

**EFFECT OF A RAPIDLY FERMENTABLE FIBER ON SATIETY, FOOD  
INTAKE, AND TOLERANCE IN HEALTHY HUMAN SUBJECTS**

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## Abstract

Epidemiological studies strongly support the role of fiber in the control of obesity. Fiber is suggested to influence mechanisms of satiety and reduce energy intake. The post-absorptive fermentation of fiber in the large intestine may be linked to the satiating effects observed. The following work focuses on an intervention study using a rapidly fermentable fiber to examine this relationship.

In this study we hypothesized that a short chain fructooligosaccharide (scFOS) would increase satiety and decrease energy intake at a subsequent meal with a dose-dependent response. Additional aims were to determine its influence on 24-hour energy intake, gastrointestinal (GI) tolerance, and breath hydrogen response. Healthy men and women participated in this randomized double-blind, crossover study. On three separate occasions subjects consumed 0 g, 5 g, or 8 g of scFOS in a beverage and proceeded to use visual analogue scales (VAS) to rate satiety over four hours. *Ad libitum* energy intake was then assessed. Subjects later consumed a consistent dose in solid form. Energy intake over 24 hours, GI tolerance, and breath hydrogen measures were obtained. Contrary to our hypothesis no significant differences were observed in satiety or energy intakes. As expected, breath hydrogen response indicated significant fermentation within four hours of scFOS ingestion; however, this did not influence tolerance, as GI symptoms did not differ significantly between treatments.

This study provides evidence that not all types of fiber significantly influence satiety. The physiological actions of one fiber type may not extend to others. It is important to increase the specificity with which health benefits are assigned to specific

fiber types, and to conceptualize fiber as a complex group of substances with diverse actions rather than as a single nutritional entity.

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**Chapter One**  
**LITERATURE REVIEW**

## **Fiber Definitions**

The definition of dietary fiber has been highly debated. Multiple definitions have been proposed and utilized by scientific and regulatory agencies worldwide.<sup>1</sup> From a historical perspective, initial definitions of the 1950s focused solely on the constituents of the plant cell wall deemed to constitute unavailable carbohydrates.<sup>2</sup> Classification in the 1970s recognized associations with promotion of health and disease prevention.<sup>3,4</sup> Nondigestibility and non-absorption in the small intestine were recognized soon after.<sup>5-7</sup> Later definitions were based upon analytical techniques used to isolate and quantify fiber, those approved by the Association of Official Analytical Chemists (AOAC).<sup>8,9</sup> Despite this evolution in the understanding of fiber, controversies over the definition of dietary fiber continue to involve debates over natural or intact substances versus processed substances, the nature and extent of physiological effects associated with possible subcategories of dietary fiber, and the problems with current analytical methods.

Recently, the consensus has developed, based on clear scientific evidence, that the definition of dietary fiber should be based on the physiological properties of food constituents, not merely on their physicochemical characteristics. This shift away from analytic classification is reflected in the definition developed by the Codex Alimentarius Commission (Codex) and numerous other definitions, including those of the United States Institute of Medicine, the Agence Française de Sécurité Sanitaire des Aliments, AACC International (formerly the American Association of Cereal Chemists), the Health Council of the Netherlands and Food Standards Australia New Zealand. Each of these definitions is based on the physiological properties of non-digestion and non-absorption

in the small intestine, coupled with one or more desirable health effects; however, definitions do vary considerably in regards to which physiological effects are emphasized and with what importance.

### **United States Fiber Definition**

In recognition of the problematic reliance on analytically based definitions, and in support of the consensus to develop a physiological based definition, the United States Institute of Medicine (IOM) published a redefined approach to fiber classification in 2001.<sup>1</sup> This definition names *total fiber* as the sum of two distinct but interrelated forms of fiber: *dietary fiber* and *functional fiber*. Dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants, while functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans.

To clarify and expand, this approach classifies dietary fiber as a mixture of polysaccharides from components of the plant cell wall or intercellular structure. These components impart physicochemical properties upon the plant matrix and remain intact against mechanical treatment. This definition also includes lignin. Lignin is not a carbohydrate but rather a phenylpropane polymer that is covalently bound to fiber polysaccharides and alters the physiological effects of the dietary fiber. Lignin exists in only small quantities in the food supply and is the sole dietary fiber-associated substance included in the definition.

Functional fibers are defined as those synthetically manufactured, isolated or extracted by chemical or enzymatic means from natural sources. Functional dietary fibers must demonstrate measureable physiological benefit. Current established physiological properties of functional fibers include attenuation of postprandial blood glucose concentrations, decreased blood cholesterol concentrations, and improved laxation. Naturally occurring polysaccharides and oligosaccharides, extracted and modified from plant sources, make up the majority of current functional fiber. These fibers are increasingly used in food processing in the form of resistant starches, polydextrose and galacto- and fructo-oligosaccharides (GOS, FOS).

Therefore, in the context of the IOM definition, dietary fiber is that which occurs naturally and is intact in foods while fiber that is extracted or isolated from natural sources, or synthesized and then added back to foods, is termed functional fiber. For instance, cellulose in cereals is a dietary fiber; however, when consumed as a supplement for laxation, it is termed a functional fiber.

Also of note, food labels in the United States continue to list dietary fiber in terms of its solubility (soluble or insoluble). This labeling procedure reflects the attempt to assign physiological effects to types of fiber based on this property. For instance, health claims assert that oat bran, a soluble fiber, lowers cholesterol levels, while insoluble fiber such as wheat bran is often associated with laxation. The American Dietetic Association (ADA) maintains that the scientific substantiation of these associations is not strong.<sup>10</sup> More recent studies suggest that viscosity and fermentability may be more effective parameters by which to evaluate the physiological effects of fiber.<sup>11</sup>

## **International Definitions**

In an effort to achieve a universally recognized definition of dietary fiber, the Codex adopted an official definition in June 2009.<sup>12,13</sup> Under this definition dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans. These must fall into one of several categories including 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; or synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities. The definition also makes provisions for the inclusion of lignin and for national authorities to hold the power to include carbohydrates with three to nine monomeric units.

## **Fiber Types**

Fiber encompasses a diverse set of materials and substances with different chemical and physical properties. These properties dictate the physiological effect of fiber and thus introduce significant variation between fiber types.

Most basically, dietary fiber consists of: non-starch polysaccharides that make up the plant cell wall including cellulose, hemicellulose,  $\beta$ -glucan, and pectin; oligosaccharides including inulin/fructans; cereal bran; and, gums and mucilages.<sup>14</sup>

Analogous carbohydrates not recovered by alcohol precipitation include resistant starch, FOS, GOS, dextrans, polydextrose and modified celluloses. Lignin, a substance directly associated with dietary fiber is also included. Brief descriptions of commonly occurring natural, synthetic, and/or isolated fiber components are found in **Table 1-1**. This list is limited to plant-derived fibers, as high fiber foods consumed in a Western diet contain a negligible amount of animal polysaccharides.

### **Fiber Intake**

It is the position of the ADA that the public should consume adequate amounts of fiber from a variety of plant foods.<sup>10</sup> This position is based on clinical evidence that adequate fiber intake may reduce risk factors for the development of chronic disease. However, despite this evidence, fiber intake in developed countries continues to be suboptimal.

Dietary Reference Intakes recommend 14 grams of fiber per 1,000 kilocalories of energy consumed. This recommendation is based on fiber intakes observed to protect against coronary heart disease (CHD).<sup>10</sup> This is the equivalent of 25 g/day for women and 38 g/day for men. Appropriate intakes for children, the elderly, and the critically ill are not clear and current recommendations are also based on 14 g/1,000 kcal. Current fiber intakes in the United States fall at half that recommended—approximately 15 g/day.

**Table 1-1.** Common constituents of total fiber

<b>Substance</b>	<b>Characteristics</b>	<b>Sources</b>
Cellulose	Unbranched, linear NSP of glucose units	Vegetables, fruits, cereal bran
Hemicelluloses	Linear or branched NSP of non-glucose units	Cereal grains
$\beta$ -glucans	Branched glucose polymers	Oats, barley, rye, wheat
Pectins	NSP $\alpha$ (1,4) linked-galacturonic acid	Fruits, vegetables, legumes, sugar beet
Oligosaccharides, Fructans	Polydisperse carbohydrate of $\beta$ (2-1) fructosyl-fructose links	Chicory, Jerusalem artichoke, onions; synthetic methods
Resistant starch (RS 1-4)	Starch/starch degradation products	Legumes, banana, potatoes; synthetic methods
Gums/mucilage	Hydrocolloids; wide range of mixed polysaccharides.	Legumes, seaweed, micro-organisms
Polydextrose	Glucose polymer; Avg. DP 12	Synthesis from glucose and sorbitol; food ingredient
Indigestible dextrins	Glucose polymer, Avg. DP 15	Heat/enzymatic treatment of starch; food additive
Lignin	Chemically bound to hemicellulose	Rice and legume hulls, cereal bran, vegetables

NSP = non-starch polysaccharide; RS = resistant starch  
 DP = degree of polymerization

## **Fiber Sources**

Major fiber sources in the food supply include grain products, vegetables, legumes, nuts, soy, and fruits. Intakes are generally acquired from white flour, potatoes and legumes.<sup>15</sup> The disparity between health recommendations and that consumed evidences that increased consumption of high fiber foods including whole grains, legumes, fruits and vegetables is needed. While the ADA maintains that fiber intake is most effectively increased via consumption of high fiber, low energy dense foods, increased supplementation with novel, functional fibers has the potential to provide adjunctive fiber sources.

## **Fiber and Health**

Ample and accumulating scientific evidence supports a diversity of health benefits associated with high fiber intakes.<sup>16</sup> High fiber intakes may play a role in the prevention of cardiovascular disease (CVD) and type 2 diabetes, improved immune function, gastrointestinal health, and body weight regulation. Research in each of these areas indicates the importance of the physiological effects of fiber on health. Recent research in these areas is briefly reviewed below.

## **Cardiovascular Health**

Observational studies indicate that fiber intake is inversely associated with risk factors for CVD including hypertension, diabetes, obesity, and dyslipidemia.<sup>17-21</sup> Resultantly, significant fiber consumption is associated with lower prevalence rates for

coronary heart disease (CHD) as well as stroke and peripheral vascular disease.<sup>22-24</sup> For example, a pooled analysis of ten prospective cohort studies from the United States and Europe, revealed that CHD risk was 10-30% lower for each 10 g/day increment of total, cereal, or fruit fiber consumed.<sup>25</sup>

Fiber from cereals seems of particular importance in cardiovascular health.<sup>26, 27</sup> There is some evidence to suggest that bran in whole grains is an important factor in this relationship.<sup>28</sup> For instance,  $\beta$ -glucan, which commonly occurs in the bran of cereal grains, holds health claims of protection from CHD.<sup>29</sup> However, as inverse associations between various types of fiber and CHD risk have been identified;<sup>26,30-32</sup> which components of fiber actually confer this protection remains unclear.

Research in the area of fiber and CVD has produced a range of possible mechanisms by which to explicate the protective effects of fiber. Central to these mechanisms are the hypocholesterolemic effects of fiber, specifically of viscous fibers. Increased intraluminal viscosity in the small intestine slows absorption of nutrients and alters the binding of bile acids.<sup>33</sup> In response to changes in the reabsorption of bile acids, LDL cholesterol is removed from the bloodstream and converted to bile acids by the liver. Resultantly, plasma levels of total and LDL cholesterol are reduced.<sup>34</sup> The fermentation of fiber in the colon and subsequent production of short chain fatty acids (SCFA) including acetate, butyrate, and specifically propionate, also are suggested to contribute to the hypocholesterolemic effects observed.<sup>35, 36</sup>

In addition to its cholesterol-lowering properties, fiber may also slow the absorption of fat and carbohydrates in the small intestine to improve insulin metabolism

and glucose control,<sup>37-39</sup> lower blood pressure,<sup>37,40</sup> and improve inflammatory markers including C-reactive protein.<sup>41,42</sup> Therefore, studies confirm that dietary patterns play a significant role in the incidence of CVD and support recommendations to increase consumption of all fiber types for optimal cardiovascular health.

## **Diabetes**

Diets high in fiber may contribute to the prevention and management of diabetes by improving glucose tolerance.<sup>37-39</sup> Fiber consumption has been shown to improve postprandial glycemic control in type 1 and type 2 diabetics.<sup>43,44</sup> Inverse associations between insoluble fiber intake and diabetes risk may be explained by an increased intestinal transit time, reduced time for carbohydrate absorption in the small intestine, and decreased insulin demand.<sup>45</sup> Whereas, viscous, soluble fibers may inhibit macronutrient absorption and delay rates of gastric emptying, resulting in reduced postprandial glucose and insulin responses.<sup>46</sup>

## **Immune Function**

The gastrointestinal (GI) tract is the largest immune organ in the human body. Referred to as gut-associated lymphoid tissue (GALT), its immune function can be modified by some types of fermentable fibers termed prebiotics.<sup>47,48</sup> Prebiotics serve as substrates for healthful, non-pathogenic bacteria species in the colon effectively stimulating the growth and activity of beneficial bifidobacteria and lactobacilli.<sup>49</sup> The fermentation process by which this occurs also produces SCFA. Notably, these end

products are suggested to stimulate the immune system.<sup>50</sup> While research in this area continues to grow, current mechanisms of action to explain SCFA induced immune modulation include lowering the colonic pH to inhibit survival of pathogens and the formation of toxic compounds while also decreasing the enzymatic activity of harmful intestinal bacteria.<sup>50</sup> Furthermore, increased numbers of healthful bacteria contribute to increased competition with pathogens for epithelial binding sites and nutrients, and may be able to cross the intestinal barrier to activate immune cells elsewhere.<sup>51</sup>

Human studies, while limited have identified prebiotic related immune benefits in infants, children, and those with inflammatory bowel disease.<sup>52-55</sup> The role of fermentable fibers in the modulation of gut microbiota continues to be an active area of research in the development of intestinal defenses and immunity, and will be further addressed in the discussion of prebiotic fiber effects.

### **Gastrointestinal Health**

Fiber intake has long been associated with gastrointestinal health, specifically laxation and regularity. A variety of insoluble fiber sources increase stool weight and bulk while decreasing transit time, to promote laxation.<sup>56</sup> These effects have been shown to reduce intracolonic pressure, which may play a role in the prevention of diverticulitis, constipation, and hemorrhoids.<sup>56, 57</sup>

Recent attention has been devoted to colonic health and possible links to systemic modulations to positively influence overall health. Major focus has been on fiber intakes that enhance SCFA production and aid in the conversion of bioactive substances.<sup>36</sup> Short

chain fatty acids have been associated with reduced risk of irritable bowel syndrome (IBS), irritable bowel disease (IBD), CVD and some cancers.<sup>58-60</sup>

For instance, studies in cell lines and animals have demonstrated the preventive effects of butyrate on colon cancer and adenoma development.<sup>61</sup> In addition to being the preferred fuel source for colonic epithelial cells, butyrate is involved in regulation of cell proliferation and differentiation.<sup>48, 58, 62, 63</sup> Acetate and propionate also have been shown to induce apoptosis in colorectal tumor cells lines; however, to a lesser extent than butyrate.<sup>64, 65</sup> The mechanism of action by which this occurs remains unclear.<sup>36</sup>

Research examining high fiber diets and colorectal cancer continues to be controversial. Data from the European Prospective Investigation into Cancer and Nutrition, a large observational prospective study of a half a million people across Europe, found an inverse association with colorectal cancer risk in those with the greatest fiber intake when compared with those with the least fiber intake.<sup>66</sup> However, analysis of pooled data from 13 other similar European and American cohort studies found no association.<sup>67</sup>

Despite a lack of definitive evidence, the scientific consensus is that fiber elicits effects that could contribute to decreased colorectal cancer risk, and increased fiber consumption is advised by the ADA for its prevention.<sup>10</sup>

### **Body Weight Regulation**

Body weight regulation is key to prevention of obesity and related morbidities. The role of fiber in preventing and managing obesity in humans is strongly supported by

epidemiological and physiological studies;<sup>68,69</sup> populations that report high fiber consumption demonstrate lower rates of obesity.<sup>70</sup>

Various fiber types have been shown to reduce short-term energy intake in human trials.<sup>71-73</sup> A review of studies examining the role of fiber in energy regulation indicated that consumption of an additional 14 g/day of fiber for greater than two days was associated with decreased energy intake and weight loss of 1.9 kg over approximately four months.<sup>69</sup> Consistent with this conclusion, data from a prospective cohort of female nurses in the United States, found an inverse association between increased fiber intake and weight gain.<sup>74</sup> Weight reductions have been even greater in similar studies of overweight or obese individuals.<sup>69</sup>

Suggested mechanisms underlying the role of fiber in weight regulation include decreased macronutrient absorption, increased satiety, and modulation of gut hormones. Certain fiber types form viscous gels in the small intestine to slow absorption of carbohydrates as they impede accessibility to digestive enzymes and intestinal mucosa. The bulking and viscosity of these fibers is believed to elicit a feeling of fullness and satiation. Lower postprandial glucose and lipid levels also result and fiber intake may influence fat oxidation and fat storage as well.<sup>75</sup> Additionally, studies have linked fiber intake to satiety related hormones.<sup>76, 77</sup>

## **Satiety**

Many of the effects of fiber are not easily measured. Satiation and satiety are two such subjective parameters lacking clear quantifiable biomarkers. Both are physiological

processes stimulated by food intake and are involved in the reduction of hunger. While closely related, the concepts describe two distinct responses.<sup>78</sup> The sensation of fullness that results in the cessation of food consumption during the course of eating is defined as satiation. This feeling develops during the course of a meal and tends to trigger the end of the meal. In contrast, satiety is the sensation of fullness that occurs between meals. The degree of satiety influences the timing of a subsequent meal and may reduce food intake at the next meal. Therefore, a food with a high degree of satiety would be more likely to produce a longer interval between periods of food consumption. Degrees of satiety may influence the type of food consumed as well as the amount.

Biomarkers of satiety remain limited. Those currently utilized include short-term blood glucose levels and levels of various hormones suggested to be involved in energy regulation.<sup>79</sup> Subjective ratings remain a predominant method of assessment, as they are non-invasive and easily obtained. Subjective ratings in the form of visual analogue scales have been shown to be effective means to measure appetite and predict food intake in within-subject-studies (crossover design).<sup>80-83</sup> However, despite well executed attempts to control environments humans eat for many reasons beyond feelings of hunger; habits, emotions, mood, stress, time of year, and palatability may play a role in introspective measures of appetite. Food form also seems to play a role in satiety. Comparisons of macronutrient matched solid food forms to beverage food forms evidence a weaker satiety response to beverages and greater subsequent energy intake.<sup>84-87</sup> Texture may play a significant role in this observation as negative associations have been reported between viscosity and intake.<sup>88</sup> Mechanisms to explain these differential responses remain

unclear.<sup>85,86,89</sup> However, both solid and liquid *fiber-containing* food forms have been shown to enhance satiety when compared to matched controls with comparable nutrient profiles.<sup>90-92</sup> This research poses questions into whether *fiber-containing* beverages can override the differential appetite response of food form.

The satiating effects of fiber have been examined in short-term studies testing a variety of fiber types, doses, and food forms. These are summarized in **Table 1-2**. The results of these studies have varied largely based on fiber type tested. Additionally, these studies are difficult to compare due to variations in measurement techniques, timing of measurements and/or treatments (may not reflect regular eating patterns) and study duration.

### **Mechanisms of satiety**

Fiber works via various pre- and post absorptive physiological mechanisms to promote satiety.<sup>69, 75</sup> Intrinsically most fibrous foods have a high volume, low energy density, and are less palatable than non-fibrous foods. As a result, fiber may act as a physiological barrier to energy intake by displacing calories and nutrients from the diet prior to absorption.<sup>93</sup> In effect this concept of time-energy displacement is predicated on enhanced satiety due to prolonged ingestion time of high fiber foods with low energy intake. The bulking and viscous properties of many fibers cause them to be processed more slowly. A viscous gel matrix results in the intestinal tract impeding access to digestive enzymes and inducing a feeling of fullness more quickly than after consumption of low fiber foods. For example, apples with natural fiber have been shown to be

significantly more satiating than fiber-free apple juice even when both test foods provided equivalent carbohydrate content.<sup>94</sup>

Satiety has also been linked to glycemic response. Increased intraluminal viscosity, decreased gastric emptying, and slowed macronutrient absorption lower postprandial glucose response and insulin secretion.<sup>75</sup> Additionally, the low glycemic index of high fiber meals have been shown to improve the postprandial glucose response of a subsequent meal. Termed the “second meal effect,” proposed mechanisms of prolonged glucose absorption involve reduced competition between fat and glucose oxidation secondary to decreased serum non-esterified fatty acid (NEFA) levels.<sup>95</sup>

Post-absorptive gut metabolism is also theorized to contribute to feelings of satiety. Short chain fatty acids are suggested to modulate motility and sensitivity in the upper gastrointestinal tract via influence on the secretion of hormones implicated in satiety and appetite regulation including ghrelin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY).<sup>76,96-102</sup> Research to substantiate this link is limited. However, some animal studies do indicate that ingestion of fermentable fibers up-regulate production and secretion of anorexigenic gut peptides including GLP-1 and PYY.<sup>76, 103</sup> Both peptides are produced in the intestinal L-cells in response to macronutrient ingestion and are proposed to play a regulatory role in digestion/absorption and influence postprandial satiety.<sup>101, 102,104-106</sup>

While human data is limited, GLP-1 levels were observed to increase in response to fermentable fiber ingestion in a study of subjects with GERD.<sup>107</sup> Furthermore, peripheral infusions of GLP-1 in physiological doses reduce gastric emptying rates in

obese human subjects and decreased subjective ratings of hunger and prospective food intake.<sup>108</sup> A meta-analysis on the effect of GLP-1 on *ad libitum* food intake in humans also evidenced reduced energy intakes in lean and overweight subjects; however, energy reductions were dose dependent with greatest effect observed at levels beyond a physiological dose.<sup>109</sup> Increased PYY levels, concomitant with weight loss, have also been observed in a study of overweight and obese humans after fermentable fiber supplementation.<sup>77</sup> Nonetheless, the effects of specific fiber types on gut peptide production and secretion remains largely unclear.

### **Functional Fiber**

The concept of functional foods most basically refers to foods with proven capability relevant to health and the reduction of disease risk. Features used to establish functionality include a food or food ingredient's ability to elicit beneficial effects on target functions beyond nutritive value, and evidence in the form of human nutrition trials demonstrating enhanced well-being/reduced disease risk.<sup>110</sup>

Fibers with established physiological functions are termed functional fibers. As previously described, they are extracted/isolated from natural sources or synthesized, then added back to foods. Functional fibers are common added ingredients in the food supply, providing bulk and texture. For example, inulin has been used as a fat-replacement and FOS as a sugar-replacement.<sup>48</sup> Functional fibers are often recognized as natural food ingredients and fiber, as they occur in a variety of commonly consumed food products.<sup>111</sup>

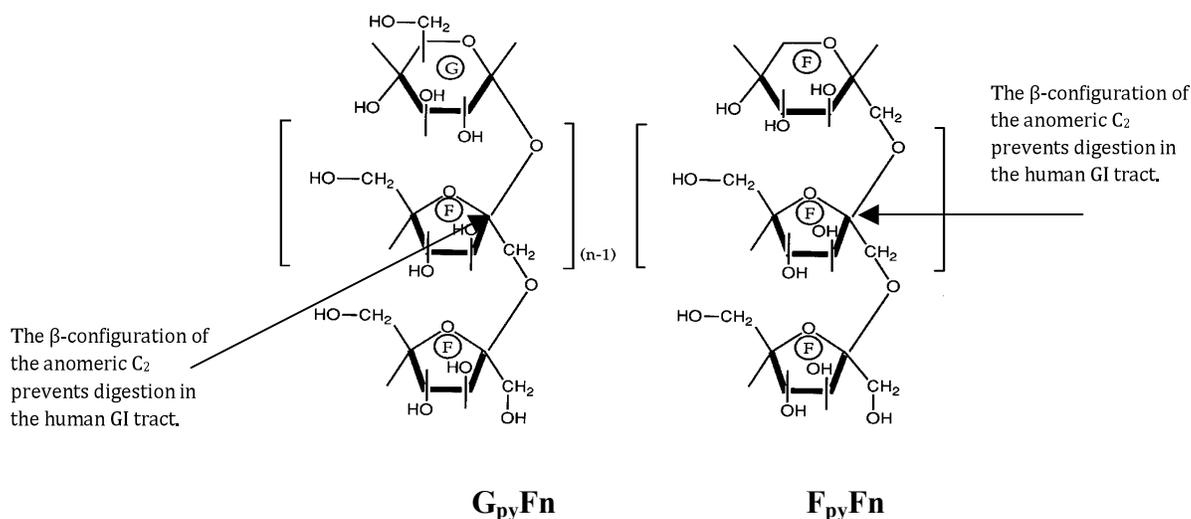
As reliance on processed foods in the diet continues to grow, functional fiber provides an adequate method to increase the fiber intakes of consumers.

### **Fructooligosaccharide (FOS)**

Fructooligosaccharides are low-digestible carbohydrates that may be extracted from natural sources, but more commonly are synthetically produced and added to foods.<sup>111</sup> Fructooligosaccharides (also referred to as oligofructose) are a class of carbohydrates with low degrees of polymerization (DP) and low molecular weights, termed oligosaccharides.<sup>111</sup> This group of short-chain carbohydrates is water-soluble and possesses a sweet taste. Resultantly, FOS is used in the food industry as a low calorie sugar substitute, but may also function similar to soluble fiber. In terms of natural sources, fructooligosaccharide are of plant origin, originating from multiple mono- and dicotyledonous families.<sup>112</sup> Many of these plant species are commonly consumed in the form of vegetables including asparagus, garlic, leek, onion, artichoke, scorzonera (European herb with edible roots) and chicory roots.<sup>113</sup> Short chain fructooligosaccharide (scFOS) can also be produced by enzymatic conversion of sucrose. Synthetically, fructose molecules can be added to sucrose using the transfructosyl activity of the fungal  $\beta$ -fructofuranosidase from *Aspergillus niger*. The product consists of sucrose, glucose and fructose (average DP, 3.6) and is identical to molecules naturally occurring in foods.<sup>111</sup>

Chemically, FOS is a  $\beta$ -fructan, a carbohydrate in which one or more fructosyl-fructose links form the majority of osidic bonds, and is primarily composed of beta (2-1)

fructosyl-fructose linkages.<sup>111, 114</sup> In scFOS, these linkages bond one molecule of sucrose to 1-3 molecules of fructose. Fructooligosaccharides are composed of  $\beta$ -D-fructofuranoses attached by  $\beta$  (2-1) linkages. The chain begins with a  $\beta$ -D-glucopyranosyl or  $\beta$ -D-fructofuransyl, both in the pyranose configuration<sup>111</sup> (**Figure 1-1**).



**Figure 1-1.** Chemical Structure of FOS.  $n$  is the degree of polymerization or the number of  $\beta$ -D-fructofuranose; G and F stand for glucose and fructose, respectively; G<sub>py</sub>F<sub>n</sub> is  $\alpha$ -D-glucopyranosyl- $[\beta$ -D-fructofuranosyl] $_{(n-1)}$ -D-fructofuranoside; and F<sub>py</sub>F<sub>n</sub> is  $\beta$ -D-fructopyranosyl- $[D$ -fructofuranosyl] $_{(n-1)}$ -D-fructofuranoside.

Adapted from Roberfroid et al. *Annu. Rev. Nutr.* 1998. 18 (119).

Notably, the  $\beta$ -configuration of the anomeric C<sub>2</sub> in the fructose monomers induces resistance to hydrolysis by human digestive enzymes specific for glycosidic linkages.<sup>111</sup>

A critical review of the composition and sources of FOS found that it neither passes through the glycolytic pathway nor is stored as glycogen in the manner of digestible starches or sugars. Upon ingestion neither fructose nor glucose components are observed in the portal blood further evidencing resistance to digestion in the upper part of the

gastrointestinal tract.<sup>115, 116</sup> These findings are supported by both *in-vitro* and *in-vivo* data.<sup>111</sup> Fructooligosaccharides pass to the large intestine where they are fermented to produce a functional prebiotic effect. This physiological function is also evidenced by *in vitro* and *in vivo* studies.<sup>117</sup>

### **FOS Intake**

Fructooligosaccharides are part of edible plants, and therefore are already commonly consumed in the United States. It is estimated that average per capita daily intake of FOS ranges from 1-4 gram per day.<sup>118</sup> Because FOS contributes only 1.5-2.0 kilocalories per gram of energy (due to colonic fermentation) it seems an effective method to increase intake of fiber with decreased energy density. Numerous toxicology studies support the safety of FOS asserting that it is not mutagenic, carcinogenic, or teratogenic.<sup>112</sup> Some studies indicate that gastrointestinal symptoms may result from intake; however, these appear to be dose dependent and thus in appropriate doses can be avoided.<sup>111</sup> A review of clinical research studies exploring this area, found that FOS is well tolerated at levels of 15 g/day.<sup>119</sup>

### **Prebiotic Effect**

Fructooligosaccharide are established prebiotics. Prebiotics constitute a dynamic and rapidly expanding area of research. A prebiotic is classified as a food ingredient that displays resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; that is fermentation by intestinal microflora; and that

stimulates the growth and/or activity of healthful intestinal bacteria.<sup>48</sup> Formally, a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health.<sup>49</sup>

As previously stated, *in vitro* and *in vivo* studies confirm that in its passage through the gastrointestinal tract, FOS never produces glucose or fructose.<sup>120</sup> It remains intact to undergo fermentation by colonic bacteria and subsequently produce lactic acid, SCFA and gases: methane, hydrogen and carbon dioxide. In addition, *in vivo* studies evidence that this fermentation leads to the beneficial growth of the bifidobacteria and lactobacilli populations;<sup>112, 121, 122</sup> therefore, FOS may enhance the proliferation of probiotics or beneficial microbes to maximize sustainable alterations in microflora of the colon.<sup>123</sup> Importantly, prebiotics, if combined with compatible probiotics, have potential therapeutic utility. For example, animals fed diets with added prebiotics exhibit increases in mucosal immunoglobulin production, mesenteric lymph nodes, Peyer's patches, and altered cytokine formation and lymphocyte numbers in the spleen and intestinal mucosa.<sup>124</sup> These alterations contribute to increased immunity.

Mechanisms by which fermentable prebiotics exert immune effects involve increased beneficial bacteria numbers and the production of SCFA.<sup>51</sup> Beneficial bacterial strains provide increased competition with pathogenic bacteria for intestinal binding sites and nutrients, may activate immune cells by crossing the intestinal barrier and can influence host defense via production of antibacterial substances that inhibit the growth and survival of pathogens.<sup>51</sup> Furthermore, the production of SCFA is believed to acidify

the colonic environment to further inhibit survival of pathogens. Additionally, butyrate is thought to hinder effects of proinflammatory cytokines, influence lymphocyte activation and inhibit cell proliferation.<sup>14</sup>

### **Animal Studies**

FOS has demonstrated the ability to decrease energy intake, promote weight loss, and improve lipid profiles in obese Zucker rats.<sup>125</sup> Additional rat studies demonstrate that FOS ingestion increases the secretion of satiety hormones including glucagon-like peptide-1 (GLP-1).<sup>126,127</sup> Mechanisms to explain these findings are suggested to involve increased production of SCFA. Short chain fatty acids have been shown to stimulate secretion of GLP-1 and its precursor proglucagon mRNA in rodents<sup>106,128</sup> and dogs,<sup>129</sup> as well as increase the secretion of PYY in rats.<sup>130</sup>

### **Intervention Studies**

Recent studies examining the satiety value of isolated fibers have produced interesting, yet inconsistent results. For instance, an 8 g dose of viscous fiber powder from fenugreek, an isolated fiber added to a beverage, increased reports of satiety in obese subjects.<sup>91</sup> And, in a study comparing the effects of four different isolated fibers when added to a muffin, resistant starch and corn bran enhanced short-term satiety while polydextrose did not.<sup>92</sup> However, a pilot study comparing the appetite effect of fermentable fiber (pectin and  $\beta$ -glucan) against nonfermentable fiber (methylcellulose) supplements, reported no role for either in promoting satiety or reducing hunger or food

intake.<sup>131</sup> In contrast to findings in animals, post satiety reports after the 27 g daily doses indicated that nonfermentable fiber was more, rather than less satiating than fermentable fiber.<sup>132</sup> These and similar studies indicate that not all fibers influence satiety equally and that intervention studies are necessary to identify what fiber types are most effective and furthermore what properties of that fiber type are responsible for satiation. Current studies specific to FOS and appetite sensations have yielded mixed results. These studies are reviewed below.

A pilot study was the first to assess the effects of FOS on satiety and energy intake in humans.<sup>133</sup> In a single-blind, crossover design, ten healthy adults completed two 2-week experimental phases receiving either two daily doses of 8 g of FOS or placebo (dextrin maltose) in the form of a supplement. A washout period was included between phases. Hunger, satiety, fullness and prospective food intake were measured using previously validated visual analogue scales (VAS) at the end of each experimental phase. Energy intake was also measured. The FOS treatment was shown to significantly increase satiety after breakfast and dinner meals in subjects. Reductions in hunger and prospective food intake were observed after dinner as well. Total energy intake during the FOS phase was also significantly lower than during the placebo phase. Findings indicate that FOS may promote a negative energy balance by increasing satiety and reducing energy intake.

In continuation, a similar study sought to examine the effects of FOS alone or in combination with  $\beta$ -glucan (a fermentable fiber found in oats and barley) on appetite ratings and energy intake.<sup>131</sup> Twenty-one healthy adults were included in a 4-way balanced order, double-blind crossover study to investigate the effects of these fibers

using meal-replacement bars. Subjects consumed one of four meal-replacement bars (control, 1.2 g  $\beta$ -glucan, 8 g FOS, or 1.2 g  $\beta$ -glucan + 8 g FOS) at breakfast on two consecutive days. Subjects also consumed a second dose (identical bar) on day one of the trial. Appetite scores were measured using electronic VAS and subsequent *ad libitum* meal intake was recorded. In contrast to the pilot study, neither fiber nor combination thereof was shown to affect appetite ratings or energy intake during the course of the two days tested suggestive of added FOS eliciting no short term effects on these parameters.

In addition to appetite sensations the effects of FOS supplementation on body weight and satiety hormone concentration in otherwise healthy overweight and obese adults (body mass index (BMI) > 25) has been examined.<sup>77</sup> Forty-eight subjects randomly consumed 21g FOS or a placebo (dextrin maltose) for 12 weeks within a double-blind trial. Treatments were in the form of powder added to a beverage. Body composition, satiety hormone concentrations (ghrelin, PYY, GLP-1) food intake and appetite ratings (VAS) were measured. Findings revealed that weight loss (primarily of body fat) was significantly higher in the FOS group. Ghrelin concentrations were significantly lower and PYY concentrations significantly greater in the FOS group coinciding with an expected lower energy intake in this group. No significant difference in GLP-1 concentrations was observed and despite a reduction in energy intake in the FOS group, subjective appetite ratings were not significantly different between the groups. The FOS group also exhibited a reduction in postprandial glucose response. Thus, findings indicate that long-term FOS supplementation may play a role in energy reduction in overweight and obese populations but did not influence appetite sensations.

Based on evidence that colonic fermentation of nondigestible carbohydrates influences gastric and esophageal motility in healthy patients, the physiological responses to FOS administration has been studied in patients with GERD.<sup>107</sup> In the course of two 7-day periods nine subjects received 6.6 g FOS or placebo three times daily after meals. Breath hydrogen concentrations and plasma concentrations of GLP-1, PYY, and CCK were monitored. GLP-1 plasma response was observed to be significantly higher after FOS consumption than the placebo compared to baseline. A meta-analysis of the effects of GLP-1 infusion in humans revealed reduced energy intakes in both lean and overweight individuals.<sup>109</sup> Physiological concentrations of GLP-1 have been shown to significantly reduce gastric emptying rates in obese individuals, this playing a key role in proposed mechanisms of appetite regulation and food intake.<sup>108</sup>

## **Conclusions**

As the prevalence of overweight and obesity continues to increase in developed countries, the need for nutritional interventions grows. Human trials indicate that individuals with high intakes of fiber may weigh less and be at reduced risk for chronic disease. Despite convincing scientific evidence and recommendations for increased fiber consumption, individual intakes are suboptimal. However, consumer dependence on processed foods has steadily increased. Therefore, the development of novel functional fibers may provide an effective way to increase the fiber content of commonly consumed foods and from a public health perspective benefit the general population.

Fructooligosaccharides are one such novel fiber currently used in the food supply. Research in the area indicates FOS may play a role in appetite control; however, mechanisms of action are largely unclear and short-term human trials have produced inconsistent results. Studies conducted thus far indicate that the effects of FOS are likely dose dependent and interrelated to its prebiotic effect. It is clear that the long-term efficacy of FOS necessitates further examination.

**Table 1-2.** Effects of fiber on satiety and energy intake

<b>Study</b>	<b>Design</b>	<b>Subjects</b>	<b>Intervention</b>	<b>Form</b>	<b>Measures</b>	<b>Major Findings</b>
Cani et al. (2006) <sup>38</sup>	Randomized Single-blind Crossover	Healthy M/F N=10	FOS – 16g/d for 2 wks w/ washout	Supplement	VAS, energy intake	↑ satiety post B/D; ↓ hunger, pros. food intake after D; ↓ total energy intake and post B/L
Chow et al. (2006) <sup>39</sup>	Randomized Double-blind Crossover	OW/OB Diabetic M/F N=99	Guar gum - 9g	Nutrition bar	VAS	↑ fullness; ↓ hunger, pros. Food intake post L
Delargy et al. (1997) <sup>40</sup>	Randomized Crossover	Healthy M N=15	Psyllium gum -22g Wheat bran - 22g Psyllium gum/wheat bran – 3 g	Cereal	VAS, energy intake	↓ short term energy intake w/ wheat bran
Freeland et al. (2009) <sup>25</sup>	Randomized Crossover Two part	Healthy M N= 17,16 (N= 9 both)	Wheat bran – 41 g w/ and w/o 41g glucose	Cereal	VAS, energy intake	↓ energy intake comparable to equal wt. of glucose
Hamedani et al. (2009) <sup>27</sup>	Randomized Crossover	Healthy M/F N=32	Corn/wheat bran -26g	Cereal	VAS, energy intake, glucose	↓ energy intake, ↓ postprandial glucose response
Howarth et al. (2003) <sup>41</sup>	Single-blind	Healthy M/F N=11	Pectin + β-glucan – 27g Methylcellulose -27g daily for 3 wks w/ washout	Supplement	VAS, energy intake, body wt	No effect on appetite or food intake
Levine et al. (1989) <sup>42</sup>	Randomized Single blind	Healthy M/F N=14	5 cereals (0g-39g wheat or corn bran fiber/serving)	Cereal	Energy intake	↓ short term energy intake with ↑ fiber content

Study	Design	Subjects	Intervention	Form	Measures	Major Findings
Mathern et al. (2009) <sup>24</sup>	Single-blind Randomized Crossover	OB M/F N=18	Fenugreek – 4g, 8g	Beverage	VAS, energy intake, glucose, insulin	↑ satiety, fullness; ↓ hunger, pros. food intake w/ 8g fenugreek
Parnell et al. (2009) <sup>43</sup>	Randomized Double-blind Crossover	OW/OB M/F N=48	FOS - 21g/d for 12 wks	Beverage	VAS, energy intake, glucose, insulin, hormones, body wt.	↓ energy intake, body wt., glucose, insulin, ghrelin; ↑ PYY
Peters et al. (2009) <sup>131</sup>	Randomized Double-blind Crossover	Healthy M/F N=21	FOS - 8 g/d; β-glucan - 1.2 g/d; FOS – 8 g + β-glucan – 1.2 g/d for 2 days (second dose on day 1)	Meal replacement bar	VAS, energy intake	No effect on appetite or food intake
Samra et al. (2007) <sup>26</sup>	Randomized Crossover Two-part	Healthy M N=16,15	Wheat bran - 33g	Cereal	VAS, energy intake, glucose	↓ short term energy intake and ↑ fullness; ↓ postprandial glucose response
Willis et al. (2009) <sup>36</sup>	Double-blind Randomized Crossover	Healthy M/F N = 20	Resistant starch - 8g Corn bran - 9.6g β-glucan + oat fiber – 9.4g Polydextrose - 9.5g	Muffin	VAS	↑ satiety w/ resistant starch, corn bran

**Abbreviations:** M-males, F-females, OW-overweight, OB-obese, VAS-visual analogue scales (appetite rating), B-breakfast, L-lunch, D-dinner, ↑ - increase, ↓ - decrease

## **Chapter Two**

### **EFFECT OF A SHORT CHAIN FRUCTOOLIGOSACCHARIDE ON SATIETY, ENERGY INTAKE, AND TOLERANCE IN HEALTHY HUMAN SUBJECTS**

## Overview

**Background:** Colonic fermentation is theorized to influence satiety and food intake.

Short chain fructooligosaccharides (scFOS) are rapidly fermented fibers that can be easily added to foods to impact these parameters.

**Objective:** The objective of this study was to evaluate the short-term satiety response of scFOS and its ability to decrease food intake.

**Design:** In a double-blind crossover design, 20 healthy human subjects were randomly assigned to consume two separate doses of 0 (control), 5 g, or 8 g of scFOS, the first at breakfast in beverage form, and the second prior to dinner in solid chews. Visual analogue scales (VAS) were completed to assess satiety between breakfast and lunch. *Ad libitum* food intake was measured at a lunch meal and over 24 hours. Breath hydrogen measures were obtained and gastrointestinal tolerance was assessed.

**Results:** No enhancements in satiety were found. Food intake was lower after scFOS treatments; however, differences were not significant. Breath hydrogen measures, used as markers of fermentation, indicated significant dose dependent increases within four hours of ingestion; however, all treatments were well tolerated with no significant differences in gastrointestinal symptoms.

**Conclusions:** These results indicate that rapidly fermented scFOS is well tolerated but does not enhance short-term satiety. Fermentation of scFOS may play a more important role for other digestive benefits, such as prebiotic properties.

## Introduction

Fiber intakes are associated with increased satiety and lower body weight.<sup>69, 134</sup> Populations that report high fiber consumption demonstrate lower rates of obesity.<sup>70</sup> Enhanced satiety may play a key role in this relationship.<sup>75</sup> Soluble fibers mediate postprandial glucose response and delay gastric emptying rates via an ability to form viscous mixtures in the gastrointestinal (GI) tract. This in turn induces feelings of fullness and increase satiety.<sup>68, 79</sup> However, beyond glycemic control, intestinal fermentation of fibers may also influence satiety.<sup>135</sup>

Short-chain fructooligosaccharide (scFOS) are a form of novel fiber that demonstrates rapid fermentation in the colon with the subsequent production of short-chain fatty acids (SCFA).<sup>49,111</sup> Additionally, scFOS are endowed with prebiotic properties, and therefore stimulate the growth of health promoting bacteria in the colon.<sup>49</sup>

Longer chain FOS has demonstrated the ability to decrease energy intake, promote weight loss, and improve lipid profiles in rats.<sup>125</sup> Additional animal studies demonstrate that FOS ingestion increases the secretion of satiety hormones including glucagon-like peptide-1 (GLP-1),<sup>126,127</sup> GLP-1, secreted in response to carbohydrates, lipids, and mixed meals,<sup>136-139</sup> is involved in appetite regulation and energy intake.<sup>104,105,140</sup> Interestingly, GLP-1 secretion is stimulated by SCFA.<sup>106, 128,141</sup> Intravenous GLP-1 administration in ranges from physiological to supraphysiological doses in human subjects resulted in increased satiety and reductions in energy intake.<sup>108,140</sup> GLP-1 plasma levels have been shown to increase significantly after FOS ingestion in a single human study as well.<sup>107</sup> Mechanisms to explain these observations remain unclear.

A recent pilot study in healthy human subjects demonstrated enhanced satiety and reduced energy intake after consumption of 8 g FOS supplements twice daily for two weeks.<sup>133</sup> In contrast, consumption of 8 g of FOS in a meal-replacement bar one to times a day for two days did not affect appetite rating or energy intake.<sup>131</sup> A study in overweight adults also failed to demonstrate an enhanced satiety response after receiving 21 g of FOS daily for 12 weeks; however, reductions in energy intake and body fat were observed.<sup>77</sup>

To our knowledge, no study to date has examined the effects of scFOS on satiety. Therefore, we sought to test two different doses of scFOS against a control to determine effects on satiety response and energy intake in healthy human subjects. We hypothesized that scFOS would increase satiety and decrease energy intake at a subsequent meal with a dose-dependent response.

## **Methods and Materials**

### **Experimental Design**

The study was a randomized, double-blind, crossover design of three single meal treatments of a test breakfast with 0 g (control), 5 or 8 g of scFOS added to a hot cocoa breakfast beverage. A second dose, consistent with the first, was consumed as a snack prior to dinner. This dose was in the form of three solid chocolate flavored candies. Fiber doses were based on their characterization as an excellent fiber source (10 g/d) and that observed to be a tolerable dose (16 g/d). Subjects completed all three treatments in a randomized order.

## **Subjects**

The University of Minnesota Institutional Review Board Human Subjects Committee approved all aspects of this research. Participants were recruited via fliers posted around the University of Minnesota campus. An initial phone screening was used to determine if subjects met inclusion criteria. Healthy men and women between the ages of 18-64 and with a BMI between 18-27 were included in the study. All subjects demonstrated spoken and written English literacy and provided written, informed consent after review of study protocol and procedures (Appendix A).

Exclusion criteria included smoking, vegetarianism, history of disease or gastrointestinal conditions affecting digestion/absorption and use of medications that could influence the outcome of the study. Exclusions also included pregnancy, lactation, recent weight fluctuations, menstrual irregularity and regular fiber intakes greater than 15 g/d.

Twenty subjects (10 men, 10 women) were recruited for this trial based on sample size estimates for statistical power.<sup>80</sup>

## **Protocol**

Subjects reported to the testing site in a fasted state (12 hours) on three separate occasions. Visits were scheduled at least one week apart. Subjects arrived to the testing site at 7:15 am and remained seated in a quiet testing room for the duration of the study, approximately 4.5 hours. Subjects were allowed to read, use laptops, or work quietly. Women participated during the follicular phase of their menstrual cycle based on

previous research to indicate that women have a higher spontaneous energy intake in the luteal or premenstrual phase compared to the follicular or postmenstrual phase.<sup>142</sup>

Subjects followed a low-fiber lead in diet and abstained from alcohol and excessive vigorous exercise for 24 hours prior to a visit. In addition, subjects were instructed to maintain current exercise levels and not to initiate a weight loss program for the duration of the study. Physical conditions and location of the room were consistent for all visits.

Upon arrival, subjects were instructed on how to complete computerized visual analogue scales (VAS) to record satiety, hunger, fullness and prospective food consumption. Subjects completed four baseline VAS and provided a baseline hydrogen breath sample by exhaling into a mouthpiece connected to a sample holding bag. Breakfast was then served with the treatment beverage. Subjects were asked to consume the breakfast within 10 minutes.

Additional VAS were completed at 15, 30, 45, 60, 90, 120, 180, and 240 minutes from baseline. In order to assess the palatability of the test beverage, five additional VAS were completed at the 30-minute interval. Subjects provided a hydrogen breath sample and then consumed an *ad libitum* buffet lunch 240 minutes after baseline. Subjects were presented with a tray consisting of three large plates filled with five personal pizzas cut into various sizes and randomly arranged. Food was offered in excess to minimize external food intake cues. Subjects were instructed to eat until they were comfortably satisfied. Portions eaten were weighed and measured for energy content. Final satiety and palatability VAS were completed and subjects were instructed on how to complete 24-hour food records for the remainder of the day. Subjects were also instructed to consume

three candy chews consistent with their breakfast treatment two hours prior to their dinner meal and complete a subjective gastrointestinal assessment over the course of 24 hours.

### **Treatment Descriptions and Meal Contents**

All three test beverages and candy chews looked similar and were nearly identical in macronutrient content (**Table 2.2**). Meal contents were kept consistent across trials with the exception of the fiber content of breakfast. The scFOS investigated in this trial was Nutraflora<sup>®</sup>. The fiber powder was mixed with 8 ounces of hot water. A plain bagel (Lender's<sup>®</sup>) and 1-ounce plain cream cheese (Philadelphia<sup>®</sup>) were also served with the hot coca beverage for breakfast. Breakfast was approximately 500 kilocalories. Lunch consisted of buffet portions of French bread pepperoni pizza (Stouffer's<sup>®</sup>) and bottled water (Dasani<sup>®</sup>). Complete nutritional facts for the pizza lunch can be found in Appendix D.

### **Visual Analogue Scales**

Subjective appetite sensations were assessed using previously validated computerized visual analogue scales (VAS).<sup>80</sup> One hundred millimeter lines were displayed in the same order at each measured time point. The lines were flanked on either side by two opposing statements and measured 0-100mm from left to right. Ratings of **hunger** (0 = I am not hungry at all – 100 = I have never been more hungry); **satisfaction** (0 = I am completely empty – 100 = I cannot eat another bite); **fullness** (0 = not at all full

– 100 = totally full); and **prospective food consumption** (0 = nothing at all – 100 = a lot) were assessed. Only one question was displayed on the screen at a time and subjects were asked to point and click along the scale at the point that matched perceived feelings. Subject could return to questions and review or edit their response prior to saving their response at each time interval. Once saved, responses could not be reviewed.

Five additional questions were used to assess palatability. Visual appeal, smell, taste, and overall pleasantness were scored as good (0 mm) and bad (100 mm). Aftertaste was scored as much (0 mm) versus none (100 mm).

Additionally, subjective gastrointestinal tolerance was assessed using similar structured VAS. Questions compared changes in gastrointestinal activity to normal baseline behavior. Assessments included stool frequency (a count), stool consistency (0 = diarrhea – 100 = hard stool/constipation), degree of intestinal bloating and flatulence (0 = minimal – 100 = excessive). Participant's ratings were converted to a numerical scale (0-100) measuring from the left. Area under the curve (AUC) was calculated. All VAS utilized in the study can be found in Appendix C.

### **Sample Size**

Sample size for this study was determined based on previously published research. Flint et al. determined that if the effect parameters of interest are limited to fasting and mean appetite ratings, a sample size of 18 subjects is sufficient to accurately test a hypothesis using a power of 0.8.<sup>80</sup> Twenty subjects completed the study to maintain power in the event of subject drop out.

## **Randomization**

A Williams design balanced for carry-over effects was utilized for subject randomization (Appendix B). There were six sequences of treatments and three or four subjects assigned to each sequence. Rows represent subjects; columns represent treatment periods. The sequences were divided evenly between ten men and ten women. Subjects were assigned to sequence in the order of enrollment.

## **Energy Intake**

Food intake was recorded 24 hours from the start of each study visit. Subjects were provided food records at each study visit. Food records were analyzed using the dietary analysis program, Nutrition Data System for Research (NDSR).<sup>143</sup> Analysis included nutrient information including: total energy, carbohydrate, fat, protein, and fiber intake.

## **Breath Hydrogen Measures**

Two breath samples were collected at each visit. Collection occurred at baseline and 240 minutes post intervention. Subjects were instructed to exhale directly into a sample bag via a mouthpiece. All measurements were done with the same apparatus. Breath hydrogen was analyzed as a measure of colonic fermentation. Sample analysis was performed with a Quintron Microanalyzer (Quintron Instruments, Milwaukee, WI).

## **Statistical Methods**

Baseline demographic characteristics were reported by gender as means and

standard errors. Area under the curve (AUC) was calculated for VAS response. The AUC was evaluated by the trapezoidal rule. The fiber treatments were compared by AUC for satiety, hunger, fullness and prospective food intake using a mixed effects linear model with a random subject effect to model within-person correlation between repeated AUC measurements; this model was also used to check period and carryover effects. Pair-wise comparisons were used to compare treatment means. Data are presented as mean AUC  $\pm$  SEM. Statistical analysis was performed with SAS version 9.1.2.<sup>144</sup>

## **Results**

### **Demographic Characteristics**

Twenty subjects (10 men, 10 women) completed the study. Two subjects were Asian and three were Hispanic. The mean age of males was  $28 \pm 2$  (range 20-47) and females  $28 \pm 4$  (range 18-60). Mean BMI was  $22.7 \pm 0.7$  (range 19.5-25.8) and  $21.2 \pm 0.7$  (range 18.4-24.6) for males and females, respectively. Baseline demographics are summarized in **Table 2-1**. There were no difference between men and women. Gender, age, and BMI did not differ between treatment sequences.

### **Appetite Sensations**

There were no differences between treatments in baseline VAS scores. **Figure 2-1** gives the mean AUC for all appetite sensations tested (hunger, satisfaction, fullness, prospective food intake) for each treatment. **Figures 2.2-3** illustrate VAS scores at measured time points by treatment. Lower scores are equated to less hunger and lower

prospective food intake, while higher scores indicate greater sensations of fullness and satisfaction.

Prospective food intake increased significantly with dose of scFOS (test for linear trend  $P = 0.026$ ). Feelings of hunger ( $P = 0.12$ ) and prospective food intake trended toward a lesser response to the control when compared with scFOS treatments. Feelings of satisfaction ( $P = 0.06$ ) and fullness ( $P = 0.10$ ) also trended to greater response to the control when compared to scFOS interventions.

### **Palatability**

Sensory evaluation of the test beverages found no difference between treatments. The contents of the buffet lunch were also kept consistent and no significant differences in palatability of the meal were reported between visits.

### **Energy Intake**

There were no differences between treatments in food intake 240 minutes post intervention or 24 hour energy intake as shown in **Figure 2.4**.

### **Gastrointestinal Tolerance**

All doses of scFOS were well tolerated. Any issues that did arise were minor and there were no reports of any adverse side effects of the interventions. There was a significant increasing linear trend in combined scores of gastrointestinal symptoms with increasing dose of scFOS ( $P = 0.046$ ). Flatulence increased slightly with total 16 g scFOS

(P = 0.055).

### **Breath Hydrogen Response**

There were no baseline differences between treatments in breath hydrogen. Breath hydrogen showed a significant increasing linear trend with scFOS dose (P < .0001). Both 5 g and 8 g of scFOS produced significantly within-group increases from baseline breath hydrogen (P < 0.001). Breath hydrogen did not show an association with any measures of gastrointestinal intolerance. (**Figure 2.4**)

### **Discussion**

Fiber is proposed to modulate the increasing prevalence of overweight and obesity.<sup>134, 145, 146</sup> However, there is limited information on the independent effects of isolated, fermentable fibers and energy regulation. The present study sought to examine the relationship between scFOS and its influence on satiety and energy intake. Contrary to expectations based on current theory of colonic fermentation, our findings indicated no role for acceptable amounts of scFOS in short-term satiety and energy intake. Significant fermentation was observed within four hours of scFOS ingestion as evidenced by breath hydrogen excretion.

Our findings were inconsistent with animal models in which longer chain FOS has been shown to reduce energy intake via increases in SCFA or satiogenic gut hormones, namely, GLP-1.<sup>126, 127</sup> Consistent with animal data, otherwise healthy subjects with gastroesophageal reflux disease (GERD) demonstrated significant increases in

plasma GLP-1 levels after seven days.<sup>107</sup> Subject consumed 6.6 g of FOS three times daily after meals during this period. Increases in GLP-1 were also observed in a pilot study of healthy subjects receiving 16 g/d of a related prebiotic fiber for two weeks.<sup>147</sup> Furthermore, infusions of GLP-1 in physiological doses have been shown to reduce gastric emptying rates (GER) and decrease hunger ratings in obese subjects.<sup>108</sup> A meta-analysis of GLP-1 on *ad libitum* energy intake concluded that GLP-1 infusions reduced energy intake in both normal weight and overweight subjects.<sup>109</sup> Reductions in GER were suggested to contribute to increased satiety induced by GLP-1.

The 10-16-g/d scFOS supplementation ingested by our subjects may have been insufficient to influence gut hormones including GLP-1. Animal studies generally provide a dose of 10% of total dietary intake.<sup>59</sup> In contrast, scFOS treatments in our study provided < 5%. Studies indicate that FOS is well tolerated by the gastrointestinal system at levels of 15 g/d; as the amount increases increased gastrointestinal symptoms are more likely to occur.<sup>119</sup> Therefore, the dose utilized in our study seems at the high end of what human subjects can tolerate. The two studies observing significant increases in GLP-1 in human subjects utilized higher doses and/or administered treatments for longer periods of time.<sup>107, 147</sup> Notably, these studies also consisted of small sample sizes. A larger study of overweight subjects consuming 21g of FOS daily for 12 weeks failed to demonstrate any change in GLP-1 levels.<sup>77</sup> Subjects also reported negative side effects, which deterred them from consuming the treatment compared to the control. In contrast, all scFOS treatments were well tolerated in our study.

A recent pilot study in which human subjects consumed 8 g of FOS or a control twice daily for two weeks significantly increased satiety after breakfast and dinner.<sup>133</sup> Total energy intake per day was also 5% lower than with the control. While a similar dose was used in our study, the discrepancy in results may be explained by the failure of FOS to automatically exert a beneficial influence on satiety and energy regulation. Influences on energy regulation may be delayed and thus require a longer duration of study. While breath hydrogen measures indicated that scFOS was undergoing fermentation in the colon within hours, its full fermentation and production of SCFA may have continued for several more hours. Resultantly, the effects of scFOS may not be immediate, but rather most apparent in the days following its ingestion.

Consistent with our findings, subjects receiving 8 g of FOS in meal-replacement bars twice daily for one day followed by once the consecutive day demonstrated no effect on short-term appetite or energy intake.<sup>131</sup> Additionally, a study examining the satiety effects of specific fermentable fiber supplements against nonfermentable fibers supplements, found no role for the short-term use of either in increasing satiety.<sup>132</sup> The aforementioned study of overweight subjects consuming 21 g/d of FOS also reported no difference in appetite ratings despite reductions in energy intake and decreased body weight.<sup>77</sup> This study suggests that reduced energy intakes may not be associated with subjective perceptions of increased satiety. Our findings, while not significant, did demonstrate reduced energy intake after scFOS treatments. Significant differences may have been obtained if study conditions were of a longer duration.

It is also feasible that the food form utilized in our study limited our ability to enhance satiety. Visual analogue scales were completed only after ingestion of the first scFOS treatment. This treatment was consumed in the form of a hot beverage. Comparisons of macronutrient matched solid food forms to beverage food forms evidence a weaker satiety response to beverage food forms and greater subsequent energy intake.<sup>84-87</sup> Furthermore, in an acute setting fiber properties such as viscosity may play a greater role in satiety than fermentation. Examination of the effect of soluble fibers on satiety in the form of beverages evidenced a greater satiety response as viscosity of the fibers increased.<sup>148</sup> Beverage viscosity also is reported to be inversely related to postprandial hunger.<sup>149</sup> Additionally, fiber may act differently when removed and isolated from an intact plant structure; therefore, FOS may not influence satiety to the same degree as other types of fiber.

In conclusion, our findings are suggestive of no role for acceptable amounts of scFOS in short-term satiety and energy intake. Breath hydrogen measures evidenced rapid fermentation indicating that the prebiotic properties of scFOS may provide physiological benefits beyond satiety. Coupled with the research reviewed, future directions include continued study of the physiological effects of scFOS and other specific fiber types and the potential for implications on energy regulation. Long-term studies may be helpful in more fully elucidating these effects and mechanisms of action. Also, as accumulating evidence suggests the importance of gut microflora on overall health, the prebiotic effects of scFOS also require extensive study.

**Table 2-1.** Baseline Demographic Characteristics

Subjects	Age (Mean $\pm$ SEM)	BMI (Mean $\pm$ SEM)
Males (N = 10)	28 $\pm$ 2	23 $\pm$ 0.7
Females (N= 10)	28 $\pm$ 4	21 $\pm$ 0.7
Pooled (N = 20)	28 $\pm$ 2	22 $\pm$ 0.5

**Table 2-2.** Nutrient composition of treatments<sup>1</sup>

Treatment	Total	scFOS	Energy	Carbohydrate	Protein	Fat	Sodium
	<i>g</i>	<i>g</i>	<i>kcal</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>mg</i>
Bev Control	271	0	128	21.3	4.3	2.1	366
Bev 1	276	5	136	26.5	4.3	2.1	366
Bev 2	279	8	141	29.3	4.3	2.1	366
Sol Control	19.5	0	67	11.4	0.4	2.9	0.2
Sol 1	19.5	5	57	13.5	0.3	2.1	0.2
Sol 2	19.5	8	51	14.7	0.2	1.6	0.1

Bev = beverage, Sol = solid

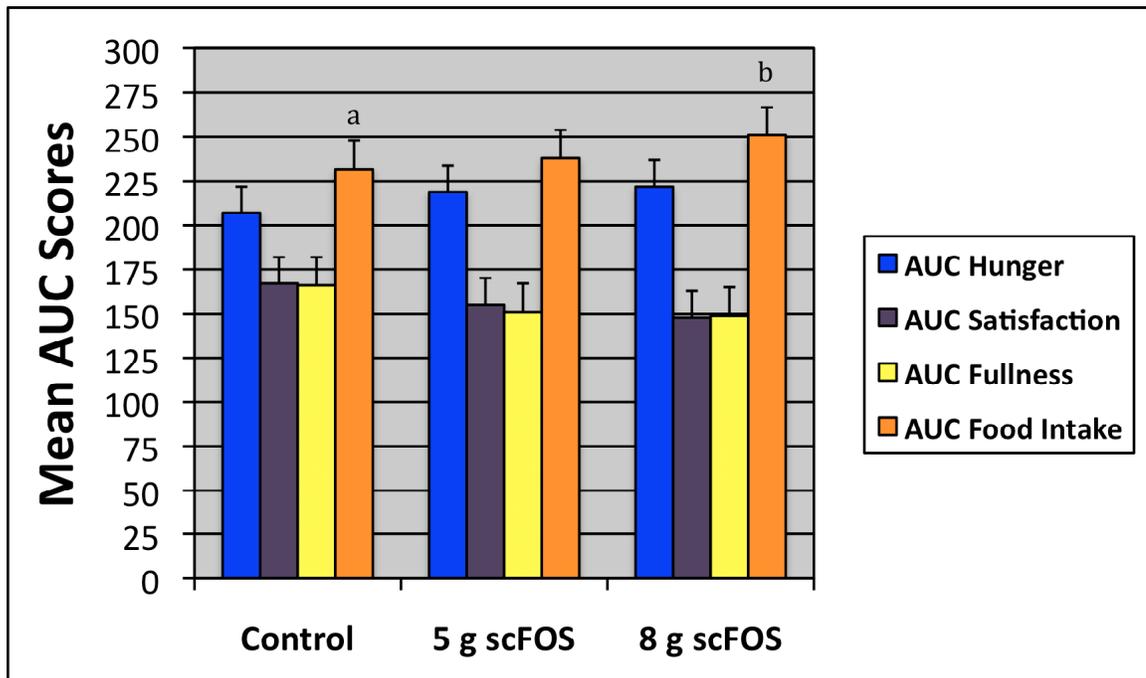
<sup>1</sup>Analysis was completed at GTC Nutrition; Golden, CO. Fiber content was analyzed using AOAC methods.

**Preparation: (1 serving = 1 packet hot chocolate mix)**

- Empty the packet into cup.
- Gradually stir 8 oz. (1 cup) of hot (not boiling) water into mix

**Preparation: (1 serving = 3 solid chews)**

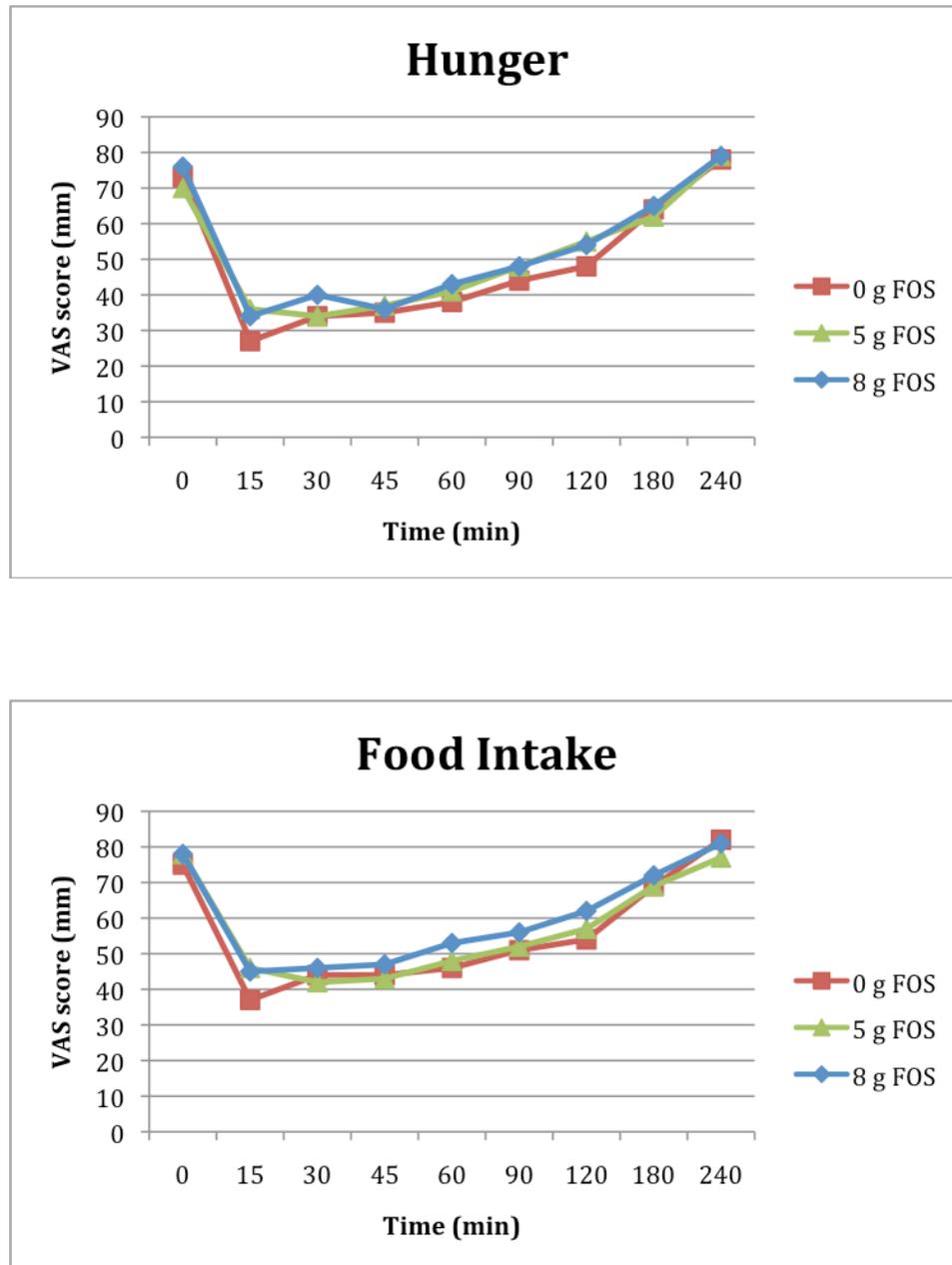
## Mean AUC Satiety Scores



**Figure 2-1.** Mean AUC scores  $\pm$  SEM for each treatment and each VAS questions (N=20 for each bar). AUC was calculated by the trapezoidal rule. The fiber treatments were compared by AUC for satiety, hunger, fullness and prospective food intake using a mixed effects linear model with a random subject effect. Pair-wise comparisons were used to compare treatment means. Data are presented as mean AUC value  $\pm$  SEM. Within each question, treatments with letters above were significantly different at  $P < 0.05$ .

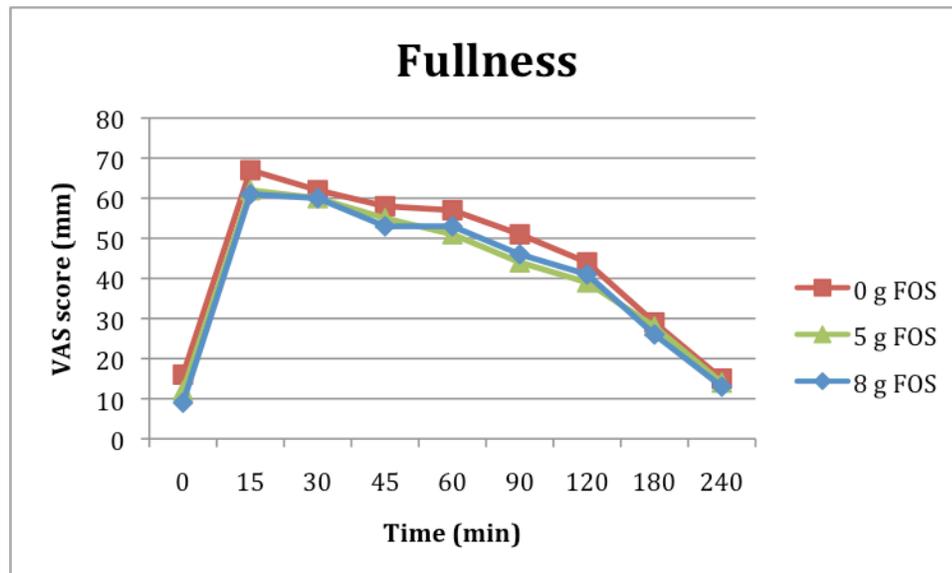
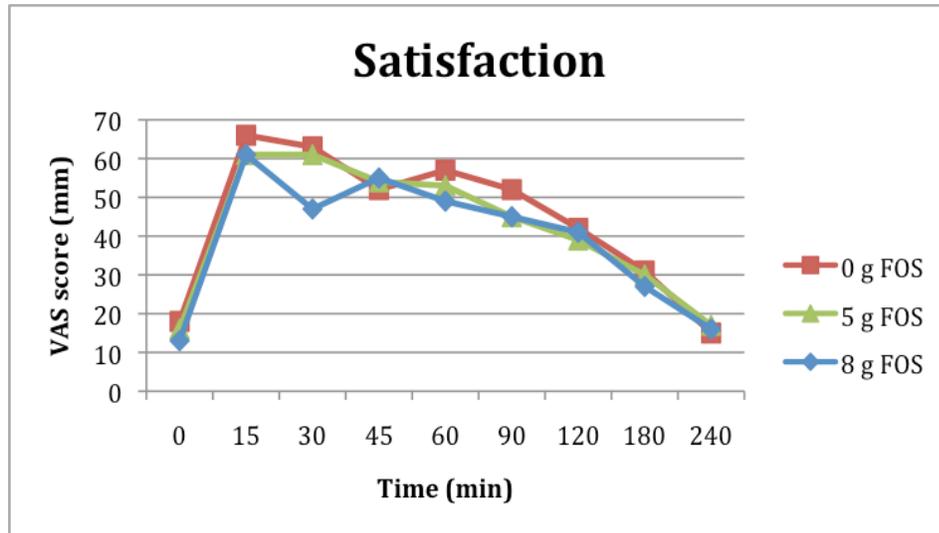
Treatments without a letter were not significantly different.

## Changes in Hunger and Prospective Food Intake



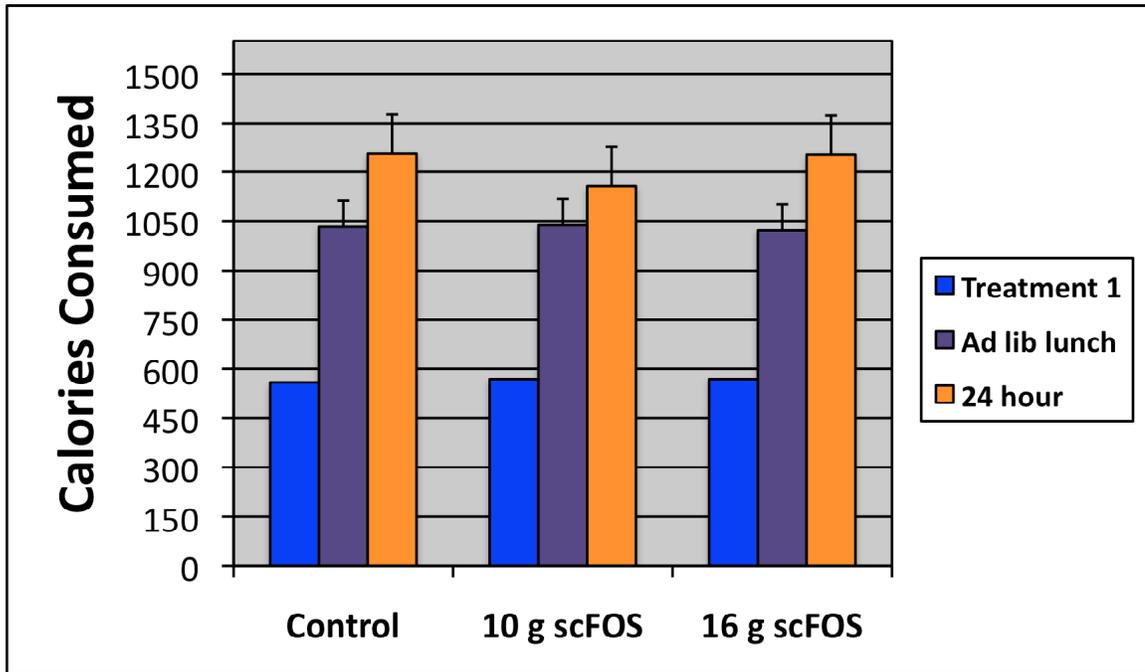
**Figure 2-2.** Changes in appetite sensations based on mean VAS score at each time point for each question (N=20 for each line). Lower means less hunger and less intake.

## Changes in Satisfaction and Fullness



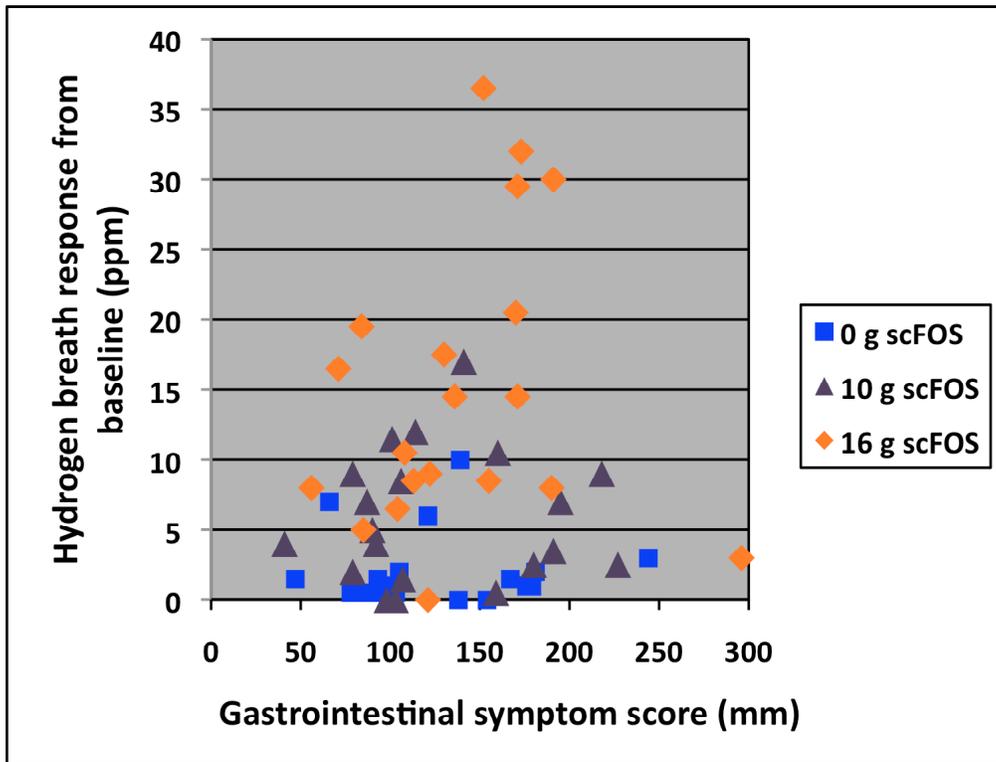
**Figure 2-3.** Changes in appetite sensations based on mean VAS score at each time point for each question (N=20 for each line). Higher means more satisfied and more full.

## Mean Calories Consumed



**Figure 2-4.** Mean  $\pm$  SEM calorie intake during the 24-hour intervention day. There were no differences between treatments for intake at the buffet lunch or over the remainder of the day.

## Gastrointestinal Tolerance and Breath Hydrogen Response



**Figure 2.4.** Total gastrointestinal symptom scores based on VAS ratings by treatment (N=20) plotted against 240 minute change in hydrogen breath response from baseline (parts per million). Breath hydrogen response was indicative of colonic fermentation.

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## **Appendix A. Fiber Study Consent Form**

### **SATIETY RESPONSE OF scFOS CONSENT FORM**

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

Joanne Slavin, PhD, RD in the Department of Food Science and Nutrition is 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.

#### **Description and Purpose of the Study**

You are being asked to participate in a study of fiber and its effect on satiety and gastrointestinal tolerance. The fiber you will consume is already used in food products and is safe to consume. Approximately 20 subjects will participate in this study. The study consists of three visits. All visits are necessary to complete the study itself. You are selected for this study because you are a man or woman in good health.

At each visit you will consume breakfast and lunch meals.

#### **Study Procedures**

At all three visits, you will be provided breakfast and a beverage with or without 5 or 8 grams of fiber. These treatments will be given in a random order. You will also be asked to complete assessments of hunger, satiety, and palatability at predetermined time intervals and will provide breath samples at baseline and 4 hours after breakfast. You will then consume an *ad libitum* lunch 4 hours after breakfast. After leaving the study site you will consume 3 chews with or without 5 or 8 grams of fiber 2 hours prior to your evening meal. These treatments will also be given in a random order. Additionally, you will record all food intake for 24 hours and complete an assessment of gastrointestinal tolerance.

#### **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. Possible risks of additional fiber include gas and bloating. You may discontinue participation in the study if you experience undesirable side effects.

#### **Benefits Associated with the Study**

There is no direct benefit to 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. If you think you have suffered a research-related injury, let the investigators know right away.

#### **Compensation for Participation**

Study related visits and the meals for the study will be provided at no cost to you. You will be compensated \$300.00 if you complete the entire study and \$25.00 for each completed scheduled visit in the event that you are unable to complete the study.

**Confidentiality and Document Review**

The results of this research study may be presented at meetings or in publications, so absolute confidentiality cannot be guaranteed. However, your identity will not be disclosed in these presentations. Data will be kept for 1 year after collection, to complete the analysis of data and reporting of the information in the scientific literature.

**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.

**Contacts and Questions**

You may ask any questions you have now.

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 Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St SE, Minneapolis, MN 55455; 612-625-1650.

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 \_\_\_\_\_ Date \_\_\_\_\_

## Appendix B. Randomization

<b>Sbj#</b>	<b>Study Visit</b>		
	<b>1</b>	<b>2</b>	<b>3</b>
1	B	C	A
2	A	C	B
3	C	B	A
4	C	A	B
5	B	A	C
6	A	B	C
7	A	B	C
8	C	A	B
9	A	C	B
10	B	A	C
11	C	B	A
12	B	C	A
13	C	A	B
14	C	B	A
15	A	C	B
16	A	B	C
17	B	C	A
18	B	A	C
19	C	B	A
20	B	C	A

- **Men = Subjects 1-10**
- **Women = Subjects 11-20**

### **Treatment Codes:**

A = 0 g control

B = 5 g scFOS

C = 8 g scFOS

**Appendix A.** Visual Analogue Scales (100 mm)

**Satiety Questions**

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

## Palatability Questions

Visual appeal

Good \_\_\_\_\_ Bad

Smell

Good \_\_\_\_\_ Bad

Taste

Good \_\_\_\_\_ Bad

Aftertaste

Much \_\_\_\_\_ None

Overall Pleasantness

Good \_\_\_\_\_ Bad

## Gastrointestinal Tolerance Questions

Stool Frequency: \_\_\_\_\_ (number of stools in last 24 hours)

Stool Consistency

Diarrhea \_\_\_\_\_ Hard  
stool/constipation

Degree of intestinal bloating

Minimal \_\_\_\_\_ Excessive

Degree of flatulence

Minimal \_\_\_\_\_ Excessive

## Appendix D. Nutrient Composition and Preparation Method for Pizza Lunch

### Nutrition Facts

*Stouffer's Pepperoni French Bread Pizza*

Serving Size 1 pizza (159 g) (5.63 oz)

Servings Per Container 2

### Amount Per Serving

**Calories** 410    Calories From Fat 180

#### %Daily Value\*

<b>Total Fat</b> 20g	<b>31%</b>
Saturated Fat 7g	<b>34%</b>
Trans Fat 0g	
<b>Cholesterol</b> 25mg	<b>8%</b>
<b>Sodium</b> 810mg	<b>34%</b>
<b>Total Carbohydrates</b> 43g	<b>14%</b>
Dietary Fiber 4g	<b>15%</b>
Sugars 5g	
<b>Protein</b> 15g	

### 5 pepperoni pizzas provided:

- 2,050 calories
- 100 grams of fat
- 215 grams of carbohydrate
- 75 grams of protein

### Pizza Preparation

1. Preheat oven to 350°F
2. Remove 5 pizzas from box and plastic bag
3. Place pizzas on baking sheet on center rack in oven
4. Cook 25-30 minutes
5. Remove pizzas from oven and cut into various sized pieces
6. Place pizza slices on three separate plates
7. Weigh plates and pizza
8. Record weight prior to serving subjects
9. Weight back plates and any uneaten pizza after 20 min lunch period
10. Determine weight of pizza eaten by subtracting weight of remaining pizza and plates from original total weight recorded previously
11. Use above nutritional information to calculate energy intake