of approximately 30% soluble fiber.[288]

Fiber blends have the potential to provide a number of advantages over the use of a single fiber source, as they can allow achievement of a range of physiological effects. Inclusion of fermentable fibers promotes SCFA production, and fibers that increase butyrate production can be selected to benefit colon health. In addition, fibers vary in the rate of fermentation because of a number of characteristics such as molecular weight, chain length, and structure. Combining fibers with a range of fermentability (quick to slow) could be used to sustain SCFA production along the entire length of the colon. Although the use of slowly fermented fibers has mainly been studied in relation to reduced risk for colon cancer, the benefits associated with increased butyrate and total SCFA production (energy for colonocytes, antidiarrheal effects, reduced inflammation) are of great interest in clinical nutrition. For this reason, combination of AG with other prebiotic fibers may be especially beneficial because of its slower rate of fermentation.

Use of fiber blends may also be beneficial for increasing tolerance. Relatively high doses of fiber are needed to meet recommendations for daily fiber intake. Supplementation with single fiber sources, both soluble and insoluble, has been shown to cause GI side effects, such as bloating, flatulence, and abdominal pain.[204, 278, 289] By combining multiple fibers in lower doses, it may be possible to achieve desired benefits without exceeding the tolerance level for any one fiber. For example, *in vitro* studies suggest that GI tolerance is improved when FOS/inulin blends are used compared with these fibers alone.[290, 291] Furthermore, combinations of different fibers may result in synergistic beneficial health effects. Combination of FOS and AG in a 1:1 ratio has been

shown to reduce GI side effects compared with FOS alone, while at the same time conferring a synergistic prebiotic benefit.[258, 292]

Current Research with Fiber Blends

A number of fiber blends have been developed by the healthcare industry for use in clinical nutrition products. One fiber blend is a 100% soluble, 70:30 blend of FOS and inulin (Prebio[1]; Nestlé, Vevey, Switzerland), which has primarily been studied in pediatric populations. In a prospective study of preadolescent cancer patients (n = 67), supplementation with 1.2 g/d of the 70:30 blend for 1 month resulted in significantly increased lactobacilli in stool compared with a control group.[293, 294] Similarly, a dose of 2.5 g/d for 3 weeks was effective at restoring fecal bifidobacteria levels in children (n = 140) following antibiotic treatment.[295] Lactobacilli also tended to increase in the group consuming the fiber treatment, although this was not significant.[295] This blend may also have beneficial effects on immune response. In infants, supplementation with 1.7 g/d of the 70:30 blend for 10 weeks was shown to enhance IgG antibody response to vaccination.[267] Although these studies benefit from relatively large sample sizes, the lack of a crossover design is a major limitation. This is especially true for studies examining gut bacteria levels because these are known to vary greatly by individual. However, preliminary evidence indicates low doses of this blend may have beneficial effects in children.

Research with this blend in adults is less clear. Elderly individuals (n = 60) were given a supplement of macronutrients, vitamins, *Lactobacillus paracasei*, and 6 g of the

70:30 blend or placebo for 4 months. Supplemented individuals had increased natural killer cell activity and significantly fewer infections than the placebo group.[296] However, because of the complexity of the supplement, it is not possible to attribute these effects specifically to fiber. Earlier research by the same group found no immunological effect when 6 g/d of the 70:30 blend was added to the diets of elderly individuals already receiving a nutrition supplement.[297] Additional studies using a crossover design and controlled diets are required to better understand the effects of this fiber blend on outcomes in an adult population.

Another popular blend is a mixture of 6 fibers: soy polysaccharide, cellulose, AG, FOS, inulin, and RS (Nutricia Multi Fibre; Nutricia, Zoetermeer, The Netherlands). The reported proportion of each fiber varies among studies, but the blend used for tube feeding provides roughly equal amounts of soluble and insoluble fiber, whereas the oral supplement contains 60% soluble fiber. Results from trials using this blend in children have been variable, but the main benefit seems to be a reduction in laxative use. In boys aged 1–36 months (n = 144) with dehydration and diarrhea, short-term addition of 1 g 6-fiber blend/100 mL to an oral rehydration solution did not alter 48-hour stool output or duration of diarrhea.[298] Similarly, in a randomized crossover design, the same blend or a fiber-free formula was given to children on home tube feeds (n = 25). After 6 weeks, there were no differences in stool frequency, diarrhea, or constipation; however, when the formula was consumed for 6 months, constipation occurred less frequently, and laxative use was less in children consuming fiber.[299] A reduction in laxative use was also reported in chronically sick children (n = 60) receiving a nutrition supplement that provided 4 g 6-

fiber blend/d for 12 weeks.[300] Overall, this blend appears to be more effective with longer term interventions.

Few studies have examined the effects of this 6-fiber blend on microbiota in the pediatric population. In a randomized crossover design, children receiving long-term EN (n = 20) receiving a 6-fiber blend-enriched feed for 3 months experienced a significant increase in the proportion of bifidobacteria in stool compared with a control formula.[301] Although this suggests a prebiotic benefit, the baseline proportion of bifidobacteria appeared to differ between treatments, so a statistical comparison of baseline levels would have been useful for better understanding the relationship. Baseline bacteria concentrations are often correlated with the magnitude of response to treatment, so it would have been helpful to address whether these differences impacted the treatment effect.

This fiber blend appears to have a number of benefits in adults. In healthy volunteers (n = 10) consuming liquid diets, supplementation with 30 g 6-fiber blend per day resulted in transit time similar to a self-selected diet, whereas a fiber-free feed significantly slowed transit, suggesting a normalizing effect of the blend on GI transit.[302] Schneider et al.[303] examined the effectiveness of a 6-fiber blend-enriched formula on SCFA production and microbiota in patients (n = 15) on total EN for dysphagia. In a crossover design, patients received a fiber-free enteral formula for 7 days, followed in random order by the fiber-free formula or formula supplemented with 15 g/L of the 6-fiber blend for 14 days each. Following the fiber treatment, there was a significant increase in butyrate and total SCFA compared with baseline and the control, as well as a

significant increase in total fecal bacteria counts; however, there were no changes in bifidobacteria or lactobacilli in stool samples. This may due to the relatively low levels of prebiotic fibers this blend provided (between 2.4 and 3.8 g of FOS and inulin per day). Although these studies are strengthened by using a crossover design, they are limited by small sample size. Larger studies are needed to confirm these results and determine if they are generalizable to other patients. However, this research does show that addition of fiber can alter SCFA production and gut microbiota in patients undergoing long-term EN.

Supplementation with this fiber blend has also been used during inflammatory conditions. Patients with severe acute pancreatitis (n = 30) were randomly assigned to receive a fiber-free enteral formula or the same formula supplemented with the 6-fiber blend to provide 24 g fiber/d. The median duration of hospital stay was significantly shorter, and all prognostic indices (C-reactive protein [CRP] values, Acute Physiology and Chronic Health Evaluation [APACHE] II score, computed tomography [CT] score) normalized earlier in the fiber group.[304] This suggests a fiber blend may be beneficial for improving the acute phase response and suppressing inflammation. Unfortunately, additional measures, such as SCFA production and bacterial counts, were not taken to help clarify the mechanism.[304]

A patented blend of 75% insoluble and 25% soluble fibers (oat, soy polysaccharide, acacia gum, and carboxymethylcellulose) combined with FOS (Jevity FOS; Abbott Laboratories, Hoofddorp, The Netherlands) has also been studied in a number of clinical trials, but has had minimal effects on bowel function. Critically ill patients receiving EN and antibiotics (n = 44) were randomized to receive the fiber-

supplemented formula or control.[305] These groups were again divided so that half received pectin and half received placebo. Significantly fewer patients in the fiber-free/placebo and fiber/pectin groups experienced diarrhea as compared with the fiber/placebo group.[305] Although there was a trend toward a lower incidence of diarrhea in the fiber/pectin group, the small sample size in each arm of the study may have prevented this from reaching significance. In a multicenter, randomized controlled study, children aged 1–6 years requiring tube feeding (n = 94) were randomized to receive standard formula without fiber or an energy-dense formula with the 5-fiber blend for 21 days.[306] Although there were no significant differences in stool consistency or GI tolerance among treatments, the formula with added fiber showed the greatest improvement in GI symptom scores and tended to result in more formed stools as compared with the other groups.[306]

A pilot study by Wierdsma et al.[307] evaluated the effect of the 5-fiber blend on gastrointestinal quality of life (GIQLI) and gut microbiota in home-living tube feed–dependent adult patients. Patients were randomized to receive a fiber-free formula (n = 10) or a similar formula supplemented with 17.6 g/L fiber (n = 6) for 6 weeks. GIQLI scores and number of bifidobacteria in stool significantly decreased compared with baseline in the fiber-free group but remained stable in the fiber-supplemented group.[307] This suggests the fiber blend may be beneficial at preventing the decline in healthy bacteria associated with fiber-free feeds; however, the fiber-supplemented group was very small and there was high variability in bacteria counts, so these results should be interpreted with caution.

A blend of FOS and pea fiber has been used in a number of studies and appears to have benefits on laxation and gut health. In a randomized crossover design, healthy participants (n = 10) consumed a standard formula or the same formula supplemented with 5.1 g/L FOS and 8.9 g/L pea fiber (average intake was 9.5 g/d FOS and 16.5 g/d pea fiber) for 14 days each. Fiber supplementation blunted the reduction in fecal bacteria normally associated with liquid diets and significantly increased bifidobacteria and reduced clostridia compared with baseline.[289] Fiber consumption also increased total SCFA, acetate, and propionate compared with the fiber-free formula.[289] A second randomized crossover study also examined a blend of FOS and pea fiber in children with compromised gut function (n = 14). In random order, children received a fiber-free formula or one supplemented with 3.5 g/L FOS and 3.8 g/L outer pea fiber for 2 weeks each.[308] Stool frequency did not differ between treatments, but use of the fiber-containing formula resulted in improved stool consistency.[308] Fiber reduced the proportion of both hard and watery stools, suggesting a normalizing effect on bowel function.[308] The fiber formula was well tolerated and reduced flatulence in a subset of children with neurological disorders.[308] In a recent study, surgical ICU septic patients (n = 34) receiving broad-spectrum antibiotics and total EN were randomized to receive a fiber-free formula or one supplemented with 15.1 g/L fiber from FOS and pea fiber for up to 14 days.[309] Mean diarrhea score in the fiber group was significantly lower than in the fiber-free group, showing this fiber blend can be effective at reducing diarrhea in critically ill patients.[309]

A number of other blends have been tested in humans, with the primary benefits on laxation. Hospitalized geriatric patients (n = 172) were randomized to a standard formula or formula supplemented with 30 g fiber (33% insoluble: cellulose and hemicellulose A; 67% soluble: pectin, hemicellulose B, inulin) for an average of 28 days.[310] Fiber supplementation resulted in fewer watery stools and less laxative use compared with control, suggesting a moderating effect on bowel function.[310] In a crossover design, medically stable residents of a chronic care facility (n = 10) were randomized to receive a fiber-free formula or one supplemented with 28.8 g/d of a 50/50 blend of soy and oat fiber for 10 days.[311] Fiber significantly increased fecal weight and bowel frequency but had no effect on transit time.[311]

Conclusion

Current research highlights the potential for fiber blends to provide a variety of health benefits, including reduction in diarrhea and/or constipation, promotion of SCFA production, maintenance of healthy gut microbiota, and enhanced immune function. These benefits are especially important in the clinical nutrition setting, where gut function may be altered and patients may be prone to opportunistic infections. However, design characteristics such as subject population, choice of end points, duration of fiber supplementation, and definitions of diarrhea/constipation vary among the available studies and make comparisons among different fiber blends difficult. Other differences in enteral formulas, such as the presence of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols may also contribute to diarrhea, but this has not yet been

well studied.[312] In addition, many of the current studies are limited by small sample size and lack of crossover design. This increases variability and makes finding an effect more difficult and also limits the ability to generalize results to a larger population.

Overall, combination of fibers with varying fermentability and solubility seems to be most effective at providing a range of benefits, as well as high tolerance. However, it is important to balance the benefit of providing a greater variety of fiber sources with providing a high enough dose to elicit the desired effect while keeping GI side effects to a minimum. The design of fiber blends should be guided by current research on effective dose and tolerance for the individual fiber components. Additional research is needed to identify synergistic effects of fiber from various sources. Randomized, controlled, crossover trials with larger sample sizes are needed to better understand the physiological effects of these fiber blends. Future studies should compare different mixed-fiber blends to determine the optimum combinations and doses of fiber to produce the desired end points in a variety of patient populations.

Table 2-1. Recommendations for use of fiber in enteral nutrition

Source	Recommendations
Fiber Consensus Panel[225]	• To prevent EN induced diarrhea in post surgical and in critical ill patients, supplementing EN with PHGG is effective (Recommendation A) • Fermentable and viscous fibers (e.g. oat β-glucan) are effective for glycemic control, but the available studies make it difficult to ascertain to what extent fiber supplementation contributes to the beneficial effects of the diabetes formulas (No Recommendation) • Short-term studies showed that soy polysaccharides or soy polysaccharides combined with oat fiber, increased daily stool weight and frequency. There is only one pilot study showing a beneficial effect of adding soy polysaccharides to control bowel habits in patients on long-term enteral feeding (Recommendation C)
ESPEN[226]	• A fiber intake of 15-30 g/d is advisable for patient on EN • In non-ICU patients or those requiring long-term EN, a mixture of bulking and fermentable fiber is the best approach • Dietary fiber can contribute to normalization of bowel function in elderly patients • In acute illness, fermentable fiber is effective in reducing diarrhea in patients after surgery and in critically ill patients (guar gum and pectin are superior to soy polysaccharides)
SCCM and ASPEN[228]	• If there is evidence of diarrhea, soluble fiber containing or small peptide formulations may be utilized (Grade E) • Soluble fiber may be beneficial for the fully resuscitated, hemodynamically stable critically ill patient who develops diarrhea. Insoluble fiber should be avoided in all critically ill patients. Both soluble and insoluble fiber should be avoided in patients at high risk for bowel ischemia or severe dysmotility (Grade C)

EN, enteral nutrition; PHGG, partially hydrolyzed guar gum; ESPEN, European Society for Clinical Nutrition and Metabolism; ICU, intensive care unit; SCCM, Society for Critical Care Medicine; ASPEN, American Society for Parenteral and Enteral Nutrition

Table 2- 2. Classification of fiber based on three physicochemical characteristics

Soluble • Acacia gum • PHGG • Inulin • FOS • Pectin • Hemicellulose A • Oat fiber	Insoluble • Cellulose • Soy polysaccharide • Resistant Starch • Hemicellulose B
Fermentable • Acacia Gum • PHGG • Inulin • FOS • Soy polysaccharide • Resistant Starch • Pectin	Non-fermentable • Cellulose • Outer Pea Fiber
Viscous • Pectin • Some gums (e.g. guar gum)	Nonviscous • Cellulose • Outer pea fiber • Soy polysaccharide • Resistant starch • PHGG • Inulin • FOS

FOS, fructo-oligosaccharides; PHGG, partially hydrolyzed guar gum

Chapter Three

FIBER INTAKE INCONSISTENTLY ALTERS GUT HORMONE LEVELS IN HUMANS FOLLOWING ACUTE OR CHRONIC INTAKE*[2]

*Publication Citation

Klosterbuer AS, Greaves KA, Slavin JL. Fiber intake inconsistently alters gut hormone levels in humans following acute or chronic intake. *Journal of Food Research.* 2012; 1:255-273.

[2] This research was supported by the Kellogg Company.

Chapter Synopsis

Diet composition affects the release of gut hormones involved in the regulation of appetite and energy intake. While some research suggests high fiber foods cause greater satiety than low fiber foods, few studies have measured gut hormone levels as a mechanism by which fiber may influence appetite. A review of the literature was conducted to better understand the effect of fiber on gut hormone concentrations in humans, with specific focus on peptide YY, glucagon-like peptide-1, cholecystokinin, and ghrelin. Considerable variation was found in study design, population, fiber type and level. Few studies reported a significant effect of fiber on gut hormone levels, and data suggest caloric load may have a more significant influence on gut hormone release. While it is possible that circulating gut hormones are not the mechanism by which fibers influence satiety, it is also possible that variability in study design prevents definitive conclusions around this relationship.

Introduction

A variety of peptides are released from the gastrointestinal (GI) tract in response to the nutritional state. These gut hormones are considered to be important factors in the control of appetite and satiety. The strength and timing of postprandial gut hormone release is clearly influenced by macronutrient distribution and total meal composition. Certain macronutrients are thought to be more satiating due to their ability to influence gut hormones; however, the impact of fiber on this relationship is not clearly understood. While some research suggests high fiber foods result in greater satiety than low fiber foods, few studies have measured circulating gut hormone response after fiber intake in humans. Therefore, a review of the literature was conducted to better understand fibers' impact on gut hormone concentrations in the blood. Although many peptides and hormones are released from cells in the GI tract and may influence satiety (e.g. oxyntomodulin, pancreatic polypeptide, glucose-dependent insulinotropic polypeptide, leptin, adiponectin, enterostatin, glucagon, insulin, amylin), only four – peptide YY (PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), and ghrelin – were chosen for this review due to their relatively well established effects on appetite.

Peptide YY

PYY is a 36-amino-acid polypeptide synthesized and secreted from the L-cells of the terminal ileum.[313] Upon release, the molecule undergoes cleavage by the enzyme dipeptidyl peptidase IV (DPP-IV) to yield a truncated peptide, PYY_{3-36}, which is the predominant circulating form in the fed and fasted state.[314] PYY_{3-36} binds with high

affinity to the Y2 receptor, located throughout the central nervous system (CNS) and vagal afferents.[315] PYY is thought to inhibit appetite by acting centrally on homeostatic centers in the hypothalamus to reduce expression of neuropeptide Y (NPY), an orexigenic peptide. Neural reflexes are also important, since PYY concentrations increase before nutrients reach the site of PYY release and vagotomy abolishes the appetite suppressing effect of PYY.[316] PYY also activates the ileal brake, which slows gastric emptying and nutrient absorption, and may extend feelings of satiety.[65]

Plasma PYY levels rise within 15 minutes of a meal, and peak approximately an hour after nutrient ingestion.[317] The magnitude of PYY release depends on both the caloric load and macronutrient content of the meal. When balanced for total energy, meals high in fat and protein appear to increase PYY more than carbohydrate-rich meals.[318-320] Intravenous infusion of PYY has been shown to significantly increase satiety and reduce energy intake in humans.[313, 321-323] However, many studies use pharmacological doses which can lead to side effects such as nausea and vomiting, which may interfere with appetite ratings. Researchers have reported lower fasting and postprandial PYY concentrations in obese participants compared to lean individuals, and this is reversed following bariatric surgery.[321, 324] This suggests PYY may play an important role in energy homeostasis, and has led to interest in PYY as a potential antiobesity therapeutic agent.

Glucagon-like Peptide-1

GLP-1 is formed from the cleavage of proglucagon, and is released primarily from the L-cells of the distal small intestine. Further N-terminal truncation is required to produce the biologically active form, $GLP\text{-}1_{7\text{-}36}$.[105] GLP-1 undergoes rapid degradation by DPP-IV and only 10-15% reaches the systemic circulation intact.[138] GLP-1 receptors are expressed in the gut, brainstem, hypothalamus, and vagal afferent nerves. It is thought that GLP-1 may access the CNS directly via the area postrema, which lacks a blood-brain barrier, but the significance of this pathway is unknown.[114] GLP-1 exerts several physiological effects that may influence appetite. As an incretin hormone, GLP-1 amplifies the insulin response to glucose ingestion and inhibits the release of glycogen from the liver.[36, 111, 151] In the GI tract, GLP-1 inhibits gastric and pancreatic exocrine secretion, as well as gastric emptying, which may enhance satiety.[36, 111, 157]

Upon eating, plasma GLP-1 levels increase within 10-15 minutes and peak by 40 minutes.[145] This initial increase occurs prior to nutrients reaching the small intestine, and is likely mediated by neural inputs. GLP-1 release is proportionate to the number of calories consumed. Additionally, when matched for energy, meals high in carbohydrates and protein seem to be more potent stimulators of GLP-1 secretion than high fat meals.[149, 150] In humans, GLP-1 infusion has been shown to increase satiety and decrease food intake in healthy normal weight and obese participants, as well as individuals with type 2 diabetes.[139-141, 325] A meta-analysis by Verdich et al. concluded that infusion with physiological doses of GLP-1 reduced energy intake by an average of 12%.[325]

Cholecystokinin

CCK is released primarily from I-cells in the duodenum and proximal jejunum, but small amounts are also produced by neurons in the GI tract and nervous system.[326, 327] CCK is formed by selective processing of its precursor, proCCK, which results in multiple bioactive forms ranging in size from 8 to 58 amino acids.[328, 329] All isoforms show affinity for the CCK receptor, located on the gallbladder, pancreas, and stomach, as well as in the hindbrain and hypothalamus.[330] CCK-induced satiation appears to be mediated neuronally via activation of vagal afferents in the stomach and duodenum.[331] In addition, CCK slows gastric emptying, which may increase stomach distension and causes greater satiety.[332]

Plasma CCK typically increases within 15 minutes of a meal, and the duration of elevation depends both on caloric load and macronutrient content. When matched for energy, fat and protein appear to be stronger stimuli for CCK release than carbohydrates.[326] In humans, infusion with CCK reduces meal size and duration, and has been estimated to suppress energy intake by an average of 22.5%.[43] There also appear to be gender differences in the CCK response, with women experiencing greater CCK elevation than men; however, it is not clear if this corresponds to differences in appetite sensations between genders.[333, 334]

Ghrelin

Ghrelin is a 28-amino-acid peptide hormone originating primarily from the stomach, with lesser amounts formed in the small intestine and other organs.[335]

Circulating ghrelin is present in both an acylated and non-acylated form, but only the acylated form binds the ghrelin receptor and is considered biologically active.[336] Ghrelin receptors are widely distributed throughout the body in tissues such as brain, stomach, intestine, pancreas, and heart.[337] Ghrelin is thought to interact with NPY and agouti-related peptide (AgRP)-expressing neurons of the arcuate nucleus of the hypothalamus through vagal afferents or more directly via the bloodstream.[36] NPY and AgRP are orexigenic peptides and promote food intake.[36]

Ghrelin is unique in that it is the only peripheral hormone known to be a powerful stimulant of appetite and food intake.[111] Plasma ghrelin levels increase markedly prior to a meal, suggesting a role in meal initiation.[115] In general, nutrient intake suppresses plasma ghrelin levels. While caloric load is the most important determinant of the magnitude and duration of ghrelin suppression, macronutrient composition of the meal also plays a role. When matched for total energy, lipids appear to be less effective than carbohydrates or protein at suppressing ghrelin.[118-120] Peripheral infusion with ghrelin increases energy intake and hunger in humans.[113] In addition, there is evidence that obese individuals may be more sensitive to the effects of ghrelin.[338] Ghrelin may also play a role in long-term weight regulation as levels increase with weight loss and decrease with weight gain.[116]

Summary

The presence or absence of food in the GI tract causes the release of a number of peptides that act to optimize the digestive process and regulate appetite and energy

expenditure. Levels of these hormones are influenced by meal composition, caloric load, body weight, gender, and other factors. This study aimed to determine the effect of fiber intake on circulating gut hormone levels in humans.

Methods

PubMed/Medline was used to identify original research and review articles on September 27, 2010. The following key words and search terms were used: (dietary fiber OR fiber OR fibre OR whole grain OR complex carbohydrate) AND (gut hormones OR ghrelin OR peptide YY OR peptide tyrosine tyrosine OR PYY OR glucagon-like peptide-1 OR glucagon like peptide OR GLP OR GLP-1 OR cholecystokinin OR CCK). All searches were limited to human studies, English language, and peer-reviewed publications. References from original research and review articles were scanned to identify other potentially relevant studies.

The following inclusion criteria were used: Adults (19+ years); healthy individuals of any body weight; clinical trials; measurable fiber level and type; outcome data for PYY, GLP-1, CCK, or ghrelin; attrition rates ≤20%; and studies completed between 1990 and the present. Exclusion criteria included infants; children; people <19 years of age; people with diabetes, hyperlipidemia, hypertension, hypercholesterolemia, or any other health disorders. Studies that used descriptive (retrospective or prospective) study design only, or studies without a measurable fiber intervention were excluded, as were studies with an attrition rate >20%. Studies that met the inclusion criteria were further examined for relevance, validity, and quality by evaluating sample population and

size, study design (crossover vs. parallel), randomization, blinding, choice of control, and appropriateness of statistical analyses. These characteristics were organized into tables. Studies that lacked a control were excluded at this level of evaluation.

Results

Effect of Fiber on Peptide YY Concentrations

The PubMed search generated a list of 27 publications, including 22 original research articles and 5 review articles (Figure 3-1). Eleven primary research articles were relevant to the research question, of which 9 were obtained from the PubMed search and 2 were discovered by examining the reference lists from the review articles. Nine of the 11 relevant publications met the quality criteria and were included in the final analysis. More than 11 types of fibers were studied and doses ranged from 3.8 to 27 g. Fiber was primarily supplied via a supplemented grain product (e.g. bread, muffins, or cereal) which was consumed alone or as part of a mixed meal. Alternatively, fiber was provided as a powder added to a beverage. General study characteristics and outcomes are summarized in Table 3-1.

Three studies examined the effects of β-glucan fibers on PYY response. Two studies used randomized, crossover designs and controls matched for calories and macronutrients. The first measured 3 g barley β-glucan in 14 normal weight volunteers.[339] Area under the curve (AUC) measured over 3 hours was significantly higher following β-glucan intake compared to control. In a similar study, 3 doses (2.2-5.5 g) of oat β-glucan were tested in 14 overweight men and women.[340] PYY levels were

compared to control at individual time points (AUC was not compared). The highest dose of β-glucan resulted in significantly higher PYY levels after 4 hours compared to control. A dose response effect was observed for increasing levels of β-glucan. Although these studies are limited by small sample size, they suggest a dose of 3-6 g β–glucan may raise postprandial PYY levels.

One study examined the effects of chronic β-glucan supplementation on PYY levels. In a parallel design, overweight women (n=66) consumed a low calorie diet supplemented with 0, 5-6 or 8-9 g β-glucan for 3 months.[341] Total fasting PYY decreased in all groups compared to baseline, but the decrease was significantly less for the high dose compared to control. However, it is not possible to distinguish the effects of fiber supplementation from the effects of caloric restriction and weight loss on gut hormone levels.

Two randomized, crossover studies examined the effect of wheat and/or oat fiber on PYY response. Juvonen et al. tested 10 g wheat or oat bran alone, 5 g of each in combination, and a control and found no differences in PYY response among treatments.[342] Similarly, Weickert et al. tested 10.5 g of added wheat or oat fiber in 14 women and found that postprandial PYY $AUC_{0\text{-}300}$ was blunted following wheat fiber, while PYY levels after oat fiber did not differ from control.[343]

Other fiber sources were tested in single studies, but at varying doses. In a randomized, crossover design, subjects (n=20) consumed 0, 4, 8, or 12 g of a mixed fiber (pectin, barley β-glucan, guar gum, pea fiber, and citrus fiber).[81] $PYY_{3\text{-}36}$ $AUC_{0\text{-}60}$ did not differ among treatments; however, many samples fell below the assay detection level.

In another randomized crossover study, subjects (n=16) consumed 4 isoenergetic meals with varying amounts of psyllium (6.2-27 g) and soy protein or a bread control.[344] The high fiber meals caused a longer elevation of PYY levels compared to control, but this was only significant at 90 min and 120 minutes after the meal; AUC_{0-120} did not differ.

Two studies using parallel designs examined chronic consumption of a fiber source. Subjects (n=10) consumed 16 g/d of an inulin/oligofructose blend or control for 2 weeks, at which point postprandial PYY was measured in response to a free choice buffet breakfast.[187] Mean total PYY levels were compared at individual time points (AUC was not measured). Plasma PYY was significantly increased 10 minutes after breakfast in subjects who had been consuming the inulin treatment compared to control. Another study examined the effect of increasing doses (5 to 10 g) of a functional fiber blend consumed for 3 weeks.[345] Following intervention, fasting PYY was significantly higher in the supplemented group compared to control, but only in a subset of individuals with BMI <23. In addition, PYY levels at week 3 were not different from baseline.

Overall, the available evidence does not show a clear effect of fiber on PYY levels. Acute feeding studies reported that small amounts of β-glucan or large amounts of psyllium increased postprandial PYY, while wheat and oat bran and a mixed fiber blend did not increase PYY compared to control meals. Chronic, daily consumption of β-glucan combined with energy restriction was shown to decrease fasting levels of PYY, while a mix of inulin and oligofructose or a functional fiber blend had little effect on fasting PYY levels. In general, the available studies are limited by sample size and study

design. The wide variety of fiber types and doses used make it is difficult to discern an overall relationship between fiber and PYY response.

Effect of Fiber on Glucagon-Like Peptide-1 Concentrations

The PubMed search generated a list of 53 publications, including 37 original research articles and 16 review articles, meta-analyses, or letters to the editor (Figure 3-1). Nineteen primary research articles were relevant to the research question, of which 17 were obtained from the PubMed search and 2 were discovered by examining the reference lists from the review articles. Of the 19 relevant publications, 16 met the quality criteria and were included in the final analysis. Many types of fiber were evaluated, with doses ranging from 1.7 g to 29 g fiber. In 11 studies, the fiber was provided as a supplemented grain product (most commonly bread), while in the other 5 studies, a powdered fiber supplement was mixed with a beverage or other test product. General study characteristics and outcomes are summarized in Table 3-2.

Several studies tested multiple types and amounts of fiber, but only one combination showed a positive impact on GLP-1 levels. In a randomized, crossover design, subjects (n=15) consumed 7 test meals with varying levels (9.9-81 g) of dietary fiber plus resistant starch (RS) from various forms of barley, oats, and modified corn starch or a low fiber control.[346] Test meals were consumed in the evening, and GLP-1 was measured the next morning following a standard breakfast. The total GLP-1 AUC_{0-120} was significantly higher than control following consumption of the test meal containing 20.2 g fiber + RS from ordinary barley. There were no other differences

among treatments. This suggests the source of fiber may be more important than the dose, since other treatments with similar amounts of fiber + RS had no effect. Another study that compared 5.5 g whole wheat barley to control found no differences in GLP-1 $AUC_{0\text{-}300}$ following the test meal.[347]

Additional studies have evaluated the effect of fiber from other whole grain sources on GLP-1 response. Two crossover studies compared various types and doses (6.1-29 g) of rye bread to a low-fiber white bread matched for available carbohydrates. In both studies, GLP-1 $AUC_{0\text{-}180}$ did not differ among treatments.[348, 349] A high fiber rye bread (whole meal rye bread enriched with rye bran) providing 29 g fiber caused significantly greater GLP-1 values compared to control at 150 and 180 minutes postprandially.[349] However, this product was also higher in energy, fat, and protein, so it is unclear if fiber was responsible for the observed effects. A rye bread enriched with β-glucan (17.1 g fiber, including 5.4 g β-glucan) also increased GLP-1 compared to control later in the postprandial period (120 and 150 minutes).[348] In a randomized, crossover design, Weickert et al. examined the effect of 10.5 g wheat or oat fiber compared to control and found no differences among treatments in GLP-1 measured as $AUC_{0\text{-}300}$ or individual time points.[350] Similarly, ancient wheat Einkorn (4-6 g) did not alter GLP-1 $AUC_{0\text{-}180}$ compared to a modern wheat bread.[351] However, fiber differences between control and treatment were minor and the sample size was small (n=11).

Psyllium was tested in two randomized, crossover trials. In the first, subjects (n=10) consumed a meal with added psyllium (1.7 g) and/or fat or an unsupplemented meal matched for available carbohydrates.[352] GLP-1 $AUC_{0\text{-}240}$ was significantly higher

than control in the meal with added psyllium and fat, but this effect was likely due to caloric differences between meals. AUC was not different between control and the low fat psyllium treatment, which was matched for calories.[352] In a later study, isoenergetic meals with varying levels of psyllium (6.2-27 g) and protein were compared to an unsupplemented control.[344] GLP-1 $AUC_{0\text{-}120}$ did not differ among treatments, but GLP-1 concentrations decreased below baseline following consumption of the high fiber, high protein treatment, indicating a negative effect of fiber and/or protein on GLP-1 levels.

Two studies tested pea fiber, either alone or as part of a mixed fiber blend. In a study by Raben et al., subjects (n=10) consumed a meal supplemented with 25.5 g pea fiber or low fiber control matched for energy and macronutrients.[77] There were no differences in GLP-1 between treatments when measured as $AUC_{0\text{-}240}$ or at individual time points. Willis et al. examined the effect of muffins supplemented with 0, 4, 8, or 12 g of a mixed fiber (pectin, barley β-glucan, guar gum, pea fiber, and citrus fiber) and found that GLP-1 $AUC_{0\text{-}60}$ was significantly higher for the 0 g dose than the 4 and 12 g doses, which again suggests a potential suppressive effect of fiber on GLP-1.[81]

Three randomized, crossover trials measured GLP-1 response to fiber dissolved in a test beverage. Two studied the effect of a preload of guar gum (2.5 g) + galactose or water (control), followed by a test meal.[353, 354] In both studies, GLP-1 levels were increased compared to control between 30 and 60 minutes postprandially. However, this is not a useful comparison since GLP-1 is known to increase as a result of caloric load. The fiber treatment contained 200 kcal and was compared to a non-caloric control. In another study, there were no differences in GLP-1 $AUC_{0\text{-}360}$ between a beverage

containing 24 g inulin + 56 g high fructose corn syrup (HFCS) and beverages with 80 g or 56 g of HFCS alone.[355]

Three studies with parallel design have evaluated the effect of chronic fiber supplementation on GLP-1 levels. Consumption of oat β-glucan (5-6 or 8-9 g/d) as part of a reduced calorie diet led to a reduction in fasting GLP-1 levels after 3 months.[341] Values were not different from a control group on the same low calorie diet, suggesting that weight loss has a greater effect on gut hormone levels than fiber. In another study, subjects consumed increasing doses (5 to 10 g) of a novel functional fiber or control for 3 weeks.[345] There were no differences in fasting GLP-1 levels at the end of the treatment period. Similarly, fasting GLP-1 levels were not different in subjects receiving 16 g/d of an inulin/oligofructose blend or control for 2 weeks.[187] However, GLP-1 was elevated compared to control at 10 minutes following a standard meal in subjects who had consumed fiber; AUC was not evaluated. These studies suggest chronic fiber intake independent of weight changes does not impact GLP-1 levels. In addition, due to the parallel design, these studies must be interpreted with caution, given the interindividual variability in gut hormone levels.

The available research suggests that fiber does not increase GLP-1 levels compared to control. Most studies were limited by sample size or design. Only one study reported an increase in GLP-1 AUC following fiber intervention (20.2 g ordinary barley), and other studies with similar types or doses of fiber found no effect. High doses of fiber (17-29 g) from rye bread significantly increased GLP-1 between 2 and 3 hours

after a test meal, but at no other time points. Other fiber interventions showed no effect on GLP-1 concentrations when matched for calorie content.

Effect of Fiber on Cholecystokinin Concentrations

The PubMed search generated a list of 64 publications, including 47 original research articles and 17 review articles, meta-analyses, or letters to the editor (Figure 3-1). No additional articles were discovered from examination of review article reference lists. Eleven primary research articles were relevant to the research question, of which 9 met the quality criteria and were included in the final analysis. Fiber came from a variety of sources, but β-glucan sources were the most common; fiber doses ranged from 3.7 to 35.5 g fiber. While most studies provided fiber as part of a mixed meal, one used a fiber-supplemented liquid formula. Control meals were generally well matched to the treatment meals in terms of energy and macronutrients. General study characteristics and outcomes are summarized in Table 3-3.

Several studies evaluated CCK response to supplementation with fibers containing β-glucans. Test cereals containing varying amounts of oat β-glucan (2.16-5.65 g) were compared to a low fiber cereal in a randomized, crossover design.[340] There was a significant dose response for women (n=7), but the combined sex analyses showed no differences in CCK AUC_{0-240}. A similar gender effect was observed in subjects consuming mixed meals containing 7 g (control) or 20 g fiber (added fiber in the high fiber meal was primarily from oats).[356] In women, the high fiber meal elicited a significantly higher mean CCK response compared to control, while the CCK response

between meals did not differ in men. In another study, male volunteers (n=11) consumed pasta made from barley with high β-glucan content (15.7 g fiber; 5 g β-glucan) or control.[357] CCK AUC_{0-360} did not differ, but the pattern of CCK response was different. While CCK concentrations returned to baseline by 3 hours after the low fiber meal, CCK levels did not return to baseline until 6 hours following the high fiber meal.

In a chronic study using a parallel design, consumption of oat β-glucan (5-6 or 8-9 g/d) as part of a reduced calorie diet for 3 months did not alter fasting CCK levels compared to control in women.[341] Another chronic study evaluated addition of 20 g partially hydrolyzed guar gum (PHGG) to a very low calorie formula diet in obese women (n=25).[358] Women received PHGG during either week 3 or 5 of the diet. Following a meal challenge using the formula diet, average CCK concentrations did not differ between treatment and control.

Additional randomized, crossover trials have evaluated different types of fiber or types of carbohydrate. In a small study, men (n=10) consumed a test meal with 12 g fiber from bean flakes or a low fiber meal matched for energy and macronutrients.[359] The bean flake meal produced almost twice the CCK AUC_{0-360} response, which was statistically significant. Pasman et al. compared the effect of isoenergetic meals containing complex or simple carbohydrates in 26 male volunteers.[360] The complex carbohydrate meal contained 6.7 g of fiber, provided primarily by rye bread. There was no difference in CCK response between the meals when measured as AUC_{0-240} or at individual time points.

Two studies compared meals that differed in glycemic index. The first found that consumption of a low fiber (2.4 g), high glycemic index meal resulted in a significantly greater CCK $AUC_{0\text{-}480}$ compared to a high fiber (35.5 g), low glycemic index meal in female volunteers (n=22).[361] In contrast, when matched for fiber content (29-30 g), consumption of a low glycemic index meal resulted in significantly greater CCK $AUC_{0\text{-}420}$ in men (n=12).[362]

The available evidence indicates that fiber does not have a consistent effect on CCK levels. In a small, but well designed study, fiber from bean flakes caused a clear increase in CCK compared to control, but the results are applicable only to men. Acute consumption of fiber from oats may increase CCK in women only, while chronic intake of fiber has no effect. In addition, meals varying in type of carbohydrate yielded inconsistent effects on CCK. Most studies were limited by small sample size, and may not be representative of the general population since they were conducted in certain genders, BMI ranges, or individuals on a reduced calorie diet.

Effect of Fiber on Ghrelin Concentrations

The PubMed search generated a list of 51 publications, including 40 original research articles and 11 review articles, meta-analyses, or letters to the editor (Figure 3-1). Twenty-three primary research articles were relevant to the research question, of which 19 were obtained from the PubMed search and 4 were discovered by examining the reference lists from the review articles. Of the 23 relevant publications, 19 met the quality criteria and were included in the final analysis. A variety of individual fibers and

fiber blends were studied, with doses ranging from 2 to 52 g fiber. Twelve studies provided fiber as a supplemented grain product or as part of a mixed meal, 5 added powdered fiber to a liquid or semi-solid product, and 2 added fiber to water. General study characteristics and outcomes are summarized in Table 3-4.

Several studies measured ghrelin response to β-glucan supplementation. In a randomized, crossover design, subjects (n=14) consumed isoenergetic breads enriched with 3 g barley β-glucan or control.[339] Ghrelin $AUC_{60\text{-}180}$ was significantly lower following the fiber treatment. In contrast, there were no differences in ghrelin $AUC_{0\text{-}240}$ among subjects (n=14) consuming cereal supplemented with varying doses of oat β-glucan (2.16-5.65 g) or control matched for available carbohydrate and protein.[340] Similarly, in a 3 month parallel trial, supplementation with oat β-glucan (5-6 or 8-9 g) had no effect on fasting ghrelin levels in women on a reduced calorie diet.[341] However, it possible that any effect of fiber would have been overshadowed by the influence of weight change on gut hormone levels. Additional randomized, crossover trials using 10-10.5 g fiber from oats or wheat did not show a suppressive effect of fiber on ghrelin levels compared to an isoenergetic control.[342, 343] In fact, one study found that 10.5 g wheat fiber resulted in significantly *higher* ghrelin $AUC_{0\text{-}180}$ compared to control.[343]

A series of crossover studies examined the effects of carob fiber on postprandial ghrelin levels. In the first study, subjects (n=20) consumed a liquid meal alone or enriched with 5, 10, or 20 g carob fiber.[363] Acylated (but not total) ghrelin was significantly lower 60 minutes after the test meal for all doses of fiber compared to control. There were no other differences over the 5 hour postprandial period, and AUC

was not analyzed. In contrast, the same doses of carob fiber added to glucose water had no effect on acylated ghrelin, but the 10 g dose decreased total ghrelin compared to control.[364] In a third study, volunteers consumed calorie and nutrient matched meals with or without 50 g carob fiber, followed by an overnight fast.[365] Ghrelin was measured the next morning following ingestion of a standardized white bread. Fasting acylated (but not total) ghrelin was significantly higher following consumption of the meal enriched with carob fiber; there were no differences in postprandial ghrelin levels.

There were 9 additional acute, crossover studies with fiber and ghrelin, each testing different types of fiber. In a study by Karhunen et al., subjects (n=16) consumed isoenergetic meals with varying levels of psyllium (7.6-27 g) and protein or a low fiber, low protein control in randomized order.[344] The declines in total ghrelin, measured as $AUC_{0\text{-}120}$ after the high fiber meals were blunted and differed significantly from the low fiber meals. Similarly, in subjects (n=11) consuming a meal with 6 g arabinoxylan or control matched for energy and macronutrients, ghrelin suppression was greater following control.[366] In a study by Willis et al., subjects (n=20) consumed muffins with 0, 4, 8, or 12 g of a mixed fiber in random order.[81] There were no differences in $AUC_{0\text{-}90}$ between treatments and control, but the highest dose led to significantly higher values than the lower doses. Consumption of rye products with varying levels of fiber (6.5-12.3 g) did not alter ghrelin $AUC_{0\text{-}180}$ compared to low fiber control matched for available carbohydrates.[367] These studies suggest that fiber does not have a suppressive effect on ghrelin, and that certain fibers may actually blunt the decline in postprandial ghrelin levels.

In contrast, several studies have reported greater declines in ghrelin levels following fiber compared to control. Consumption of bread enriched with lupin kernel flour (15 g fiber) resulted in significantly lower plasma ghrelin values than a calorie-matched white bread over a 3 hour postprandial period.[368] However, the enriched bread also contained twice the protein as control, so it is unclear if the effects are due to fiber, protein, or the combination. Consumption of 6 g fiber from plums produced significantly lower ghrelin values compared to white bread, but only at 15 and 30 minutes after the meal; there were no differences in ghrelin $AUC_{0\text{-}120}$.[369] Addition of 24 g inulin to a HFCS beverage caused a significant decrease in ghrelin levels compared to control, but not until 4 hours later, after a standard test lunch was consumed.[355] This suggests that fiber may produce a 2^{nd} meal effect on ghrelin levels. Although these studies suggest a suppressive effect of fiber on ghrelin, any effects appear to be short lived.

Two studies tested the influence of different types of carbohydrate on ghrelin levels. Ghrelin response was not different when subjects consumed a high glycemic index meal or a low glycemic index meal with similar fiber content.[362] In another study, subjects consumed meals containing simple or complex carbohydrates at varying calorie levels, but with similar fiber content.[370] The decrease in ghrelin $AUC_{0\text{-}240}$ was greater for the high calorie, simple carbohydrate meal than for the high calorie, complex carbohydrate meal, which suggests carbohydrate structure may affect ghrelin levels, regardless of fiber content.

Two additional studies examined the effect of chronic fiber supplementation on fasting ghrelin levels. In a randomized, crossover design, subjects consumed 12 g/d

pullulan, RS, soluble fiber dextrin, soluble corn fiber or control for 2 weeks each. There were no differences in fasting ghrelin among treatments.[371] Similarly, consumption of a novel functional fiber for 3 weeks did not alter fasting ghrelin levels compared to a control diet.[345] However, this study was limited by parallel design.

The available evidence suggests fiber does not positively influence postprandial ghrelin levels. The majority of studies found that fiber had no effect or a negative effect on ghrelin (higher levels compared to control) over a range of fiber sources and doses. In the few studies showing a suppressive effect of fiber, lower ghrelin values were only observed at limited time points throughout the postprandial period. However, many of these studies were limited by small sample size, lack of crossover design, or use of a control that differed in variables other than fiber content.

Discussion

The available literature on fiber and gut hormones is limited in both quality and quantity. Few studies with strong design (randomized, controlled, double-blind, crossover trials) measure gut hormone levels following acute fiber intake. Therefore, to provide a more complete assessment of the literature, studies with parallel design and those that measured fasting hormone levels after chronic fiber intake were also included in this review. Gut hormone levels can be highly variable from individual to individual, so the reliability of results from those studies is unknown. There is also little consistency in the types of fibers and doses used across studies, and a wide variety of isolated fibers, synthetic fibers, and high-fiber whole foods were used. Furthermore, control treatments

differed greatly among studies and were not always appropriate for examining the effect of fiber supplementation. Since the primary outcome was gut hormone levels compared to control, the use of inappropriate control treatments could significantly alter the results. These variations make it difficult to discern the true effect of fiber on gut hormone levels.

Few studies have been conducted investigating the effect of fiber on PYY release. Only nine publications met the inclusion criteria for the current review, resulting in 20 fiber-control comparisons based on many different fiber types and levels. Of those comparisons, the influence of fiber on circulating PYY levels was seen with acute feeding of test meals containing 3-6 g barley or oat β-glucan or greater than 25 g psyllium. Generally, fat and protein, as well as calorie load of a meal, have a greater influence on release of PYY into circulation than carbohydrates.[318-320] Fiber, as a member of the carbohydrate family of macronutrients, might not be expected to influence PYY to a great extent beyond the provision of calories to a meal.

Sixteen publications investigating the effect of fiber on GLP-1 release met the inclusion criteria for the current review, resulting in 34 fiber-control comparisons based on many different fiber types and levels. Of those comparisons, influence of fiber meals on circulating GLP-1 levels were seen primarily when differences in calorie content of the products were reported. For instance, in a study of psyllium, an increase in circulating GLP-1 was found when fat, and therefore calories, was added to the test meal, but not when the meals were matched for energy.[352] Circulating GLP-1 levels are known to be influenced by calories consumed, however when calorie content of a meal is held constant, carbohydrates and proteins are potent stimulators of GLP-1 release.[149, 150] The

results of this review suggest that calories are a more potent stimulator of GLP-1 release into the bloodstream than fiber. Any effect of fiber on appetite through GLP-1 action may be mediated directly via the vagal nerve and not as a result of circulating GLP-1.

Nine publications investigating the effect of fiber on circulating levels of CCK met the inclusion criteria for the current review, resulting in only 14 fiber-control comparisons based on many different fiber types and levels. In general, the results would suggest that fibers are not efficacious in promoting higher levels of circulating CCK. These results should not be surprising as carbohydrates have not been found to be as robust in their influence on circulating CCK levels as either protein or fat. Based on this review, two areas of interest for further investigation are the influence of beans and glycemia on CCK release.[359, 362] Although only 1 study has been published examining bean flakes, the results were quite promising with twice the response, based on AUC, when compared to a control meal. The efficacious component of the bean may be the protein and/or phytonutrient co-passengers in the formulation. Glycemic index of a meal was examined by Reynolds and coworkers with a report that, when controlled for fiber content of the meal, glycemic index significantly influenced the CCK response to the meal.[362] Preliminary research has suggested that glycemia may influence appetite and satiety and this is the first report that suggests that one mechanism may be related to CCK release. More research is needed in both of these areas to confirm these early findings.

Ghrelin is known to be influenced by consumption of food. The increase in ghrelin levels between meals is generally reversed once food is consumed. Some data suggest that protein and carbohydrates are more effective than lipids at attenuating the

rise in ghrelin; however, the presence of food in the gut may be the primary precipitating factor. Nineteen publications investigating the effect of fiber on attenuating the rise in circulating levels of ghrelin met the inclusion criteria for the current review, resulting in 44 fiber-control comparisons. Although several studies examining specific time points following the meal suggest that the influence of fiber on ghrelin may be time-specific, other data suggest that inclusion of fiber in a meal may actually blunt the postprandial decrease in ghrelin. In general, data reported as AUC did not support the hypothesis that fiber suppresses ghrelin levels.

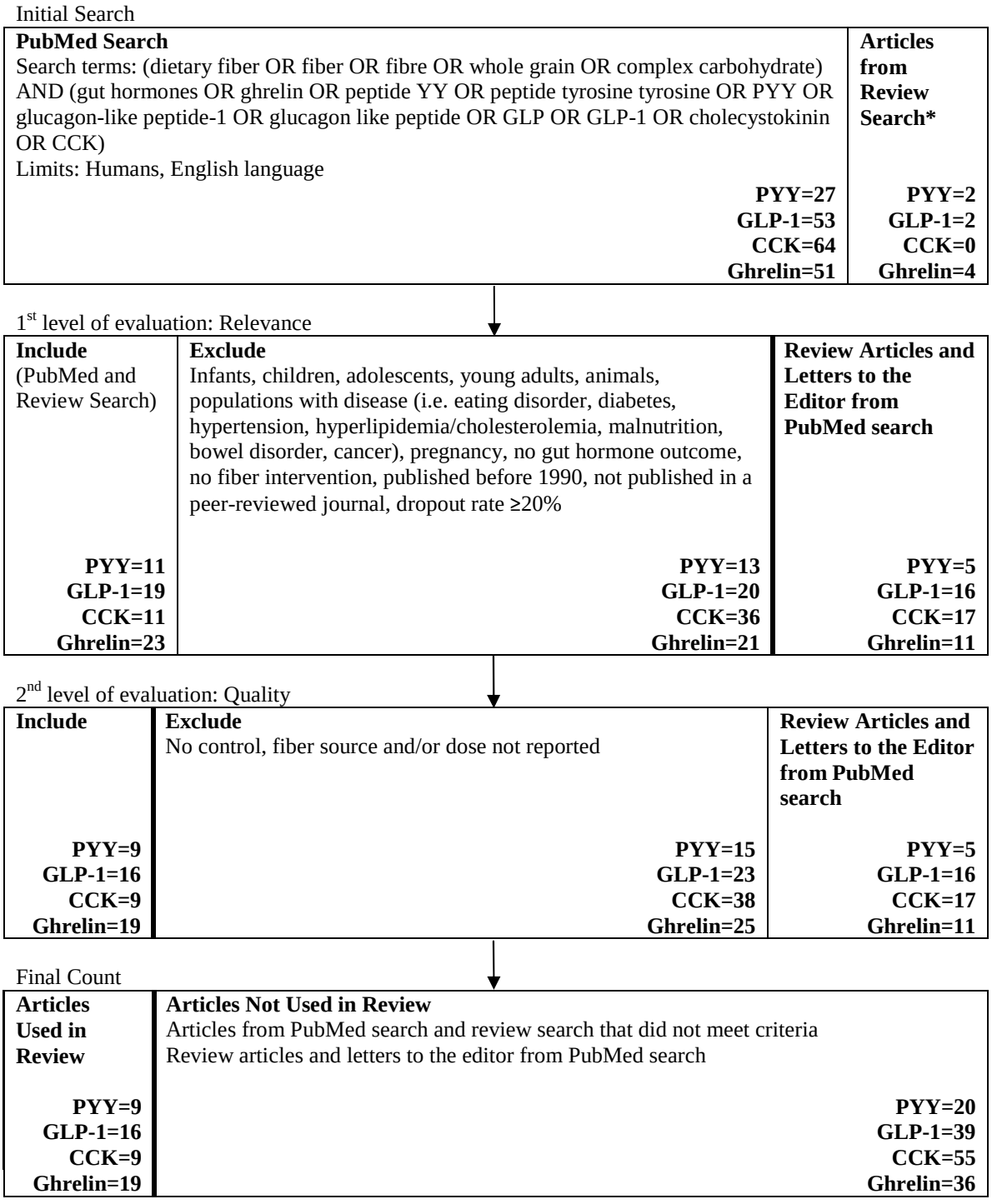
Other issues complicating gut hormone research are related to the technological aspects and limitations involved in the measurement of gut hormones. Most studies rely on more affordable, but less sophisticated techniques, such as enzyme immunoassay or radio immunoassay, for gut hormone analysis.[372] These often measure the total amount of the peptide, rather than a specific form. In many cases, only certain forms of a hormone may be bioactive, so measuring the total concentration may not be entirely informative. In addition, some studies have shown changes in one form of a peptide, but not another (e.g. acylated ghrelin vs. total ghrelin), suggesting that measuring total peptide amounts is providing an incomplete picture.[363] Furthermore, degradation of some peptides (e.g. GLP-1 by DPP-IV) both in the blood and in stored samples could lead to inaccurate measurements and interpretations. In addition, since many gut hormones bind their receptors and exert actions in the gut, measurement of these peptides in venous blood may not be meaningful in terms of their physiological effects.

The primary reason for measuring gut hormones following fiber intervention is to identify potential mechanisms by which fiber may influence appetite. However, it is important to consider the fact that individual gut hormones are not released in isolation following a meal. Instead, they are released in concert with other hormones and peptides which act together to control the digestion and absorption process and signal energy needs. Nevertheless, most studies focus on individual hormones as independent contributors to the primary outcome of appetite. Specific combinations of gut hormones have been shown to have additive effects on outcomes such as inhibition of food intake, and other synergistic relationships may exist.[315] By studying each hormone in isolation, we may be missing the bigger picture.

Conclusion

The available research does not support a consistent effect of fiber on modifying circulating gut hormone levels. While it is possible that fiber does not influence appetite via gut hormone pathways, it is also possible that the lack of consistent study design merely prevents us from forming conclusions around this relationship. Current research uses a wide variety of fiber sources with different physical and chemical properties which may influence gut hormone response. Different fiber types may influence gut hormone levels based on their physicochemical properties, but additional research is required to examine this relationship. The relationship between fiber intake and appetite may also be mediated by mechanisms not detectable with the measurement of circulating gut hormone levels.

Figure 3-1 Search process and selection criteria diagram



* Reference lists of reviews from PubMed search were examined. References that met relevance criteria were included and later examined for quality

Table 3-1. Studies measuring effect of fiber on PYY

Ref	N	X/P	C/A	Fiber Type	Fiber Dose	PYY Increase vs. Control
[339]	14	X	A	barley β-glucan concentrate	3 g	**Yes**
[340]	14	X	A	oat β-glucan	2.2 g	No
				oat β-glucan	3.8 g	No
				oat β-glucan	5.5 g	**Yes** (2-4 h after test meal)
[341]	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
[342]	20	X	A	wheat bran	10 g	No
				oat bran	10 g	No
				wheat bran + oat bran	5 g each	No
[343]	14	X	A	wheat fiber	10.5 g	No
				oat fiber	10.6 g	No
[81]	20	X	A	mixed fiber	4 g	No
				mixed fiber	8 g	No
				mixed fiber	12 g	No
[344]	16	X	A	psyllium + low protein	7.6 g	No
				psyllium + low protein	27 g	**Yes**
				psyllium + high protein	6.2 g	No
				psyllium + high protein	25.8	**Yes**
[187]	10	X	C/A	inulin/oligofructose blend	16 g x 2 wks	**Yes** (but only at 10 min after standardized non-fiber meal on day 14)
[345]	54	P	C	Functional fiber blend	5 g/d x 1 wk, then 10 g/d x 2 wks	**Yes** (in BMI <23; values not different from baseline)

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; P, parallel design; X, crossover design

Table 3-2. Studies measuring effect of fiber on GLP-1

Ref	N	X/P	A/C	Fiber Type	Fiber Dose	GLP-1 Increase vs. Control
[341]	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
[81]	20	X	A	mixed fiber	4 g	No
				mixed fiber	8 g	No
				mixed fiber	12 g	No
[344]	16	X	A	psyllium + low protein	7.6 g	No
				psyllium + low protein	27 g	No
				psyllium + high protein	6.2 g	No
				psyllium + high protein	25.8	No
[187]	10	X	C/A	inulin/oligofructose blend	16 g x 2 wks	**Yes** (but only at 10 min after standardized non-fiber meal on day 14)
[345]	54	P	C	functional fiber blend	5 g/d x 1 wk, then 10 g/d x 2 wks	No (fasting values)
[346]	15	X	A	ordinary barley	20.2 g	**Yes**
				cut ordinary barley	19.4 g	No
				ordinary barley	9.9 g	No
				high amylose barley	38.1 g	No
				high β-glucan barley	81 g	No
				resistant starch	11.5	No
[347]	10	X	A	whole wheat bread	6.3 g	**Yes**
				whole wheat barley bread	5.5 g	No
[348]	20	X	A	whole kernel rye	12.8 g	No
				whole meal rye with oat β-glucan concentrate	17.1 g	**Yes** (but only at 120 and 150 min after meal)
				dark durum wheat pasta	5.6 g	No
[349]	19	X	A	endosperm rye	6.1 g	No
				whole-meal rye	15.2 g	No
				whole-meal rye enriched with rye bran	29 g	**Yes** (but only at 150 and 180 min after meal)
[350]	14	X	A	wheat fiber	10.5 g	No
				oat fiber	10.6 g	No
[351]	11	X	A	ancient wheat Einkorn	4-6 g	No
[352]	10	X	A	psyllium	1.7 g	No
				psyllium + fat	1.7g	**Yes**

Table 3-2. Studies measuring effect of fiber on GLP-1, continued						
Ref	**N**	**X/P**	**A/C**	**Fiber Type**	**Fiber Dose**	**GLP-1 Increase vs. Control**
[13]	10	X	A	pea fiber	25.5 g	No
[353]	58	X	A	guar gum (+galactose)	2.5 g	**Yes** (but vs. water; important kcal difference)
[354]	30	X	A	guar gum (+galactose)	2.5 g	**Yes** (but vs. water; important kcal difference)
[355]	12	X	A	inulin (+HFCS)	24 g	No

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; HFCS, high fructose corn syrup; P, parallel design; X, crossover design

Table 3-3. Studies measuring effect of fiber on CCK

Ref	N	X/P	C/A	Fiber Type	Fiber Dose	CCK Increase vs. Control
[340]	14	X	A	β-glucan	2.16 g	No
				β-glucan	3.82 g	No
				β-glucan	5.45 g	No
				β-glucan + oat β-glucan concentrate	5.65 g	No (Dose response in women)
[341]	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
[356]	16	X	A	oat bran	20 g	**Yes** (women only)
[357]	11	X	A	β-glucan enriched fraction of barley flour	15.7 g, including 5g β-glucan	No (elevated above baseline for 6 hrs vs. 3 hrs in ctl.)
				barley flour naturally high in β-glucan	15.7 g, including 5g β-glucan	No
[358]	25	X	C	partially hydrolyzed guar gum	20 g	No
[359]	10	X	A	bean flakes	12 g	**Yes**
[360]	26	X	A	complex carbohydrate	6.7 g	No (vs. low fiber, simple carbohydrate meal)
[361]	22	X	A	low glycemic index meal	35.5 g	No
[362]	12	X	A	low glycemic index meal	30 g	**Yes** (vs. high glycemic meal with equal fiber)

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; P, parallel design; X, crossover design

Table 3-4. Studies measuring effect of fiber on ghrelin

Ref	N	X/P	C/A	Fiber Type	Fiber Dose	Ghrelin Decrease vs. Control
[339]	14	X	A	barley β-glucan concentrate	3 g	**Yes** (AUC_{60-180})
[340]	14	X	A	β-glucan	2.16 g	No
				β-glucan	3.82 g	No
				β-glucan	5.45 g	No
				β-glucan + oat β-glucan concentrate	5.65 g	No
[341]	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
[342]	20	X	A	wheat bran	10 g	No
				oat bran	10 g	No
				wheat bran + oat bran	5 g each	No
[343]	14	X	A	wheat fiber	10.5 g	No
				oat fiber	10.6 g	No
[81]	20	X	A	mixed fiber	4 g	No
				mixed fiber	8 g	No
				mixed fiber	12 g	No
[344]	16	X	A	psyllium + low protein	7.6 g	No
				psyllium + low protein	27 g	No
				psyllium + high protein	6.2 g	No
				psyllium + high protein	25.8	No
[345]	54	P	C	functional fiber blend	5 g/d x 1 wk, then 10 g/d x 2 wks	No
[355]	12	X	A	inulin (+HFCS)	24 g	**Yes** (after a lunch 4-6 hours after the test meal)
[362]	12	X	A	low glycemic index meal	30 g	No
[363]	20	X	A	carob fiber (in mixed meal)	5 g	**Yes** (acylated only)
				carob fiber (in mixed meal)	10 g	**Yes** (acylated only)
				carob fiber (in mixed meal)	20 g	**Yes** (acylated only)

Table 3-4. Studies measuring effect of fiber on ghrelin, continued

Ref	N	X/P	C/A	Fiber Type	Fiber Dose	Ghrelin Decrease vs. Control
[364]	20	X	A	carob fiber (in glucose water	5 g	No
				carob fiber (in glucose water)	10 g	No
				carob fiber (in glucose water)	20 g	No
[365]	19	X	A	carob fiber	45 g	No
[366]	11	X	A	Arabinoxylan	6 g	No
[367]	12	X	A	Endosperm rye bread	6.7 g	No
				Whole grain rye bread	9.6 g	No
				Rye bran bread	12.3g	No
				Endosperm rye porridge	6.5 g	No
				Whole grain rye porridge	10.1 g	No
[368]	17	X	A	lupin kernel	15 g	**Yes**
[369]	19	X	A	fiber from dried plums	6 g	**Yes** (but only at 15 and 30 min after meal)
[370]	20	X	A	low kcal meal (fiber from fruit)	14 g	No
				high kcal, simple carbohydrate	12 g	No
				high kcal, complex carbohydrate	12 g	No
[371]	20	X	C	pullulan	12 g/d x 2 wks	No (fasting values)
				resistant starch	12 g/d x 2 wks	No (fasting values)
				soluble fiber dextrin	12 g/d x 2 wks	No (fasting values)
				soluble corn fiber	12 g/d x 2 wks	No (fasting values)

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; HFCS, high fructose corn syrup; P, parallel design; X, crossover design

Chapter 4

RESISTANT STARCH AND PULLULAN REDUCE POSTPRANDIAL GLUCOSE, INSULIN, AND GLP-1, BUT HAVE NO EFFECT ON SATIETY IN HEALTHY HUMANS[3]

[3] This research was supported by Tate & Lyle Health and Nutrition Sciences.

Chapter Synopsis

Dietary fiber may increase satiety and have beneficial effects on risk factors for chronic disease. The mechanism for this is not well understood, but may be related to changes in glucose, insulin, or gut hormone concentrations. The objective of this study was to determine the effects of three novel fibers on satiety and energy intake and to assess the relationship between these outcomes and serum parameters. Twenty healthy subjects (10 men and 10 women) with normal BMI (23±2 kg/m^2) participated in this randomized, double-blind, crossover study. Fasted subjects consumed a low-fiber control breakfast or 1 of 4 breakfasts containing 25 g fiber from soluble corn fiber (SCF) or resistant starch (RS), alone or in combination with pullulan (SCF+P and RS+P). Visual analog scales assessed hunger and satiety and blood samples were collected to measure glucose, insulin, ghrelin and glucagon-like peptide-1 (GLP-1) at various intervals after the meal. Food intake was measured at an *ad libitum* lunch and for the remainder of the day. The fiber treatments did not influence satiety or energy intake compared to control ($p>0.05$). RS+P significantly reduced glucose, insulin, and GLP-1 concentrations ($p<0.05$), but neither SCF treatment altered serum parameters compared to control. In conclusion, when provided as a mixed meal matched for calories and macronutrients, these fibers have little impact on satiety. Additional research regarding the physiological effects of these novel fibers is needed to guide their use as functional ingredients in food products.

Introduction

Fiber consumption is inversely associated with body weight, body fat, and BMI in cross-sectional studies, and fiber supplementation has been shown to improve weight loss in intervention trials.[29-33] A number of review articles have summarized the ability of dietary fiber to increase satiety and reduce energy intake.[35, 38, 57] However, variability in the literature on this topic makes generalizations difficult, and it is clear not all fibers are equally satiating.[79, 374] Characteristics of the fiber (e.g. solubility, fermentability, and viscosity), dose, duration of intake, and how the fiber is consumed may all influence the level of satiety achieved.

The mechanism by which fiber may impact satiety is not clear, but may be related to changes in appetite-related gut hormones. A number of peptides, including glucagon-like peptide-1 (GLP-1), have been shown to increase satiety and decrease energy intake in humans.[325] Conversely, ghrelin is known to stimulate hunger and energy intake.[113] While many studies evaluate changes in gut hormone concentrations following intake of carbohydrates, fats, and protein, few well-controlled studies measure changes in these hormones after fiber consumption.

Fiber may also influence satiety via effects on postprandial glucose and insulin concentrations. Certain fibers can delay gastric emptying and nutrient absorption, thus slowing delivery of glucose into the bloodstream.[63, 64] Some research suggests that foods that produce a slower, sustained glucose response are associated with increased satiety,[375, 376] although not all research supports this relationship.[86, 104]

Epidemiological data indicates that high postprandial glucose concentrations are an independent risk factor for cardiovascular disease (CVD) in individuals with diabetes[377, 378] and are associated with mortality from CVD as well as all-cause mortality in non-diabetic men and women.[379, 380] Therefore, dietary strategies to reduce the glycemic response to a meal may be useful for the prevention or management of diabetes and CVD. Addition of fiber to food products may improve glycemic response and have beneficial effects on risk factors for chronic disease.[97]

Fiber intake is low in the United States, with most individuals consuming only half the recommended levels.[381] In response to this, the addition of functional fibers to new or existing food products has been a growing trend in the food industry. However, little is known regarding the physiological effects of many of these fibers. Thus, the purpose of this study was to evaluate the effects of three novel fibers on glucose, insulin, and gut hormone response and to examine the relationship between these variables and subjective measures of appetite.

Subjects and Methods

Participant Eligibility

Twenty subjects were recruited via flyers posted around the University of Minnesota campus. Subjects initially completed a telephone screen to determine if they met the inclusion criteria. Eligible subjects were English speaking, healthy men and women aged 18 to 60 years, nonsmoking, non-dieting (weight stable over the past 3 months), with a body mass index (BMI) between 18.5 and 27 kg/m^2, and with normal

fasting blood glucose. Exclusion criteria were as follows: history of disease; gastrointestinal conditions affecting digestion and absorption; use of medications; food allergies to study products; persons who did not regularly consume breakfast; restrained eaters (score >10 on the dietary restraint factor of the Three Factor Eating Questionnaire [382] (Appendix C); vegetarians; individuals who consumed more than approximately 15 g of fiber per day; or women who were pregnant or lactating. This study was approved by the University of Minnesota Institutional Review Board Human Subjects Committee. Written informed consent was obtained from all subjects prior to the start of the study (Appendix D).

Screening and Study Visits

Eligible subjects attended a screening visit at the General Clinical Research Center (GCRC). The study coordinator verified medical history and anthropometric measurements, and fasting blood glucose less than 126 mg/dL was confirmed via finger stick. Subjects were instructed to follow a low-fiber, lead-in diet and to avoid fiber supplements, alcohol, and excessive exercise for 24 hours before each study visit.

On 5 separate occasions, subjects arrived at the GCRC following a 12 hour fast. Each visit lasted approximately 4 hours and was separated by a washout period of at least 3 weeks. Women were only scheduled during the follicular phase of their menstrual cycle, so some visits were more than 3 weeks apart. At the start of each visit, an IV was placed in the antecubital vein, followed by a 10 minute break to ensure the stress of venepuncture did not alter baseline hormone concentrations.[383] Study staff then

instructed subjects on the use of computerized visual analog scales (VAS), and subjects completed baseline appetite measures. Immediately following completion of the VAS, nursing staff drew baseline blood samples for glucose, insulin, ghrelin, and GLP-1. Subjects then received a low-fiber control breakfast or 1 of 4 fiber-containing breakfasts and were instructed to consume the entire meal within 20 minutes. Participants were not allowed to consume any additional food or water for the duration of the study.

Appetite ratings were recorded by VAS and blood samples were drawn for glucose and insulin at 15, 30, 45, 60, 90, 120, and 180 minutes after completion of the test meal. Ghrelin and GLP-1 were assessed at 30 and 60 minutes after the meal. Subjects rated palatability of the test meal at the 15 minute time point. Completion of the VAS always preceded blood sampling. The IV was removed following the 180 min blood draw and subjects were then offered an *ad libitum* buffet lunch. The lunch consisted of a variety of pre-weighed food items, including sandwiches, soup, salad, fresh fruits and vegetables, dessert, and beverages (Appendix E provides a full list of items available at lunch). Subjects were instructed to eat until comfortably full. After 30 minutes, lunch items were removed and weighed to calculate energy intake. Prior to discharge from the GCRC, a registered dietitian instructed subjects on completing a detailed food record for the remainder of the day.

Test Breakfasts

Subjects consumed the 5 test breakfasts in a randomized, crossover design. Meals consisted of a muffin, hot cereal, and a fruit flavored beverage powder mixed into 250 ml

water (Appendix F provides information on preparation of the test breakfasts). The fiber treatments provided 25 g fiber from soluble corn fiber (SCF) or resistant starch (RS) alone or in combination with 5 g pullulan (SCF+P and RS+P). The control treatment contained fully digestible maltodextrin. All test products were provided by Tate and Lyle Inc. (Decatur, Ill., USA). Treatments were similar in appearance and were matched for calories, macronutrient content, and available carbohydrates (**Table 4-1**). Muffins were stored at -20°C and thawed at room temperature 2 hours prior to each subject visit.

Visual Analog Scales (VAS)

Ratings of hunger, satisfaction, fullness, and prospective food intake were assessed using a previously validated 100 mm VAS.[39] The questions appeared as follows: How hungry do you feel? Not hungry at all (0 mm) to I have never been more hungry (100 mm); How satisfied to do you feel? I am completely empty (0 mm) to I cannot eat another bite (100 mm); How full do you feel? Not at all full (0 mm) to Totally full (100 mm); How much do you think you can eat? Nothing at all (0 mm) to A lot (100 mm).

Subjects also completed five VAS questions to assess the palatability of the test breakfasts. Visual appeal, smell, taste, and overall pleasantness were rated from good (0 mm) to bad (100 mm). Aftertaste was rated from much (0 mm) to none (100 mm). Appendix A provides the VAS for satiety and palatability.

Dietary Intake Analysis

Food records were analyzed using the Nutrition Data System for Research (NDSR, version 2008, Nutrition Coordinating Center, Minneapolis, MN) program for determination of energy, carbohydrate, fat, protein, and fiber intake.

Sample Collection and Analysis

Glucose and insulin were analyzed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center. Glucose was measured by the hexokinase method (Roche Diagnostics, Indianapolis, IN) and insulin was determined by the double monoclonal antibody enzyme-linked immunosorbent assay method (Merodia AB, Uppsala, Sweden). Gut hormones were analyzed with commercially available kits from Millipore, St. Charles, MO (Total Ghrelin, Cat. # GHRT-89HK; Active Glucagon-Like Peptide-1, Cat. # EGLP-35K). Samples were collected and stored according to manufacturer's instructions (Appendix G).

Statistical Analysis

Subjects were randomized according to a Williams design that balanced treatments over visits and subjects (Appendix H). There were ten sequences, and the study was stratified so that both genders were assigned to each of the 10 sequences. Subjects were assigned to treatments in order of enrollment. The sample size for this study was chosen based on clinical research in humans.[39] The primary outcome variable

is a change on the VAS, where a difference of 10 mm is considered clinically meaningful.

Concentrations of gut hormones, glucose, and insulin are expressed as change from baseline and were compared using area under the curve (AUC), calculated using the trapezoidal rule. Change from baseline AUC for the blood parameters and *ad libitum* food intake were compared among treatments using a mixed effects linear model with a random subject effect (Proc Mixed). This procedure calculated treatment means, standard error, and statistical differences among means. Carryover and interaction terms were tested in each model but were dropped from the final models because they were not significant. Data are presented as means ± SEM. Spearman correlation coefficient tests were performed to determine relationships between selected variables. Statistical significance was achieved at $p<0.05$. All analyses were completed with SAS 9.2 (SAS Institute, Cary, N.C., USA).

Results

Subject Characteristics

Twenty subjects (10 men and 10 women) participated in this study. All 20 subjects completed all 5 study visits. The mean BMI was 23±2 kg/m^2 and the mean age was 29±8 years. Fasting values for glucose, insulin, GLP-1, and ghrelin did not differ among treatments.

Satiety-Related Questions

AUC hunger, satisfaction, and fullness were not different among fiber treatments. AUC prospective food intake did not differ for any of the fiber treatments compared to control, but SCF+P differed from SCF: subjects felt they could eat more following the SCF+P treatment than after the SCF treatment (**Figure 4-1**).

Food Intake

Energy intake at the lunch buffet and for the remainder of the day as reported by food records did not differ among treatments (**Figure 4-2**). There were also no differences in grams of carbohydrate, fat, protein, or fiber consumed during the post-intervention period (data not shown).

Glucose and Insulin

The postprandial glucose and insulin response curves are displayed in **Figure 4-3**. The RS and RS+P treatments resulted in significantly reduced AUC glucose compared to control. The glucose response following the SCF and SCF+P treatments did not differ from control or the RS treatments. AUC insulin was significantly reduced following the RS+P treatment compared to control and the SCF treatment. Glucose and insulin did not correlate with any of the subjective appetite measures, but there was an inverse relationship between AUC insulin and calories consumed at lunch and for the remainder of the day (Spearman $r = -0.37$, $p=0.0003$).

Gut Hormones

AUC GLP-1 was significantly reduced following the RS+P treatment compared to control and the SCF treatments (**Figure 4-4**). AUC GLP-1 was significantly correlated with the subjective measures of appetite. Higher concentrations of GLP-1 were associated with greater fullness (Spearman r=0.30, p=0.002) and satisfaction (Spearman r=0.30, p=0.002) and lower hunger (Spearman r=-0.25, p=0.01) and prospective food intake (Spearman r=-0.24, p=0.02). AUC ghrelin did not differ among treatments (Figure 4-4) and did not correlate with any of the subjective appetite measures.

Breakfast Palatability

Ratings for visual appeal, smell, and aftertaste did not differ among treatments. Subjects rated the taste of both SCF breakfasts similar to the control and more favorably than both RS breakfasts (**Figure 4-5**). The taste of the RS+P breakfast was the least preferred and was also significantly lower than control. Rating for overall pleasantness followed a similar pattern: both SCF breakfasts were rated as significantly more pleasant than the control and both RS treatments. The RS+P treatment had lower overall pleasantness than control.

Discussion

Novel dietary fibers are continuously being developed to increase fiber content in foods, but limited information is available regarding the physiological effects of these ingredients in humans. SCF, RS, and pullulan are glucose polymers that are resistant to

digestion but differ in physicochemical properties. SCF is formed from the hydrolysis of corn starch by heat and acid, followed by cooling to form a branched structure with both digestible and non-digestible bonds. The RS used in this study was produced from heat-moisture treated high amylose maize starch. It is insoluble and classified as a type 3 (retrograded) RS. Pullulan is produced from the fermentation of dextrin by *Aureobasidium pullulans*. It is water soluble and forms a viscous solution when dissolved.

Increased satiety is a commonly reported benefit of dietary fiber consumption. In the present study, despite providing high levels of fiber, there were no differences in any of the subjective appetite sensations or energy intake compared to the low fiber control. Our results are consistent with data showing minimal impact of RS on satiety. de Roos et al. found that supplementation with 30 g/d type 2 (intrinsically resistant) RS or type 3 RS had little effect on appetite or energy intake compared to glucose.[17] Similarly, consumption of 48 g type 2 RS divided over two meals had no effect on appetite ratings, but did reduce energy intake at an *ad libitum* evening meal.[384] Intake of two preloads containing 11.2 g type 3 RS each had no effect on satiety or food intake compared to an isoenergetic, low fiber control.[20] In contrast, Willis et al. reported increased satiety with consumption of 8 g RS.[79] Some research suggests RS may have a delayed impact on satiety mediated by colonic fermentation and production of short chain fatty acids.[346] The duration of our study may not have been long enough to capture the influence of these effects on appetite.

The effect of SCF on satiety has not been well studied. Supplementation of two carbohydrate beverage preloads with 11.8 g SCF each had no effect on appetite ratings or energy intake at a subsequent lunch compared to an isoenergetic control.[20] The amount of fiber provided was similar to the current study and suggests that SCF has minimal effects on satiety when added to a carbohydrate beverage or a mixed meal.

Interestingly, we found that prospective food intake was greater (AUC was less negative) during the postprandial period following the SCF+P treatment compared to SCF. This effect may be related to differences in the insulin responses elicited by these treatments. AUC insulin was significantly higher after SCF compared to SCF+P, and was negatively correlated with energy intake. A meta-analysis by Flint et al. found that postprandial insulin was associated with increased satiety and decreased hunger and energy intake in normal weight subjects.[93] Furthermore, there is evidence that insulin is a regulator of ghrelin suppression.[132, 133, 385] It is possible that higher insulin concentrations following SCF caused greater suppression of ghrelin over the postprandial period, and this may have altered appetite sensations. However, since ghrelin was only measured for 60 minutes after the test meal, we are unable to confirm that effect in this study. Additionally, despite lower insulin responses with the two RS treatments compared to SCF, there were no differences in appetite sensations. This indicates that additional factors are involved in regulation of satiety and energy intake.

The reduction in glycemic response following the RS treatment is consistent with other studies reporting lower glycemic and/or insulinemic responses following acute or chronic intake of RS.[384, 386-388] Addition of 5 g pullulan to the RS treatment (RS+P)

resulted in lower AUC for both glucose and insulin compared to control. Wolf et al. found that consumption of 50 g pullulan attenuated the postprandial glucose response compared to maltodextrin, resulting in 50% lower incremental AUC (iAUC). [24] A reduction in postprandial glucose and insulin was also observed following consumption of a beverage containing 25 g pullulan.[18] Although the doses used in these studies are higher than that used in the present study, this suggests that addition of pullulan to the RS meal contributed to the reduction in the glycemic and insulinemic response.

Alternatively, the SCF and SCF+P treatments did not alter the glucose or insulin response compared to the control meal. These results differ from a previous study in which subjects consumed 25 g pullulan, SCF, RS, or a 50/50 blend of SCF and pullulan mixed with a lemonade beverage.[18] The iAUC for glucose and insulin was significantly lower for all fiber treatments compared to glucose. However, these meals were not matched for available carbohydrates, so these differences likely reflect the greater availability of digestible carbohydrate in the control treatment. Our results suggest that when provided as a mixed meal matched for macronutrient and available carbohydrate content, SCF does not reduce the glucose or insulin response to a meal. However, SCF may still be useful for attenuating postprandial glucose concentrations if used to lower the available carbohydrate content of a food product. Future studies should examine this application for SCF in a mixed meal, which may be more physiologically relevant than a carbohydrate beverage.

Modulation of gut hormones is a potential mechanism by which fiber might influence satiety, yet few studies evaluate gut hormone concentrations following a mixed

meal containing fiber. We found that AUC GLP-1 following consumption of RS+P was significantly lower than GLP-1 concentrations following the low-fiber control. Others have also reported a suppressive effect of fiber on GLP-1.[61, 81, 344] These studies used viscous fibers, which may have delayed gastric emptying and nutrient absorption, resulting in fewer nutrients acting to stimulate GLP-1 release. Pullulan is a viscous fiber and therefore may have influenced GLP-1 release via this mechanism. This would also be consistent with the reduced glycemic response observed for the RS+P treatment in this study. However, to our knowledge, the effect of pullulan on gastric emptying has not been evaluated. All other fiber treatments resulted in AUC GLP-1 values that were not different from control. These results are consistent with other studies finding no effect of fiber on postprandial GLP-1 concentrations.[350-352, 386]

We also found that postprandial ghrelin concentrations were not different among treatments. Ghrelin decreases rapidly following nutrient intake, with the depth and duration of suppression related to caloric load and meal composition. In our study, ghrelin values were not yet returning to baseline at 60 minutes. Other studies have reported differences in ghrelin when measured for several hours after a test meal.[339, 355] It is possible that the time frame of measurement in this study was too short to capture differences in duration of ghrelin suppression. In general, the results of this study do not support the hypothesis that fiber influences satiety via effects on gut hormones.

The SCF and SCF+P treatments were generally rated as more palatable than the RS and RS+P treatments. However, this did not correspond to differences in appetite ratings between these treatments. This is consistent with a review paper which found that

palatability has an inconsistent effect on appetite following a test meal.[52] Our results indicate that SCF can be added to food products at high levels without negatively impacting taste, and therefore may be useful for increasing fiber in the diet.

Conclusion

Addition of 25 g fiber to a meal had no effect on subjective appetite ratings or *ad libitum* energy intake in healthy volunteers. Postprandial serum parameters varied by fiber treatment. RS, alone or in combination with pullulan, significantly reduced glycemic response compared to control. In contrast, treatments containing SCF did not alter any serum parameters compared to control. This further highlights the importance of evaluating the physiological effects of novel fibers *in vivo* in order to guide their use as functional ingredients in food products.

Table 4-1. Composition of Test Meals[1,2]

Treatment	Fiber (g)	Fat (g)	Protein (g)	Calories	Available Carbohydrate (g)	Water (g)
Control	2.8	12.7	10.4	591.3	104.9	372.1
SCF	27.8	12.6	10.3	617.1	103.9	347.8
SCF + P	27.8	12.6	10.3	614.1	103.7	347.9
RS	27.2	12.8	10.3	589.4	105.8	349.5
RS + P	27.2	12.8	10.3	586.4	105.7	349.7

[1]Nutrition content listed per test breakfast. All data provided by Tate and Lyle. Treatment materials were analyzed as dietary fiber by AOAC method 991.43 or AOAC method 2001.03.

[2]P, pullulan; RS, resistant starch; SCF, soluble corn fiber

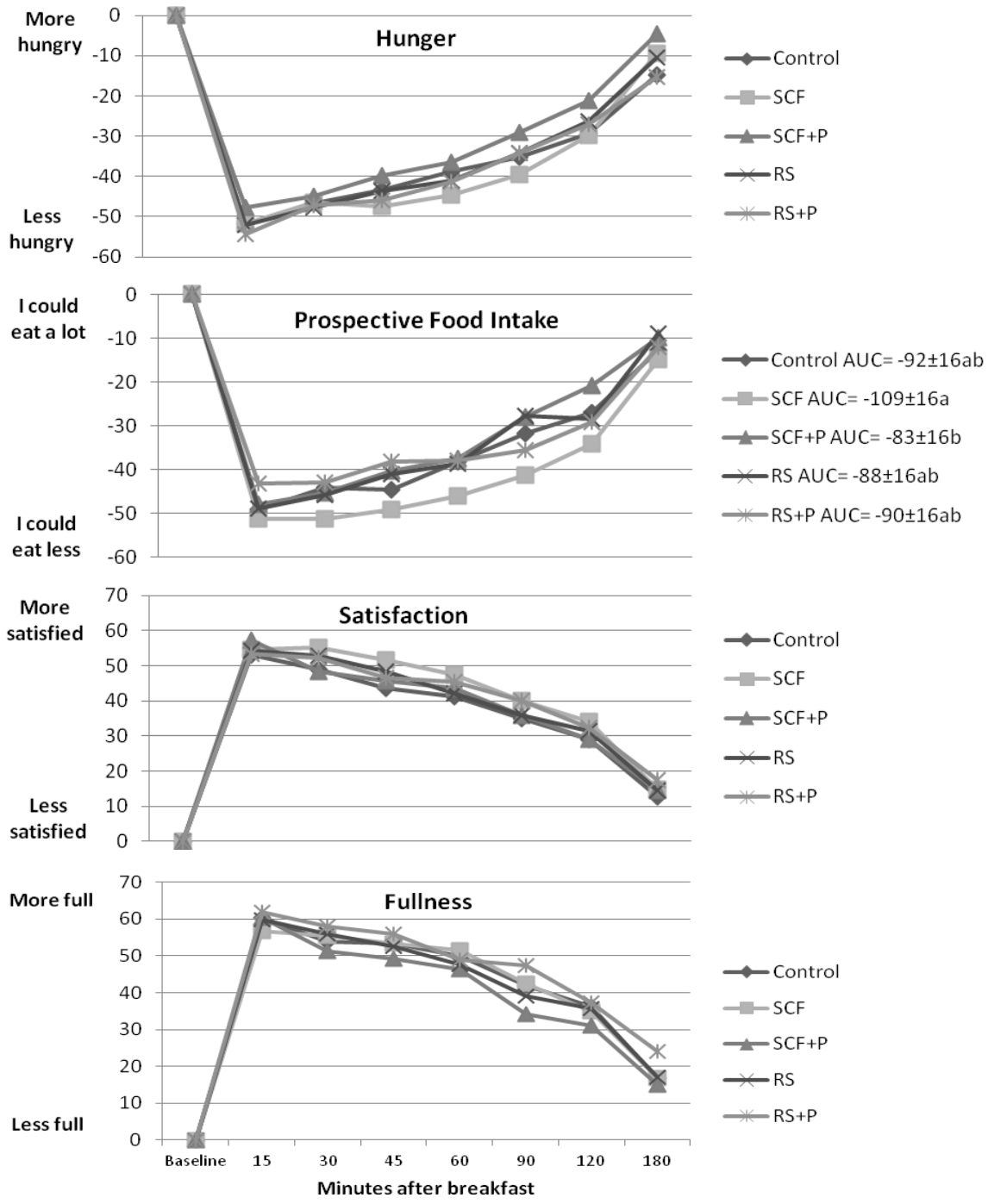


Figure 4-1. AUC for satiety-related questions, expressed as change from baseline. For prospective food intake, the numbers following the fiber treatment in the legend represent AUC score ± SEM. Treatments with different letters have statistically different AUC ($p<0.05$). AUC scores are not shown if there were no significant differences among treatments. P, pullulan; RS, resistant starch; SCF, soluble corn fiber

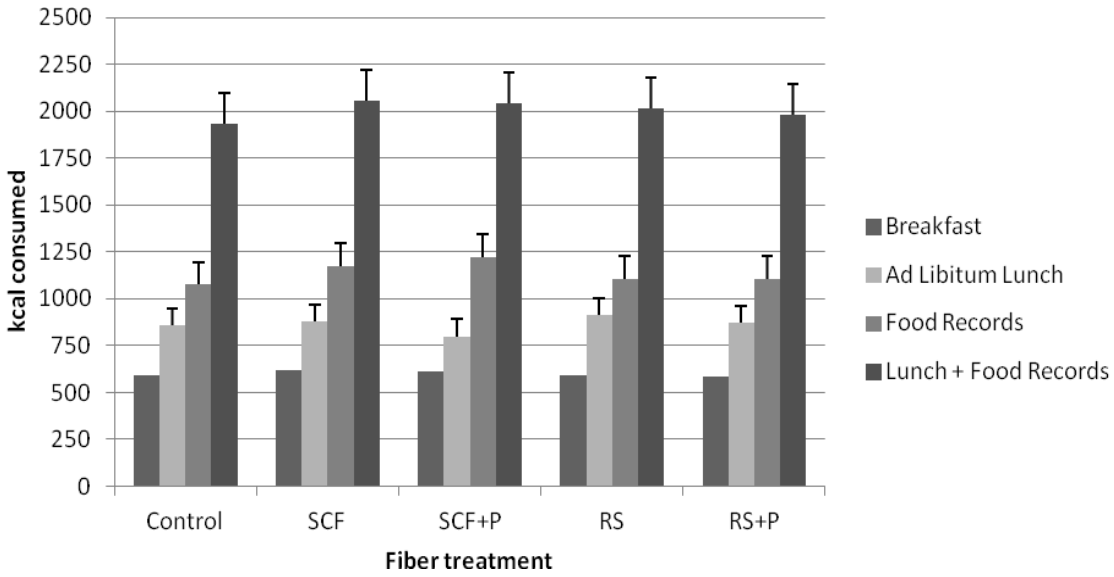


Figure 4-2. Calorie intake (mean±SEM) throughout the day of the intervention. There were no significant differences in calories consumed at the lunch buffet or the remainder of the day as reported by food records. Total intake after breakfast (lunch + food records) was also not different. P, pullulan; RS, resistant starch; SCF, soluble corn fiber

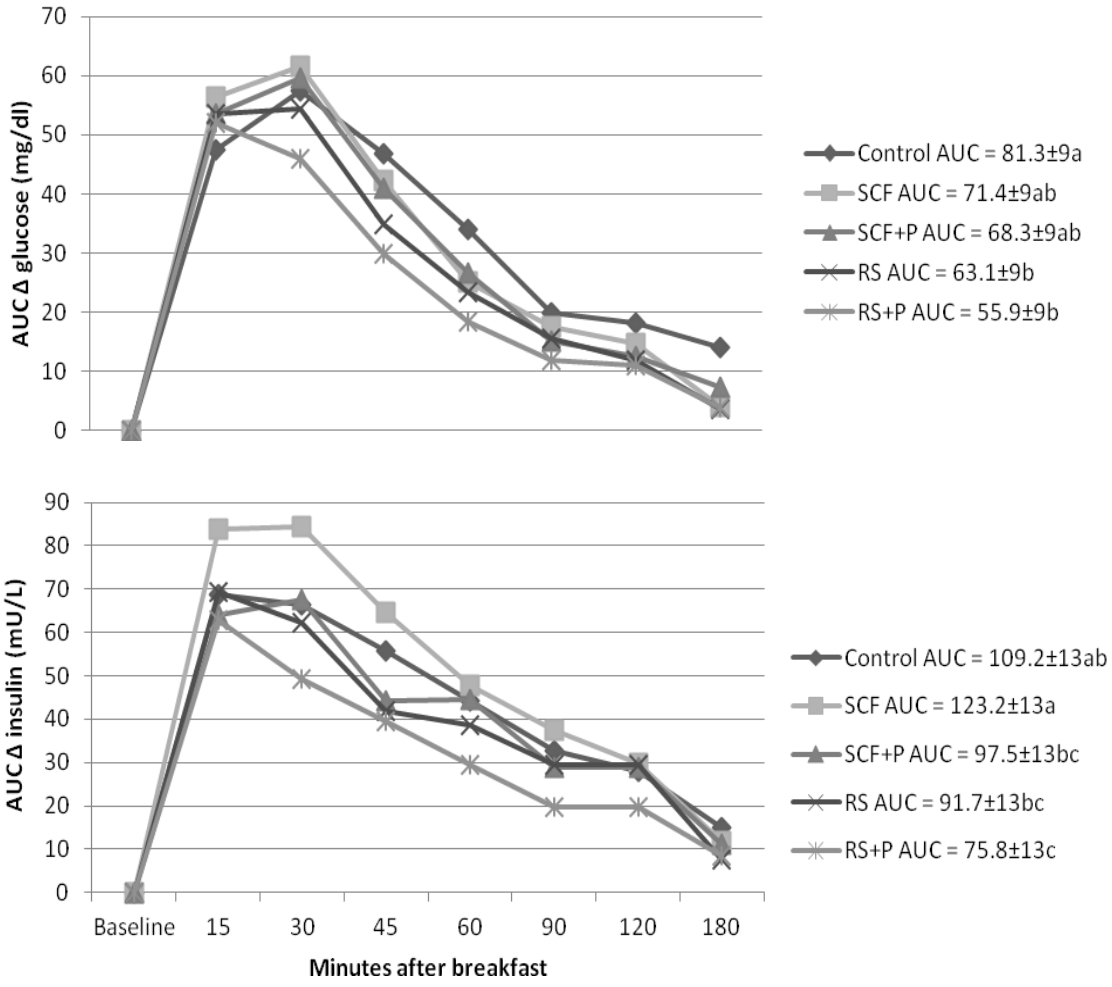


Figure 4-3. AUC glucose (top) and insulin (bottom), expressed as change from baseline. The numbers after each treatment represent the AUC±SEM. Treatments with different letters have statistically different AUC ($p<0.05$). P, pullulan; RS, resistant starch; SCF, soluble corn fiber

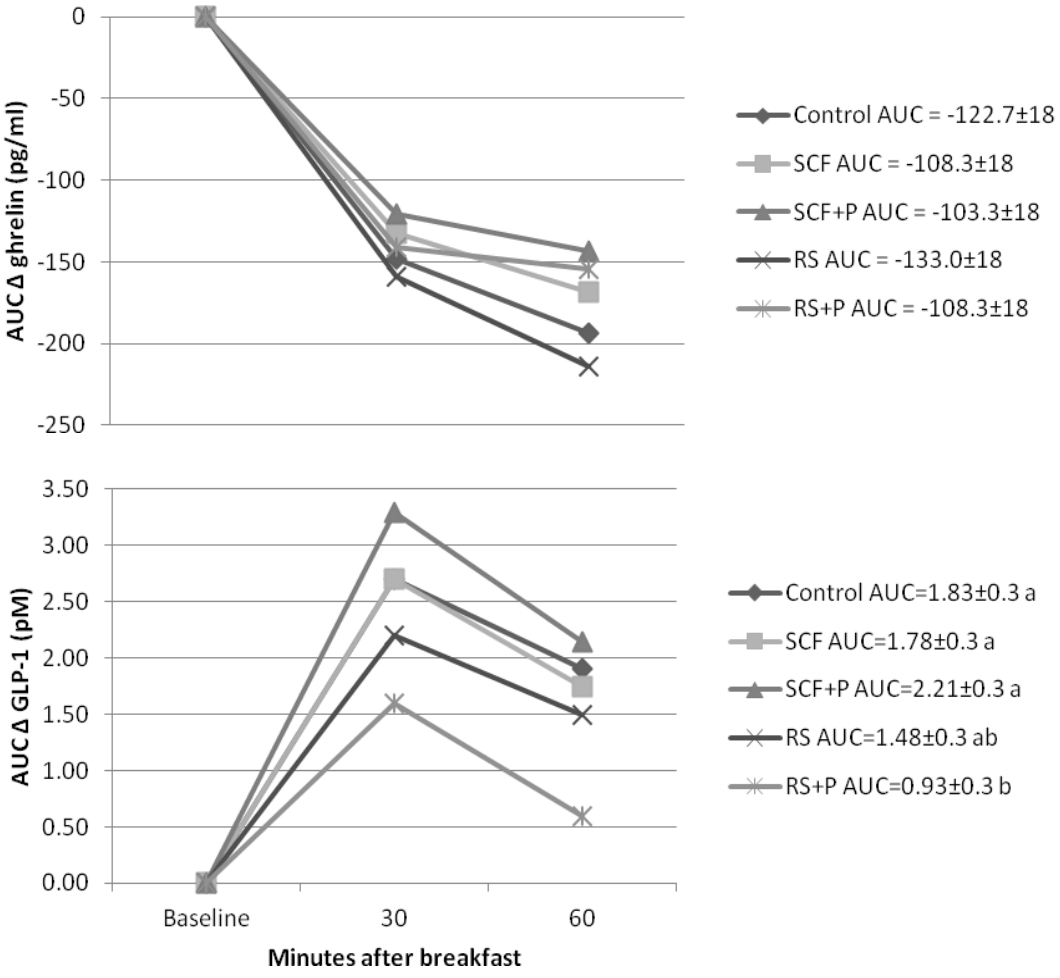


Figure 4-4. AUC ghrelin (top) and GLP-1 (bottom), expressed as change from baseline. The numbers after each treatment represent the AUC±SEM. Treatments with different letters have statistically different AUC ($p<0.05$). GLP-1, glucagon-like peptide-1; P, pullulan; RS, resistant starch; SCF, soluble corn fiber

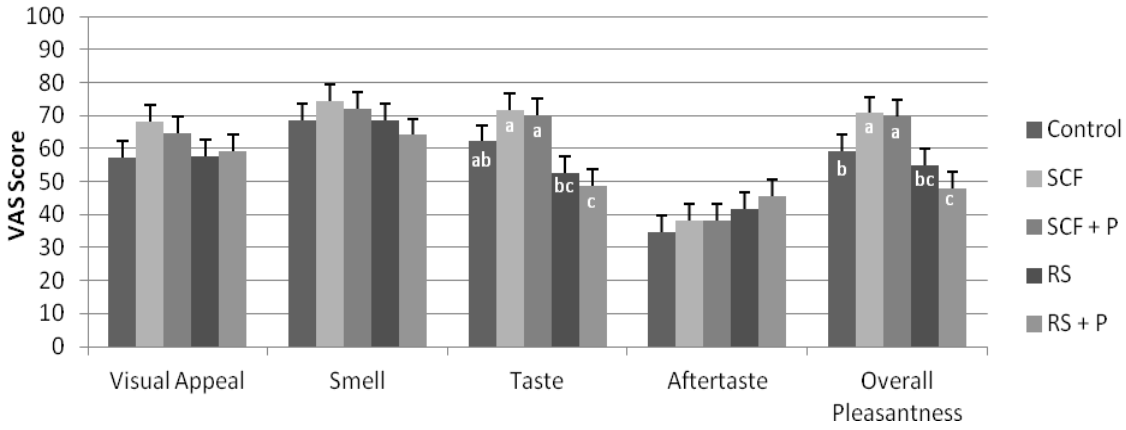


Figure 4-5. Palatability ratings (mean±SEM) for the test breakfasts. A higher score indicates better visual appeal, smell, taste, and overall pleasantness and more aftertaste. Within a palatability category, treatments with different letters have statistically different palatability ratings ($p<0.05$). P, pullulan; RS, resistant starch; SCF, soluble corn fiber; VAS, visual analog scale

Chapter 5

GASTROINTESTINAL EFFECTS OF RESISTANT STARCH, SOLUBLE CORN FIBER, AND PULLULAN IN HEALTHY ADULTS[4]

[4] This work was supported by Tate & Lyle Health and Nutrition Sciences.

Chapter Synopsis

Fiber has been shown to exert a number of benefits on gastrointestinal (GI) health, yet intake is low. Addition of novel fibers to food products may increase fiber intake and improve gut health. Our objective was to evaluate the influence of three novel fibers on GI outcomes in healthy humans. Twenty healthy participants (10 men and 10 women) with normal BMI (23±2 kg/m^2) participated in this randomized, double-blind, crossover study with 5 treatment periods. Participants consumed a maltodextrin control or 20-25 g/d fiber from soluble corn fiber (SCF) or resistant starch (RS), alone or in combination with pullulan (SCF+P and RS+P). Treatment periods were 7 days with a 3 week washout between periods. Stool samples were collected on day 7 of each period, and GI tolerance was assessed via a questionnaire on day 1 and day 6. There were no treatment differences in stool weight or consistency. SCF significantly reduced stool pH and increased total SCFA production compared to RS and control. RS+P significantly increased the percentage of butyrate compared to all other treatments. Overall, GI symptoms were minimal. SCF+P led to the highest GI score on day 1, while RS+P had the highest score on day 6. Both SCF treatments caused a significant shift in the gut microbial community. These functional fibers are generally well tolerated, have minimal effects on laxation, and may lead to beneficial changes in SCFA production in healthy adults.

Introduction

Dietary fiber exerts a number of beneficial effects on gastrointestinal (GI) health, and fiber consumption is associated with reduced risk of colorectal cancer and other forms of chronic disease.[389] Despite this, fiber intake in the United States is low, with most individuals consuming only half the recommended amounts.[390]

Many of the potential health benefits are related to fermentation of fiber by gut bacteria. Fermentation leads to production of SCFA, the most abundant of which are acetate, propionate, and butyrate. While all SCFA have metabolic significance, butyrate is considered the most important for colonic health due to its effects on promoting normal colonocyte development. Additionally, SCFA production can lower luminal pH, which may inhibit growth of potentially pathogenic bacteria.[189] Fiber may also benefit laxation by increasing stool weight and improving stool consistency.

In recent years, the role of gut microbiota in human health has received increasing attention. While many studies have focused on the concept of prebiotics and the ability of fiber to alter levels of a few select bacterial species, there is now interest in assessing how diet influences the overall bacterial community. Terminal restriction fragment length polymorphism (TRFLP) analysis is a bacterial fingerprinting technique that provides a rapid overview of interindividual differences in the gut microbial community (GMC). This technique has been used previously to identify changes in gut microbiota in response to dietary interventions.[391]

Although fiber has many potential health benefits, increasing fiber in the diet can also lead to undesirable side effects such as gas and bloating. These symptoms may act

as a deterrent for fiber intake. Tolerance is defined as a state in which there is an absence of unwanted symptoms related to fiber consumption.[392] It is of interest to identify fibers that can be added to food products without causing intolerance in order to increase overall fiber consumption.

While many Americans could benefit from increasing the amount of fiber in their diets, it is important to consider the type of fiber in order to balance tolerance and physiological benefits. Functional fibers are constantly being developed by the food industry for use as ingredients in food products. However, little research is available regarding the effects of novel functional fibers on GI health. Resistant starch (RS), soluble corn fiber (SCF), and pullulan (P) are maize-based fibers that can easily be incorporated into foods or beverages. Previous studies have shown these fibers are well fermented,[393] but research regarding their physiological effects *in vivo* is limited. Therefore, the objective of this study was to examine the influence of these fibers on laxation, GI tolerance, SCFA production, and the GMC in healthy humans.

Experimental Methods

Participants

Twenty participants (10 men and 10 women) were recruited via flyers posted around the University of Minnesota campus. Participants were initially screened over the phone to determine eligibility for the study. Eligible participants were English-speaking, healthy men and women between 18 and 60 years of age, non-smoking, not taking medications, weight stable, and had a BMI between 18.5 and 27 kg/m^2. Participants were

excluded if they had a history of cardiovascular, renal, or hepatic disease, diabetes mellitus, gastrointestinal conditions affecting digestion and absorption, were vegetarians, or consumed more than approximately 15 g of fiber per day. Participants were not taking fiber supplements or laxatives and had not taken antibiotics for at least 6 months prior to the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Minnesota Institutional Review Board Human Subjects Committee. Written informed consent was obtained from all subjects prior to any study procedures (Appendix D).

Study Design

Prior to official enrollment, participants attended a screening visit at the University of Minnesota General Clinical Research Center (GCRC) to obtain informed consent, collect anthropometric measurements, verify medical history and receive study instructions and supplies. Eligible participants were instructed to follow a low-fiber, lead-in diet and to avoid fiber supplements, excessive exercise, and alcohol for 24 hours prior to study visits. Participants were required to maintain their current activity level and were instructed not to initiate a weight loss program for the duration of the study. Prior to any study visits, participants collected a baseline fecal sample while following their habitual diet.

Participants consumed five treatments in a double-blind, crossover design with treatment periods of 7 days followed by a 21-day washout period. On Day 1 of the study,

fasted participants arrived at the GCRC and consumed either a low-fiber control breakfast or 1 of 4 fiber-containing breakfasts. Meals consisted of a muffin, hot cereal, and fruit flavored beverage. For the next 6 days, participants consumed the study products at home. Treatments were provided as cereal bars and a beverage mix, which was pre-measured into 500-ml water bottles. Participants were instructed to consume 4 cereal bars and 1 beverage over the course of each day.

The test breakfast on day 1 supplied 25 g SCF or RS alone or in combination with 5 g pullulan (SCF+P and RS+P). The cereal bars and beverage contained the same fiber treatments, but at a slightly lower dose of 20 g supplemental fiber per day. All test products were provided by Tate and Lyle Inc. (Decatur, Ill., USA). Study products were matched for macronutrient and energy content, and were consumed along with the participants' habitual diets. The compositions of the control and fiber treatments are displayed in **Table 5-1**.

SCF is produced via hydrolysis of corn starch, followed by cooling to form a branched structure. It has an average degree of polymerization of 10. The RS used in this study is classified as type 3 RS (RS3, retrograded starch) produced from heat-moisture treated high-amylose maize starch. Pullulan is a linear glucose homopolysaccharide formed via the fermentation of dextrin by the yeast *Aureobasidium pullulans*. Resistance to degradation by human digestive enzymes is due to the presence of α-1,6 bonds, as well as to steric hindrance which limits access to α-1,4 linkages.[18] The control treatment was fully digestible maltodextrin. Treatment materials were analyzed

as dietary fiber by AOAC method 991.43 or AOAC method 2001.03. Dietary fiber analyses were provided by Tate and Lyle Inc.

Stool Records and Tolerance

Prior to leaving the GCRC, participants were given instructions on completing a GI symptoms questionnaire for Day 1 and Day 6 of the study. The symptoms questionnaire assessed stool frequency and consistency, as well as GI side effects (Appendix B). Symptoms included flatulence, bloating, abdominal cramps, and stomach noises and were rated on a 10-point scale where 1=none and 10=excessive. Stool consistency was assessed on a scale of 1 to 4, where 1=liquid and 4=hard. Participants completed a daily record of study product consumption and were asked to return any uneaten study products to assess compliance. Participants were also instructed on the collection and delivery of a fecal sample on Day 7 of each treatment period. Participants collected one fresh stool sample using the Commode Specimen Collection Kit (Sage Products, Crystal Lake, Ill., USA) and sample collection bags provided by study staff. Participants delivered samples to the GCRC on ice within 2 hours of defecation.

Stool sample collection and bacteria DNA extraction

Immediately following delivery of stool samples by participants, study staff collected two pea-sized samples of fresh feces and added them to a tube containing 5 ml RNAlater (Ambion, Austin, TX). The collection tube was inverted 15 times, and samples were stored at -80°C. Frozen samples were shipped to Fred Hutchinson Cancer Research

Center (FHCRC; Seattle, WA) for analysis. Fecal samples in RNAlater were homogenized using an OMNI tissue homogenizer (OMNI Inc., Marietta, GA) and aliquoted into 300μL volumes. Fecal bacterial genomic DNA was extracted in duplicate using a QIAamp stool minikit (Qiagen, Valencia, CA) with 1 minute bead beating.[394]

Fecal Chemistry and Short Chain Fatty Acids

Stool wet weight was determined in grams by weighing the filled collection bag on a balance and subtracting the average bag weight. Stool consistency was determined subjectively by investigators and rated using King's Stool Chart.[395] Fecal samples were homogenized with a hand blender and pH was determined in an aliquot using a glass electrode at 25°C (Orion PerpHecT LogR meter, model 350; Thermo Electron Corporation, Beverly, Mass., USA).

Acetate, propionate, butyrate, and total SCFAs were extracted in duplicate and concentrations were determined via gas chromatography using the method described by Schneider et al., with minor modifications.[303] Briefly, 200 mg of stool was suspended in 1.6 ml distilled water. Two ml diethyl either and 0.4 ml sulfuric acid (50%) were added, along with 2μl ethyl butyrate as the internal standard. Samples were mixed in an orbital shaker for 45 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred to a glass test tube and residual water was absorbed using calcium chloride. Samples were filtered using a 1-ml syringe (Sherwood Medical, St. Louis, MO) and a Fisherbrand nylon filter (13 mm, pore diameter 0.2 mm; Fisher Scientific, St. Louis, MO) and frozen at -80°C until analysis via gas chromatography. Analysis was conducted

using a Stabilwax DA column (30 m, 0.52 mm internal diameter, 1-μm film thickness; Restek, Bellefonta, PA, USA) with helium as the carrier gas.

TRFLP (Terminal Restriction Fragment Length Polymorphism) Analysis

A TRFLP profile was generated for each extracted fecal bacterial genomic DNA sample using a protocol described previously, with minor modification.[394] Bacterial 16S rRNA genes were amplified with primers 11-27f and 519r (GWATTACCGCGGCGCTG). The forward primer is identical to 8-27f as described by Li *et al* except that the initial 5' AGA nucleotides were removed in order to reduce specificity and capture more GMC organisms.[394, 396]

Data Analysis

SCFA concentrations, stool weight, stool pH, stool frequency, and GI symptoms were compared among treatments using a mixed effects linear model with a random subject effect (Proc Mixed) using Statistical Analysis Systems statistical software package version 9.3 (SAS Institute, Cary, NC, USA). Carryover and period-treatment interaction terms were tested in each model, but were excluded from the final models since they were not significant. Paired *t* tests were used to determine differences in GI symptoms between Day 1 and Day 6. Data are presented as means ± standard error, adjusted for study visit. Statistical significance was set at $p<0.05$.

TRFLP profiles were analyzed with DAx software (Van Mierlo Software Consultancy, Eindhoven, The Netherlands) as previously described.[391] Non-metric

multidimensional scaling ordination (NMS) analysis was performed on the mean of duplicate P_i values using PC-ORD (MJM Software Design, Gleneden Beach, OR).[397] Permutational multivariate analysis of variance (perMANOVA) was used to test whether there was an effect of treatment on the composition of the GMC. All p-values were corrected for multiple comparisons using Bonferroni adjustments where borderline significance P=0.1/15=0.007 and significance P=0.05/15=0.0033.

To identify organisms (represented by TRFLP fragment length) that occurred uniquely in participants on different treatments, we performed indicator species analysis (ISA) in PC-ORD. We linked the TRFLP fragment lengths of the indicator peaks with gut microbial taxonomic annotations. Our reference database consisted of archived human GMC sequences from the comprehensive SILVA 102 Ref reference database of curated high-quality 16S rRNA gene sequences[398] and ~30,000 sequences generated from 10 individuals. We generated *in silico* terminal restriction fragments (TRF) from each of the 67,506 reference sequences using the Alu I and Rsa I restriction endonuclease cut site. TRF sequences with lengths (bp) matching the 2 RSA TRFLP indicator peaks (309 and 314 bp) plus or minus 2 bp were collected.

Results

Participants

All 20 participants who enrolled in the study completed all five treatments. Mean age and BMI were 29±8 years and 23±2 kg/m^2, respectively.

Stool Characteristics

Stool characteristics are presented in **Table 5-2**. Stool weight and stool consistency (investigator-reported and subject-reported) did not differ among treatments. Self-reported number of stools was greater on Day 6 following RS and RS+P compared to control ($p=0.0119$) and SCF ($p=0.0257$). Stool pH was significantly lower when participants consumed SCF compared with control ($p=0.0472$) and RS ($p=0.0457$), while pH values for SCF+P and RS+P were intermediate. These pH differences were reflected in the SCFA concentrations. Total SCFA were significantly higher for SCF compared to RS ($p=0.005$) and control ($p=0.007$), but did not differ from SCF+P or RS+P. The percentage of acetate was higher for SCF ($p=0.02$), SCF+P ($p=0.03$), and RS ($p=0.002$) compared to control. Both RS treatments resulted in a lower percentage of propionate compared to control and the SCF treatments ($p<0.0001$). The percentage of butyrate was significantly higher than all other treatments following RS+P ($p<0.001$), and was higher than both SCF treatments following RS ($p<0.01$).

GI Symptoms

GI symptoms ratings are reported in **Table 5-3**. On Day 1, participants reported greater bloating following consumption of RS+P compared to control ($p=0.0263$) and SCF ($p=0.0157$). Flatulence was highest when participants consumed SCF+P compared to control ($p=0.0271$) and SCF ($p=0.0111$), while the two RS treatments were intermediate. Abdominal cramps and stomach noise did not differ. GI score for SCF+P was significantly greater than SCF and control. On Day 6, RS+P caused greater bloating

than RS (p=0.0045), SCF (p=0.0105), and control (p=0.0045); greater flatulence than SCF (p=0.0452) and control (p=0.0023); and greater abdominal cramps and stomach noise than all other treatments. GI score for RS+P was significantly greater than all other treatments. When GI symptoms were statistically different between Day 1 and Day 6, symptoms were always rated lower on Day 6 (Table 5-3).

Gut Microbial Community

NMS analysis explained 81% and 86% of the total variation in the composition of the GMC using Alu I and Rsa I, respectively. In **Figure 5-1**, SCF and SCF+P tend to cluster at the bottom of the cloud of samples. PerMANOVA showed that there was a significant effect of treatment on the GMC measured using Rsa I ($p<0.0006$) but none using Alu I ($p> 0.05$). The GMC associated with baseline was significantly different from SCF ($p<0.001$) and SCF+P ($p<0.001$). Among treatments, the control was significantly different than SCF ($p<0.001$) and SCF+P ($p< 0.0002$). SCF was significantly different than RS ($p<0.007$), and SCF+P was significantly different than RS+P ($p<0.002$). The GMC following consumption of SCF and SCF+P were not significantly different from one another. ISA showed that Rsa I peak 309 was significantly enriched after SCF ($p<0.0006$) and Rsa I peak 314 was significantly enriched after SCF+P ($p<0.0014$). Rsa I peak 309 was identified as either *Anaerococcus vaginalis* or *Parabacteroides goldsteinii* and Rsa I peak 314 was identified as either *Parabacteroides distasonis* or *Parabacteroides merda* using an *in-silico* TRFLP

prediction program based on 16S rRNA sequences from the Silva database and human reference samples.

Discussion

Despite the relatively high dose of fiber provided in this study, few changes in stool characteristics were observed. The reduction in stool pH following consumption of SCF was minor (0.23 units), but was consistent with an earlier study in which participants consumed 21 g/d SCF for 3 weeks.[399] Walker et al. reported that a one-unit shift in pH had marked effects on bacteria populations and SCFA production *in vitro*.[400] However, the clinical significance of smaller changes in pH has not been well studied. In contrast to the present study, Stewart et al. found no differences in pH when participants consumed 12 g/d SCF[371], suggesting a higher dose may be needed for an effect. Most studies have reported a minimal effect of RS on pH.[170, 371, 401-404]

Fiber can increase stool weight via the physical presence of the fiber, the water held by the fiber, and increased bacterial mass from fermentation of the fiber.[3] In this study, the supplemental fibers had no effect on stool weight. In contrast, RS has been reported to increase stool wet weight by 0.7 to 2.7 g per g RS consumed, using doses from 25 to 55 g/d.[11, 205, 405, 406] Likewise, 21 g/d SCF was shown to increase fecal dry weight by 0.9 g per g fed.[399] However, these studies looked at stool weight over 3-5 day periods. In this study, stool weight was determined from a single sample, so it is possible any effect on laxation may have been missed due to the short collection period.

Normal stool frequency ranges from 3 times per day to 3 times per week.[166] The RS treatments led to a minor increase (0.56 stools) in the number of stools on Day 6. Most studies have reported no effect of RS on stool frequency in healthy participants when measured over the course of a week or longer.[401, 403, 406] Timm et al. reported an increase by 0.9 stools over a 5 d period for participants consuming 20 g/d SCF.[407] However, no laxative effect of SCF was found in this study. Again, this study is limited by the fact that stool frequency was only assessed for one day. In addition, this study was conducted in healthy individuals with normal bowel function. Fiber tends to have a normalizing effect on bowel frequency, after which only stool weight increases.[3] Thus, it is likely we would have seen a greater effect in constipated individuals.

SCFA are a marker of fermentation and are considered important for colonic health. We observed a significant increase in total SCFA following consumption of SCF. Previous studies have also reported higher fecal SCFA concentrations with SCF compared to control, although these differences did not reach significance.[371, 399] Changes in total SCFA concentrations mirrored differences in pH among treatments, suggesting that these acids may be a primary determinant of colonic pH. The increased percentage of butyrate following consumption of RS in this study is consistent with other reports both *in vitro* and *in vivo*.[11, 205, 408, 409] Given the growing evidence for a protective role of butyrate in colonic health, this suggests that addition of RS to food products may be beneficial.

GI tolerance is an important issue when considering fibers for addition to food products, as it may have an effect on the acceptability of the product by the consumer.

Mean symptoms scores for all treatments were low (1.6-4.4), indicating that the fibers were well tolerated overall. The SCF treatment appeared to be the best tolerated, and did not differ from control for any of the measurements. Previous studies have also found SCF to be well tolerated, with only minor increases in GI symptoms compared to control.[371, 399] RS was also well tolerated, and only differed from control for flatulence on Day 6. This is consistent with other research reporting increased flatulence with RS at doses ranging 10-39 g/d.[11, 371, 404]

Although SCF and RS were well tolerated alone, addition of pullulan led to an increase in most GI symptoms. Previous studies have also reported increased symptoms ratings following consumption of 12-50 g pullulan.[24, 371, 410] The present study used only 5 g pullulan, indicating that minor increases in GI symptoms are observed even at low levels. *In vitro* studies have found that pullulan is rapidly fermented and increases gas production.[22, 393] If pullulan is also rapidly fermented *in vivo,* this may explain the observed increase in symptoms, since gas is perceived more in the proximal bowel.[411] In contrast, RS and SCF were more slowly fermented *in vitro*[393], which may lead to improved tolerance.

GI symptoms tended to be lower on Day 6 than on Day 1. This may be related to distributing the fiber over the course of the day, rather than consuming the fiber in a single dose. This would reduce the amount of substrate available for fermentation and subsequent gas production. Research with sugar alcohols, another form of low digestible carbohydrate, has shown that ingestion of several divided doses is better tolerated than a single dose of the same amount.[392, 412] The reduction in GI symptoms from Day 1 to Day

6 may also be related to chronic ingestion of the same fiber source. Okubo et al. found that flatulence was reported at the beginning of the experiment but gradually declined within the first week of participants consuming partially hydrolyzed guar gum.[248] Others have reported habituation and adaptation of GI symptoms when fiber was consumed over a period of several weeks.[251, 413, 414]

To our knowledge, this is the first study to evaluate community-wide changes in microbiota following consumption of SCF and pullulan *in vivo.* Our analyses showed that there was a significant change in the GMC with dietary interventions. We used two fingerprinting approaches, Alu I-TRFLP, which focuses on the phylum Firmicutes, and Rsa I-TRFLP, which encompasses the phylum Bacteroidetes.[415] However, the changes with the dietary enrichment were only associated with the Rsa I-TRFLP. In particular, the relative abundance of TRFLP peaks, putatively associated as members of the Parabacteroides genus, increased in abundance when participants consumed SCF and SCF+P. These organisms are non-butyrate producing bacteria which have previously been associated with RS enrichment.[404] Surprisingly, the RS treatments did not cause an increase in these bacteria in the present study. This may be explained by the type of RS used. In the study by Martinez et al., type 4 RS (chemically modified starch) increased *Parabacteroides distasonis,* whereas type 2 RS (granular starch) had no effect; type 3 RS, which was used in the present study, was not evaluated. Walker et al. reported an increase in *Ruminococcus bromii* and *Eubacterium rectale* when overweight participants consumed type 3 RS.[416] This was not observed in the present study, and may be due to differences in microbiota between lean and overweight individuals. These studies

suggest that the effects of fiber on the GMC cannot be generalized, even within a specific class of fiber. While this study provided an initial screening of the effect of fiber intervention on the GMC, future studies could include a more thorough characterization of the GMC by sequencing the 16S rRNA genes.

Conclusion

Consumption of relatively large doses (20-25 g/d) of RS and SCF were well tolerated and had minimal effects on laxation. Further research using a longer period of bowel habit evaluation in individuals with constipation may help identify potential laxation benefits of these fibers. SCF increased total SCFA production, while RS improved the ratio of butyrate, suggesting fermentation of these fibers may have beneficial effects in the colon. Additional research is needed to further explore the effects of these fibers on gut microbiota and possible implications for human health.

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Table 5-1. Composition of the test meals.

	Treatment				
	Control	SCF	SCF+P	RS	RS+P
Test Breakfast (Day 1)					
Fiber (g)	2.8	27.8	27.8	27.2	27.2
Fat (g)	12.7	12.6	12.6	12.8	12.8
Protein (g)	10.4	10.3	10.3	10.3	10.3
Energy (kcal)	591.3	617.1	614.1	589.4	586.4
Available carbohydrate (g)	104.9	103.9	103.7	105.8	105.7
Water (g)	372.1	347.8	347.9	349.5	349.7
Cereal bars and beverage (Days 2-7)					
Fiber (g)	3.84	23.8	23.8	22.3	22.3
Energy (kcal)	642.2	668.9	668.9	639.5	639.3
Available carbohydrate (g)	135.2	135.2	135.2	133.7	133.7

SCF, soluble corn fiber; P, pullulan; RS, resistant starch

All data provided by Tate and Lyle.

Table 5-2. Stool pH, weight, number, consistency, and SCFA concentrations of healthy adults consuming soluble corn fiber (SCF; 20 g/d) or resistant starch (RS; 20 g/d) alone or in combination with 5 g pullulan (SCF+P and RS+P) or no supplemental fiber (maltodextrin control).

	Treatments					
	Control	SCF	SCF+P	RS	RS+P	SEM
Stool pH	6.70[a]	6.47[b]	6.54[a,b]	6.70[a]	6.59[a,b]	0.1
Stool weight (g)	100.1	94.3	102.0	119.0	109.6	14
Number of stools (self-reported)	1.15[b]	1.15[b]	1.37[a,b]	1.71[a]	1.65[a]	0.2
Stool consistency (self-reported)*	2.08	2.33	2.32	2.13	2.27	0.2
Stool consistency (investigator-reported)†	2.00	2.20	2.10	2.15	2.35	0.2
Total SCFA (μmol/g stool)	31.2[b]	35.5[a]	32.9[a,b]	31.1[b]	33.7[a,b]	1.7
SCFA ratio (% of total SCFA)						
Acetate	38.9[b]	40.7[a]	40.6[a]	41.3[a]	38.5[b]	0.9
Propionate	29.6[a]	29.1[a]	28.6[a]	25.6[b]	25.5[b]	0.9
Butyrate	31.6[b,c]	30.2[c]	30.9[c]	33.1[b]	36.0[a]	0.9

SCF, soluble corn fiber; P, pullulan; RS, resistant starch; SCFA, short chain fatty acid

[a,b,c]Mean values within a row with no shared superscript letters were significantly different ($p<0.05$).

*Self-reported stool consistency on Day 6 was rated on a 4-point scale (1=hard, 4=diarrhea)

†Investigator-reported stool consistency was rated on a 4-point scale (1=hard and formed; 2=soft and formed; 3=loose and unformed; 4=liquid.[395]

Table 5-3. Gastrointestinal tolerance ratings after consuming test treatment on Day 1 and Day 6 of each treatment period.

	Treatments					
	Control	SCF	SCF+P	RS	RS+P	SEM
Bloating†						
Day 1	2.40^{b,c}	2.30^{c}	3.30^{a,b}	3.10^{a,b,c}	3.50^{a}	0.54
Day 6	2.45^{b}	2.60^{b}	3.11^{a,b}	2.45^{b}	3.89^{a}	0.52
Flatulence						
Day 1	3.10^{b,c}	2.90^{c}	4.39^{a}	4.10^{a,b}	4.00^{a,b,c}	0.49
Day 6	2.25^{c*}	2.75^{b,c}	3.09^{a,b,c*}	3.40^{a,b}	3.66^{a}	0.38
Abdominal Cramps						
Day 1	2.10	1.85	2.42	2.00	2.68	0.49
Day 6	1.35^{b*}	1.75^{b}	1.61^{b}	1.65^{b}	2.89^{a}	0.42
Stomach Noise						
Day 1	3.00	2.40	3.30	2.60	2.76	0.45
Day 6	1.90^{b}	1.80^{b}	2.06^{b*}	2.05^{b}	2.94^{a}	0.33
GI Score‡						
Day 1	2.65^{b,c}	2.36^{c}	3.35^{a}	3.00^{a,b,c}	3.24^{a,b}	0.38
Day 6	1.99^{b*}	2.23^{b}	2.47^{b*}	2.39^{b}	3.35^{a}	0.33

SCF, soluble corn fiber; RS, resistant starch; P, pullulan; GI, gastrointestinal

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($p<0.05$).

*Within a gastrointestinal symptom, scores for a treatment were significantly different between Day 1 and 6 ($p<0.05$).

†Symptoms were each rated on a 10-point scale (1=none, 10=excessive)

‡ The GI score is the mean of bloating, flatulence, abdominal cramps, and stomach noise ratings (possible range 1-10)

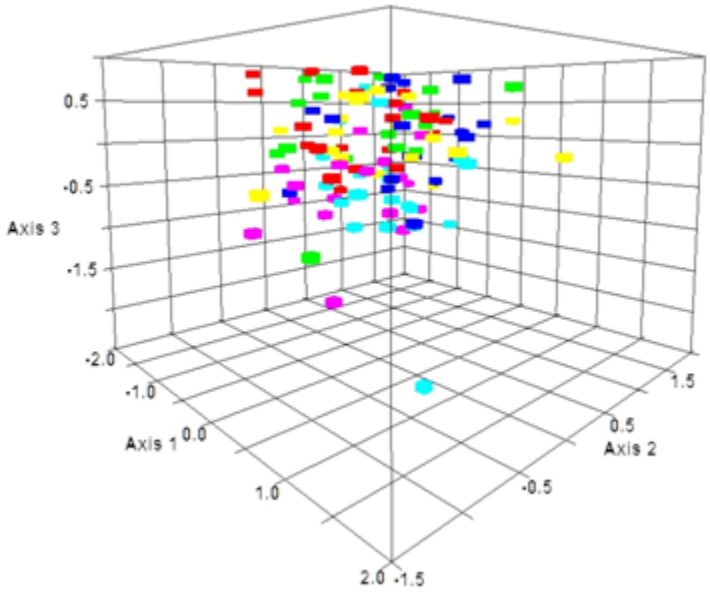

Figure 5-1. Non-metric multidimensional scaling (NMS) analysis of Rsa I-TRFLP patterns of the 16S rRNA gene from the gut microbial community of the study participants on different dietary interventions. Red = Control; Green = SCF; Light Blue = SCF+P; Fuchsia = RS; Dark Blue = RS+P; and Yellow = Baseline.

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Appendix A. 100 mm Visual Analog Scales

Questions on Satiety

How hungry do you feel?

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

How satisfied do you feel?

I am completely empty	_____	I cannot eat another bite

How full do you feel?

Not at all full	_____	Totally full

How much do you think you can eat?

Nothing at all	_____	A lot

Questions on Palatability

Visual appeal

Good _____ Bad

Smell

Good _____ Bad

Taste

Good _____ Bad

Aftertaste

Much _____ None

Overall pleasantness

Good _____ Bad

Appendix B. Gastrointestinal Symptoms Questionnaire

Symptoms Questionnaire
GCRC Protocol #1193

Satiety Study

DAY 6 of trial

DATE:_____

Please list any symptoms that are bothering you at this time. Please estimate the length of time the symptom has been present and rate its severity on a scale of 1 to 5, where 1=mild, 3= moderate, and 5=severe. If you haven't noticed anything unusual, please write "none" on the first line and leave the remainder of the form blank.

Symptom	**Duration**	**Severity (circle)**
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5
		1 2 3 4 5

Gastrointestinal Symptom Survey
DAY 6 of trial

Number of Stools today ____

Please rate the consistency of each stool you passed today
Stool Consistency (1= liquid……4=hard, circle number below)

Stool 1	1	2	3	4
Stool 2	1	2	3	4
Stool 3	1	2	3	4
Stool 4	1	2	3	4
Stool 5	1	2	3	4

If you passed more than 5 stools today, please continue rating stool consistency on the back of the page.

Please rate the amount of bloating you experienced today.

Bloating (1 = none…..10 = excessive, circle number below)

1 2 3 4 5 6 7 8 9 10

Please rate the amount of flatulence you experienced today

Flatulence (1 = none…..10 = excessive, circle number below)

1 2 3 4 5 6 7 8 9 10

Please rate the amount of abdominal cramps you experienced today

Abdominal Cramps (1 = none…..10 = excessive, circle number below)

1 2 3 4 5 6 7 8 9 10

Please rate the amount of stomach noises you experienced today

Stomach noises (1 = none…..10 = excessive, circle number below)

1 2 3 4 5 6 7 8 9 10

Appendix C. Three Factor Eating Questionnaire - Restraint Factor

TFEQ-Restraint		Score
1. When I have eaten my quota of calories, I am usually good about not eating any more	T (+1) F	
2. I deliberately take small helpings as a means of controlling my weight	T (+1) F	
3. Life is too short to worry about dieting	T F (+1)	
4. I have a pretty good idea of the number of calories in common food.	T (+1) F	
5. 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 (+1) F	
6. I enjoy eating too much to spoil it by counting calories or watching my weight	T F (+1)	
7. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat	T (+1) F	
8. I consciously hold back at meals in order not to gain weight	T (+1) F	
9. I eat anything I want, any time I want	T F (+1)	
10. I count calories as a conscious means of controlling my weight	T (+1) F	
11. I do not eat some foods because they make me fat	T (+1) F	
12. I pay a great deal of attention to changes in my figure	T (+1) F	
13. How often are you dieting in a conscious effort to control your weight? Rarely Sometimes Usually (+1) Always (+1)		
14. Would a weight fluctuation of 5 lbs affect the way you live your life? Not at all Slightly Moderately (+1) Very much (+1)		
15. Do your feelings of guilt about overeating help you to control your food intake? Never Rarely Often (+1) Always (+1)		
16. How conscious are you of what you are eating? Not at all Slightly Moderately (+1) Extremely (+1)		
17. How frequently do you avoid “stocking up” on tempting foods? Almost never Seldom Usually (+1) Almost always (+1)		
18. How likely are you to shop for low calorie foods? Unlikely Slightly unlikely Moderately likely (+1) Very likely (+1)		
19. How likely are you to consciously eat slowly in order to cut down on how much you eat? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)		
20. How likely are you to consciously eat less than you want? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)		

21. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever 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" (+1) (4) Usually limit food intake, rarely "give in" (+1) (5) Constantly limiting food intake, never "giving in" (+1)	
TOTAL SCORE *Exclude if score 11 or higher*	

Appendix D. Informed Consent

SATIETY AND GLYCEMIC RESPONSE TO RESISTANT STARCH STUDY CONSENT FORM

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

Joanne Slavin, Ph.D., RD and Abby Klosterbuer 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 Tate and Lyle, Inc.

Description and Purpose of the Study

You are being asked to participate in a study of new dietary fibers and their effects on hunger, blood glucose, and fecal chemistry. The fibers you will consume are already used in food products and are safe to consume.

Approximately 20 subjects will participate in this study. The study consists of one screening visit and five treatment visits. All 6 visits will take place at the General Clinical Research Center (GCRC) on the University of Minnesota East Bank Campus. All visits are necessary to complete the study itself. The screening visit will last approximately 30 minutes and the next 5 treatment visits will each last about four hours. You are selected for this study because you are a man or woman in good health.

At each visit, you will consume 0, 20, or 25 grams of fiber. Two fiber sources will be given, each either alone as a 20 g dose or with the addition of 5 g of another fiber source. After your visit you will be give the same fiber to consume for 6 more days. You will collect fecal samples on days 6 and 7 and complete surveys on gastrointestinal response of the fiber.

Study Procedures

At all visits, you will be given 0, 20, or 25 grams of fiber. You will also be asked to complete a survey about your level of hunger at baseline and for 3 hours after the fiber. You will be given a lunch to consume 3 hours after the fiber treatment. An IV will be placed to draw blood samples and removed before you leave the clinic. Blood samples will be drawn at baseline, 15, 30, 45, 60, 90, 120, and 180 minutes after the fiber treatment. Information from these visits will be retained in your Fairview Medical Center medical chart.

You will be given the same fiber source and instructions on how to consume the fiber for the next 6 days. On days 6 and 7 you will collect fecal samples that can be frozen for drop off at the laboratory.

You will be scheduled for your next visit at least 2 weeks later. This cycle will be repeated 5 times for a total of 5 study visits.

Risks Associated With the Study

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

Blood samples will be drawn from a vein in your arm. The risks associated with drawing blood 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

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 in 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 for the study will be provided at no cost to you.

$100.00 for each completed scheduled visit (excluding the screening visit), if you do not complete the whole study.

$500.00 if you complete the whole study, for a total of $1000.

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 1 year after the study is reported in the 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. Or you may also contact the investigator of the study, Dr. Joanne Slavin, Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108: telephone (612) 624-1290.

If you have any 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 E. Ad libitum lunch menu

Food Item	**Quantity**
Hamburger 1 hamburger bun 3 oz ground beef patty, grilled	1 item
American Cheese	1 slice
Mustard	1 packet
Ketchup	1 Tbsp
Pickles	
Lettuce Leaf	2
Grilled Cheese Sandwich 2 slices white bread 2 slices American cheese 2 tsp Promise margarine	1 item
Tomato Soup	1 serving
Chicken Noodle Soup	1 serving
Saltine Crackers	2 pkg
Potato Chips	1 serving
Lettuce Salad	1 cup
Fat Free French	1 Tbsp
Italian Dressing	1 Tbsp
Carrot Sticks	6
Celery Sticks	6
Ranch Dressing	1 Tbsp
Fresh Apple	1
Orange	1
Banana	1
Vanilla Ice Cream	1 cup
Chocolate Sauce	2 tbsp
Chips Ahoy Cookies	1 pkg
Cola	1 can
Lemon Lime Soda	1 can
Milk, Skim	1 cup
Milk, 2%	1 cup
Mineral Water	1 bottle
Yogurt, fruited	1 container
Coffee	1 cup
Tea	1 cup
Sugar	2 pkt
Half & Half	3 pkt
Equal	2 pkt

Appendix F. Test breakfast preparation – instructions for staff

Instructions for RDs:

1. Take specified treatment muffin out of freezer at 4 pm the day before subject visit
2. Thaw muffin on plate at room temperature
3. Set out specified cereal and beverage mix next to thawing muffin
4. Send kitchen staff the menu
5. Double check that buffet item weights were recorded before and after lunch
6. Calculate calorie and macronutrient intake from lunch based on information in database
7. Provide instructions for completing 24-hour food diary
8. Leave copy of intake for study coordinator

Beverage Instructions for Kitchen Staff:

- Follow instructions according to specified mix:
- Dry Mix A
    1. Weigh **5.94 g** of Dry Mix A and pour into glass
    2. Add **234.06 g** cold water from Pur filter
    3. Stir until *completely* dissolved
- Dry Mix B & C
    1. Weigh **31.5 g** of dry mix and pour into glass
    2. Add **208.5 g** cold water from Pur filter
    3. Stir until *completely* dissolved
- Dry Mix D & E
    1. Weigh **30.5 g** of dry mix and pour into glass
    2. Add **209.5 g** cold water from Pur filter
    3. Stir until *completely* dissolved

Cereal Instructions for Kitchen Staff:

1. Shake packet to assure even distribution of particles
2. Pour packet into small bowl
3. Add **110 g** whole milk
4. Stir thoroughly until most clumps disappear
5. Microwave for 1 minute
6. Stir until any remaining clumps are dissolved
7. Let cool one minute before serving

Breakfast Tray Preparation Instructions for Kitchen Staff:

- Place the following items on tray and serve:
    1. Thawed muffin
    2. Bowl of hot cereal
    3. Beverage
    4. Spoon
- Collect tray after 10 minutes.
- Alert Study Coordinator if any portion of the breakfast was not consumed.

Appendix G. Blood Samples – Collection Tube Preparation and Processing

5 mL in Red Top Tube with gel (Insulin and Glucose):

- □ Gently rock the tubes several times
- □ Let stand 30 minutes
- □ Centrifuge for 10 minutes at 3200rpm
- □ Aliquot ~2ml into GLUCOSE and INSULIN labeled screw cap vial
- □ Freeze at -20°C
- □ UMN Outreach Lab to pick up on Fridays

Insulin and glucose samples were processed at University of Minnesota Outreach Lab

2 mL in EDTA Purple Top Plasma Tube (GLP-1 – ELISA Linco Research):

- □ Add 20 microliters DPP-IV inhibitor to vacutainer tube
- □ Refrigerate empty tubes, with inhibitor added, for up to 24 hours before collection
- □ Draw blood
- □ Gently rock tube several times immediately after collection
- □ Immediately place tube back in ice bucket and keep there until centrifuged
- □ Centrifuge in refrigerated centrifuge at 3200rpm for 10 minutes
- □ Aspirate **at least** 300 microliters of plasma into 3mL screw cap aliquot tube labeled for GLP-1
- □ Place on dry ice
- □ Study coordinator will pick up and transfer to -70 freezer

2 mL in EDTA Purple Top Plasma Tube (Total Ghrelin – RIA Linco Research):

- □ Draw blood
- □ Gently rock tube several times
- □ Place tube in ice bucket and keep there until centrifuged
- □ Centrifuge in refrigerated centrifuge at 3200rpm for 10 minutes
- □ Aspirate **at least** 300 microliters of plasma into 3mL screw cap aliquot tube labeled for GHRELIN
- □ Place on dry ice
- □ Study coordinator will pick up and transfer to -70 freezer

Appendix H. Randomization Scheme

ID	**Women**				
[1]	A	B	D	E	C
[2]	A	D	B	C	E
[3]	E	B	C	A	D
[4]	B	A	E	D	C
[5]	D	C	A	E	B
[6]	D	A	C	B	E
[7]	E	C	B	D	A
[8]	B	E	A	C	D
[9]	C	E	D	B	A
[10]	C	D	E	A	B

Men

[1]	A	E	C	B	D
[2]	D	B	C	E	A
[3]	C	D	A	B	E
[4]	E	B	A	D	C
[5]	B	D	E	C	A
[6]	D	C	B	A	E
[7]	B	E	D	A	C
[8]	C	A	D	E	B
[9]	A	C	E	D	B
[10]	E	A	B	C	D

A=Control
B=SCF
C=SCF+P
D=RS
E=RS+P