GASTROINTESTINAL HEALTH AND FIBER SUPPLEMENTATION IN UNDERGRADUATE STUDENTS: A PROSPECTIVE STUDY

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my parents and sister, who have supported and believed in me throughout every aspect of my life.

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LIST OF ABBREVIATIONS

BMI	Body mass index
FDA	Food and Drug Administration
g	Grams
GI	Gastrointestinal
GOS	Glactooligosaccharides
GSRS	Gastrointestinal Symptoms Rating Scale
SD	Standard deviation

Abstract of Thesis Presented to the Graduate School
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Prebiotics are indigestible dietary fibers that positively affect the colonic microbiota leading to growth of “healthy” bacteria and possibly resistance to infection and diarrheal disease such as travelers’ diarrhea. Oligosaccharides, which are prebiotics found in high concentrations in human breast milk, promote the proliferation of bifidobacteria and lactobacilli. A group of oligosaccharides known as galactooligosaccharides (GOS), which are known to increase the growth of healthy bacteria such as bifidobacteria in young adults are of interest. Glactooligosaccharides are regarded as a functional fiber due to their ability to confer health benefits while being generally regarded as safe by the FDA.

A prospective, randomized, parallel, double blind, placebo controlled study examined the effect of GOS in maintaining digestive health in a group of 420 healthy fulltime undergraduate students (≥ 18 years old) undergoing academic stress during cold and flu season. The aim of the study was to assess whether students undergoing final exams will have better gastrointestinal health when consuming 2.5 g or 5.0 g of the fiber per day for 8 weeks. Gastrointestinal health was evaluated using the Gastrointestinal Symptom Rating Scale (GSRS). Stress and fiber had a significant effect

on loose stools and urgent bowel movement. There was a significant effect of stress seen with diarrhea, rumbling, nausea, hunger pains and stomach ache, associating higher levels of stress with more discomfort. There was also a significant fiber effect with the feeling of not emptying (stool evacuating). Overall supplementation with GOS was shown to have a significant effect on many of the symptoms assessed by GSRS.

CHAPTER 1
INTRODUCTION

Constipation, gas, bloating, diarrhea are among the many undesirable gastrointestinal symptoms. Consequently tools have been developed to assess these symptoms. One of these tools is the Gastrointestinal Symptoms Response Scale (GSRS). GSRS consists of 15 gastrointestinal symptoms that are present in gastrointestinal diseases such as irritable bowel syndrome and peptic ulcer disease [1]. The reliability of the scale was estimated by means of independent and concurrent duplicate ratings by two raters evaluating 20 cases. The scale also showed to be effective and simple to use in many clinical trials [1-2]. The scale measures stomach ache, heartburn, acid reflux, hunger pain, nausea, rumbling, bloating, burping, passing gas, constipation, diarrhea, loose stool, hard stool, urgent bowel movement and not emptying. The scale ranges from one to seven with one being no discomfort and, severe symptoms being represented by seven. Because of the importance of gastrointestinal health to the overall health of the body, it is imperative to find ways to improve gastrointestinal function and reduce these unfavorable symptoms.

Prebiotics have been acclaimed as foods that will improve gastrointestinal function and overall health. Prebiotics are classified as health-enhancing functional food ingredients [3]. Prebiotics are defined as non-digestible food ingredients that beneficially influence the host by selectively stimulating the growth and/or activity of certain bacterial species already resident in the colon, and by doing so improve the health of the host as introduced by Gibson and reviewed in McFarlane et al [3-4]. Supplementation with prebiotics can considerably modify the colonic microbiota by increasing the number of specific bacteria and thus lead to a change in the composition

of the microbiota [3]. The human colonic microbiota plays a major role in host health [5]. Due to these findings there is increased of interest in the reconstitution of the makeup of colonic microbiota to make it a more beneficial community (reviewed in McFarlane et al [4]). *Bifidobacterium spp* and *Lactobacillus spp* are perceived to have health promoting properties as reviewed in McFarlane et al [4]. Bifidobacteria have been linked to lowering of serum cholesterol levels, production of vitamins, growth inhibition of potential pathogens by producing acetate and lactate and promotion of normalization of intestinal flora following antibiotic therapy and because of this, many attempts have been made to increase their number in the colon [3, 6-7]. Prebiotics consist of fructoligosaccharides and inulin some candidate prebiotics include GOS, isomalto-oligosaccharide, lactulose, mannanoligosaccharides, nigero-oligosaccharides, oat β-glucans, raffinose, soyabean oligosaccharides, transgalactooligosaccharides and xylo-oligosaccharides [8]. Studies have shown that prebiotics have the ability to promote the growth of endogenous bifidobacteria, which then leads to their predominance in the human feces [3-4]. Prebiotics have also been shown to modulate lipid metabolism, by the use of fermentation products [4]. As a result of this fermentation, temporary unfavorable symptoms, such as gas production and bloating do occur [9]. Short chain oligosaccharides are fermented more rapidly and produce more short chain fatty acids and gas in comparison to long chain [9]. In order to benefit from the unique properties of oligosaccharides while diminishing these unfavorable side effects research in this area has become of great interest.

An oligosaccharides is a saccharide polymer that contains a small number of component sugars that typically range from three to ten units. Oligosaccharides are

found in human breast milk and are said to be the third largest component [10]. Breast milk has been shown to increase bifidobacteria and lactobacilli in the infant's colon [10]. It is thought that in the infant colon a prominent bifidobacterial count may be associated with health advantages and those infants that are breast-fed have many health benefits over formula-fed infants [10]. Oligosaccharides also found in the highest concentration in the colostrum or "babies first milk" where they enhance bifidobacteria numbers in the newborns gastrointestinal tract [10]. Moro et al, demonstrated that when infant formula is supplemented with a mixture of fructooligosaccharide and GOS at 0.4 g/dL and 0.8 g/dL, respectively, the number of bifidobacteria significantly increase in the intestine and the growth and enumeration of the bifidobacteria results in softer stool after 28 days of feeding [11].

In a study conducted by Ben et al., 371 infants were fed to breast milk and then randomized to formula supplemented with GOS (0.24 g/100 ml) or formula with no GOS. All infants were breastfed for the first four weeks after birth with only the infants that were switched to formula being randomized to one of the two formula groups [12]. After three months they were able to observe that the infants fed the formula with GOS had increased bifidobacteria, lactobacilli, acetic acid and stool frequency and a decrease in stool pH compared to those infants fed the formula with no GOS [12]. There was no difference between the group of infants breast fed and those fed formula supplemented with GOS [12]. No adverse events such as vomiting, crying or regurgitation were linked to the GOS formula [12].

GOS has been shown to increase bifidobacteria and lactobacillus in the colon and so has great potential for being considered as a prebiotic. There is no research

linking GOS with unfavorable gastrointestinal symptoms nor is there research on its effect on the symptoms using the GSRS. The makeup of glactooligosacharide varies in terms of degrees of polymerization ranging from two to eight monomeric units, and having a terminal glucose unit.

Glactooligosaccharide is made of a chain of galactose units that arise from the consecutive transgalactolysation reaction. GOS is of interest because it is said to be a functional fiber meaning it has the ability to confer health benefits and is generally regarded as safe by the FDA [13]. Due to the configuration of its osidic bonds GOS is resistant to hydrolysis or digestion in the stomach and small intestine [13]. GOS reaches the colon intact and selectively increases the growth and activity of bifidobacteria within the colon of young adults, which then may result in health benefits previously mentioned to the host [13].

Research has shown that adding GOS at 4% (wt:wt) to a commercial diet in pigs significantly increased the density of bifidobacteria and the acetate concentration compared with the control diet and the control diet supplemented with inulin [14]. These findings suggest a great prebiotic potential for the novel GOS, though these findings were in pigs [14]. Due to their metabolic and physiological similarities, pigs are known to be good models for human studies [14]. In their study Tzortzis et al. showed in vitro and in vivo that GOS can act as not only a prebiotic, but also selectivity enhances numbers of bifidobacteria. The GOS mixture showed in vitro that it has the potential to inhibit the attachment of enteropathogenic E. *coli* and S. *typhimurium*, to the colonic epithelium, and thus potentially preventing illness due to these bacteria [14].

Previous studies done in humans have used GOS supplementation. In a study by Silk et al. 44 patients with irritable bowel syndrome (IBS) completed a 12-week parallel crossover controlled clinical trial. Subjects were randomized to one of three groups 3.5 g or 7 g of GOS or 7 g of the placebo [15]. Their symptoms of IBS were monitored weekly and scored on a Likert scale [15]. They found that in the group consuming the prebiotic there was a significant increase in the number of fecal bifidobacteria, whereas in the group taking the placebo there was no difference from baseline [15]. They also found improved flatulence and bloating in subjects with irritable bowel syndrome who received 3.5 or 7 grams of this fiber [15]. The authors concluded that GOS was effective in relieving IBS symptoms and may have promise as a therapeutic agent [15].

Drakoularakou et al. conducted a placebo controlled, randomized, double blind study in a group of 159 healthy individuals who were to travel for two weeks to a place with high or low risk for developing travelers' diarrhea. Travelers' diarrhea is defined as "three to four unformed stools in 24 hours and at least one of the following symptoms such as abdominal pain, nausea, vomiting, fever, blood or mucus in the stools" [16]. Travelers' diarrhea was reduced in a group of subjects consuming 5.5 g of GOS [16]. They also found that those in the treatment group that did have diarrhea, and their duration of diarrhea was 2.4 days in comparison to the placebo group which lasted 4.6 days [16]. Glactooligosaccharide is also thought to interact with the epithelium of the host and thus prevent pathogens from adhering to epithelium of the small intestine [16]. Prebiotics, however have their limitations when it comes to treatment against infective organisms that cause diseases such as travelers' diarrhea [16]. This is mainly because

prebiotics such as GOS are fermented within the large intestine, but disease causing bacteria work mainly on the small intestine [16].

Glactooligosacharides were further investigated in a group of elderly adults by Vulevic et al. Glactooligosaccharide was administered to healthy elderly individuals. It is known that the elderly have a decreased number of bifidobacteria, and since GOS has shown to result in an increase in numbers of bifidobacteria in younger adults, the investigators decided to look at the effect of GOS administration in the elderly [5]. In this double-blind, placebo-controlled, crossover study the elderly subjects received a placebo or 5.5 g of GOS for 10 weeks. At the end of the 10 weeks they observed an increase in bifidobacteria and a decrease in less beneficial bacteria. The investigators concluded that this was a positive effect in microflora composition and GOS may be used to enhance gastrointestinal health in the elderly [5]. An area of interest that has not yet been addressed by researchers is to see if GOS can be used to enhance health when the individual is under stress.

Stress has been shown to suppress immune function in humans. In an academic stress model, 38 medical students were investigated under examination stress, and their blood was analyzed for various immune factors [17]. It was seen that psychological stress such as academic stress increases production of pro-inflammatory cytokines [17]. Cytokines are involved in humoral as well as cell mediated immunity [17]. Stress showed an increase in tumor necrosis factor alpha, interleukin 6, interleukin 1, interferon gamma, and interleukin 10 in students undergoing academic stress and greater stress perception among student showed a higher increase in these factors [17].

Studies have demonstrated that stress causes a change in colonic microbiota [17-18]. Stress decreases the number of beneficial lactobacilli and leading to an environment conducive to pathogen invasion [18]. An unfavorable change in the colonic microflora can lead to many gastrointestinal problems and is also detrimental to the overall health of the individual [18]. Knowles et al. used the academic stress model with 23 undergraduate science students to look at colonic microbiota over a time when students were put under examination stress in comparison to baseline (no stress) [18]. The total bacterial levels in the colonic microflora continuously dropped from the first exam to the fifth exam and were significantly lower in comparison to baseline levels [18]. It has also been shown that stress among many things delays gastric emptying while accelerating large bowel transit time [19]. Changes in bowel transit time affect the growth and the composition of the bacteria in the large intestine [19]. Stress also exacerbates the symptoms in gastrointestinal disorders and affects frequency and consistency of bowel movements [19]. Stress has also been shown to affect visceral sensitivity in humans [19].

It is plausible to suggest that the effects that stress elicit on the gastrointestinal microflora are in part due to cortisol which is known as the “stress hormone” [20]. In a study done by Schiffrin et al., pregnant rats were injected with either normal saline or cortisone acetate (cortisol) on days 18-21 of gestation [20]. It was then shown that the concentration of beneficial bacteria in the neonatal pup’s small intestine was significantly lower in those injected with cortisone acetate in comparison to normal saline. The total bacteria population was lower for the treatment group at each of the

given times considered. They also looked at gram negative bacteria count in the small intestine and found similar changes as they did for total bacterial count [20].

Research has demonstrated prebiotic potential for GOS, however there are many gaps to be filled regarding the function of this oligosaccharide, for example how it affects the various gastrointestinal symptoms.

No study to our knowledge has been done to assess the effect of this fiber using GSRS. Prebiotics help maintain gastrointestinal function so it is plausible that GOS before academic stress will minimize stress associated gastrointestinal symptoms. Therefore, the purpose of the proposed study is to demonstrate whether galactooligosaccharides supplemented in the diet will lead to less gastrointestinal discomfort in academically stressed undergraduate students.

CHAPTER 2
MATERIALS AND METHODS

A prospective, randomized, parallel, double-blind, placebo controlled design was used. Healthy full-time undergraduate students aged 18 years or older who had at least one cold in the past year were recruited via listservs, flyers, posters, and announcements in early fall of 2009 from the University of Florida to participate in the 8-week study. Potential subjects were excluded if they had a cold on the day of enrollment, chronic allergies involving the upper respiratory tract (chronic = taking allergy medicine daily), or immunosuppressive illnesses or treatments in the past year; would not have Internet access for the duration of the protocol; did not have at least one scheduled final exam; currently smoke; received antibiotic therapy during the previous two months prior to the start of the study; were unwilling to discontinue any immune-enhancing dietary supplements (e.g., prebiotics and fiber supplements, probiotics, echinacea, fish oil, vitamin E [>100% of the RDA or >15 mg/day]); or had an allergy to milk, simple intolerance to milk was not an exclusion.

Informed consent was obtained from the study participants. Additional consents were obtained to account for drop outs prior to randomization or for students who were screening failures. All study procedures were approved by the University of Florida Institutional Review Board. Height and weight were obtained at baseline and body mass index (BMI) was calculated. Subjects were proportionally stratified based on gender (50/50) to make sure that the same proportion of males and females participated in the eight-week study. Subjects, 427 total, were randomized to one of three treatment groups (A, B, or C) via sealed envelopes. The stratification and randomization scheme was generated by the study statistician who did not have direct contact with any

subjects. This scheme was provided to the principal investigator in two sets of sealed envelopes. Subjects and investigators were blinded to treatment groups. Subjects were given packets containing 0 g, 2.5 g, or 5 g of the functional fiber GOS (Purimmune™) provided by GTC Nutrition, Golden CO. Subjects were instructed to take one packet per day and to pour the contents of the packet into any beverage except carbonated drinks. Sucrose was added to the 0 g and 2.5 g packets so that all packets were the same weight and looked identical. The total volume in each packet was approximately one tablespoon. The fiber and sucrose easily dissolved and had a slight sweet taste. Certificate of Analysis and Technical and Quality Specifications of the fiber were obtained. The fiber has generally regarded as safe status under 21 CFR 170.30 on the basis of scientific procedures and can be added to foods available within the U.S. without prior FDA approval. The fiber is derived from milk sugar so subjects with an allergy to milk were excluded. The fiber is not made from genetically modified organisms, is dairy Kosher and Halal certified. Students were told not to share the fiber packets with anyone and to return any unused packets at the end of the protocol.

Subjects were assigned a study number that they used as their computer log-in user name. Students made up their own password. The questionnaires used throughout the eight-week protocol were administered through the University of Florida-hosted E-Learning System (Blackboard Learning System, Washington D.C.). The subjects' user number was not linked to University records; however, paper records retained by the study coordinators linked subject number to the subjects' identification and contact information. Study coordinators instructed subjects on logging in procedures and an explanation of each of the questionnaires was given to each subject.

Subjects logged on initially and completed the baseline questionnaires with the study coordinator present in case of any problems. Questions regarding demographic information were contained on short questionnaires at baseline. These questions took less than a few minutes to answer. From that point on, subjects were able to complete the online questionnaires from any location.

Daily online questionnaires asked students about level of stress on a scale from 0 (no stress) to 10 (extremely stressed), and consumption of the fiber. Study coordinators monitored subject compliance online on a daily basis and contacted subjects via phone or email to remind them to complete the questionnaires. Since the questionnaires were no longer available for the subject to fill out if they failed to complete them within the first 36 hours of their availability, the study coordinators released the questionnaires to the subjects and asked them to complete the questions to the best of their recollection. If the subject could not remember they were told to leave it blank. Subjects completed questionnaires at baseline and once a week over the course of the 8-week protocol. The questionnaires inquired about gastrointestinal symptoms. Examples of questions include: “Have you been bothered by stomach ache or pain during the past week?”, “Have you been bothered by heartburn during the past week?”, and “Have you been bothered by nausea during the past week?” [1-2]. This questionnaire would take the study participants less than 5 minutes to complete.

The figure below outlines the protocol design with starting and ending dates.

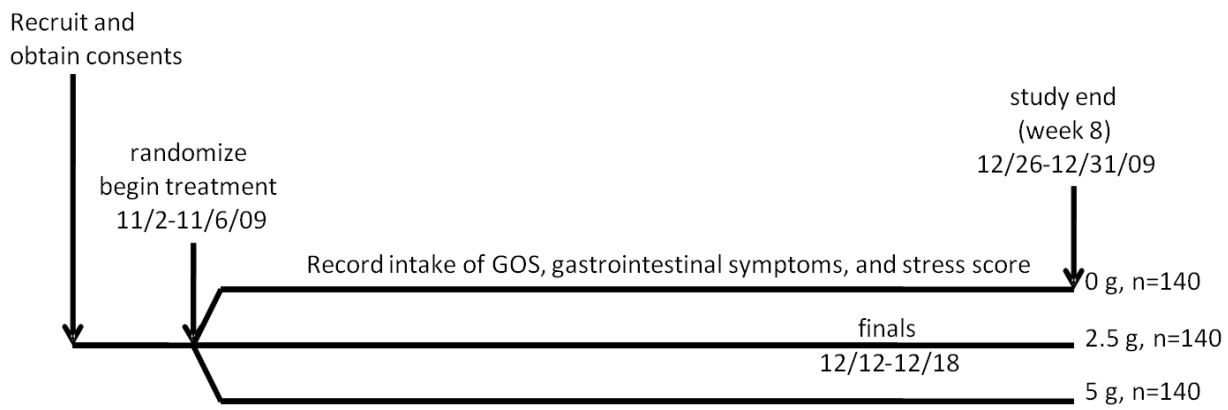

Figure 2-1. Glactooligosaccharide (GOS) and digestive health in academically stressed University students who received 0 g, 2.5 g, or 5 g of the fiber.

Gastrointestinal symptoms such as gas, bloating and diarrhea were assessed weekly using the Gastrointestinal Symptom Scale Score in order to look at the effect of GOS on the various gastrointestinal symptoms during periods of low and high stress. The daily stress level for the participants was averaged for each week and the weeks with highest and lowest average levels of stress were then identified and used for analyses. Since the gastrointestinal symptoms data did not exhibit normal distribution the data were then analyzed using nonparametric tests. First the data were graphed as box plots and then analyzed across all fiber treatment groups using a nonparametric-paired t test. A Kruskal-Wallis test was used to test differences among fiber treatment groups. A Wilcoxon matched-pairs signed ranks test was used to test for differences in gastrointestinal symptoms between periods of low and high stress. A one-way analysis of variance (ANOVA) was used to test for differences among fiber treatment groups when appropriate. Differences in gender and ethnicity among groups were analyzed using contingency tables and the chi-squared test for independence. Means were considered significant at $P \leq 0.05$.

CHAPTER 3
RESULTS

Undergraduate students were screened, consented and randomized (Figure 3-1). Out of the 427 that were randomized, nine did not complete the study. Reason for post-randomization withdrawals included: too sick to participate (group C), allergy-like symptoms (group C), physician recommendation due to previously diagnosed gastroparesis and eating disorder (group C), non-compliance (group A and B) and hospitalization resulting in the subject withdrawing from the University (group B). The distribution of gender, ethnicity and BMI was similar among all three fiber groups, however significant difference did exist in age among the different fiber groups P=0.0017 (Table 3-1).

The data are not normally distributed. The median response to most Gastrointestinal Scale Symptom questions was 1 on a scale of 1 to 7, therefore data were skewed. This right-hand skewing of data was evident after transforming data using ranks. A total of 427 participants took the fiber 22,967 out of the total 23,053 days or 96% ± 0.18 of study days, and so yielding 96% compliance among the study participants. The high stress week was identified as week 6, which was the week of final exams and the low stress week was identified as week 8, a week when students were on semester break. The high versus low stress weeks were determined by taking the average stress of all subjects throughout the course of the study and determining those weeks in which the students had the highest and lowest stress (Figure 3-2).

Significant statistical differences were observed for both the stress and fiber effects on loose stools (Figures 3-3 and 3-4) and urgent bowel movements (Figures 3-5 and 3-6). High stress was associated with a higher level of discomfort with loose stools

(Figure 3-3) and urgent bowel movement (Figure 3-5). The effect of fiber on loose stools was seen to be significant only during high stress week (Figure 3-4). Significant statistical differences were observed on the effect of fiber on urgent bowel movement when high and low stress were averaged in the three different treatment groups (Figure 3-6).

High stress was associated with a higher level of discomfort with diarrhea (Figure 3-7), rumbling (Figure 3-8), nausea (Figure 3-9), hunger pains (Figure 3-10) and stomach ache (Figure 3-11) whereas fiber showed no significant effect. Fiber effect under high stress showed significance in not emptying (Figure 3-12) whereas effect of stress did not seem to show statistical significance. Neither stress nor fiber seemed to have an effect on hard stools, constipation, passing gas, burping, bloating, acid reflux or heartburn.

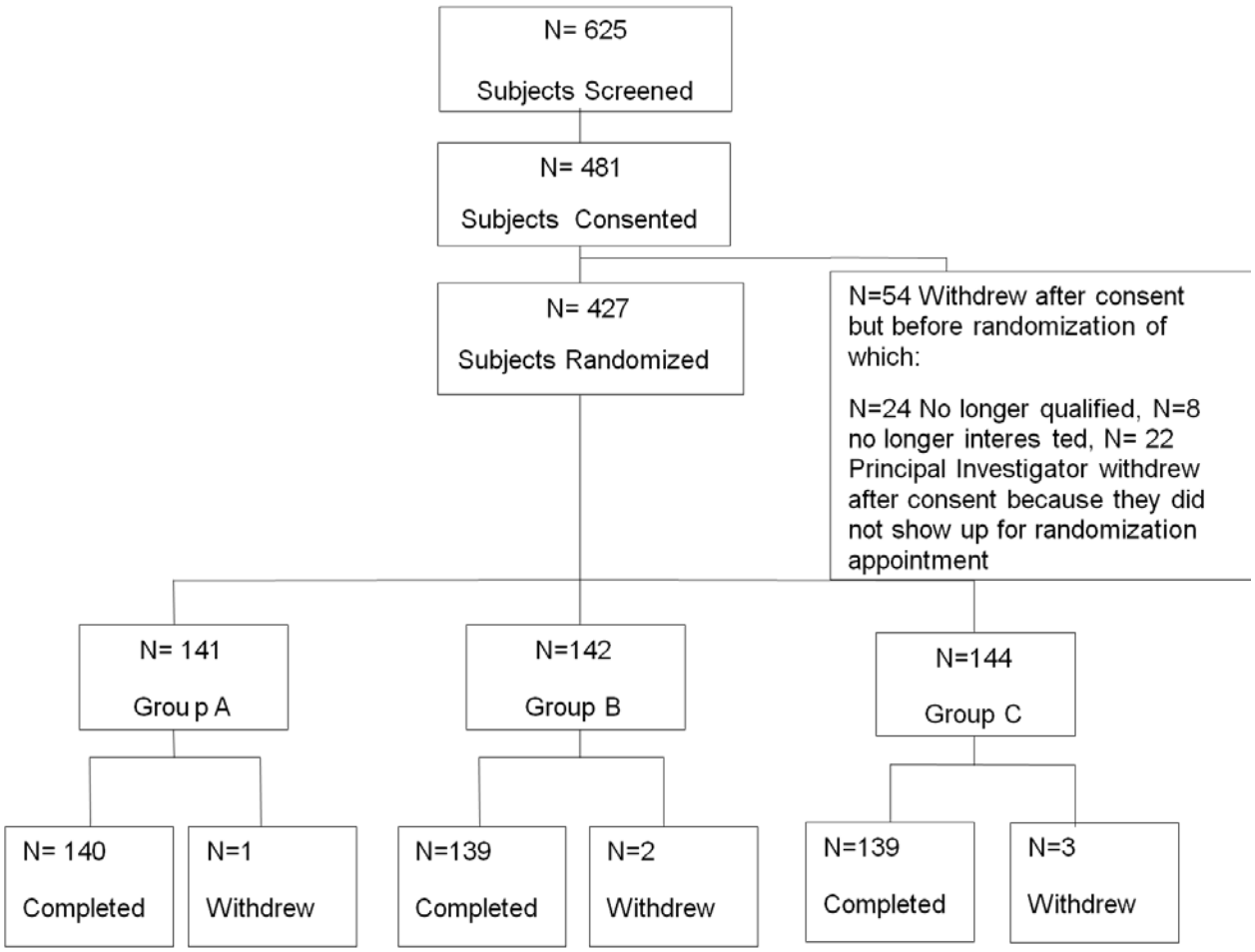

Figure 3-1. Flow chart presenting the number of subjects at each stage of the study. Group A, B, and C represent the different fiber groups.

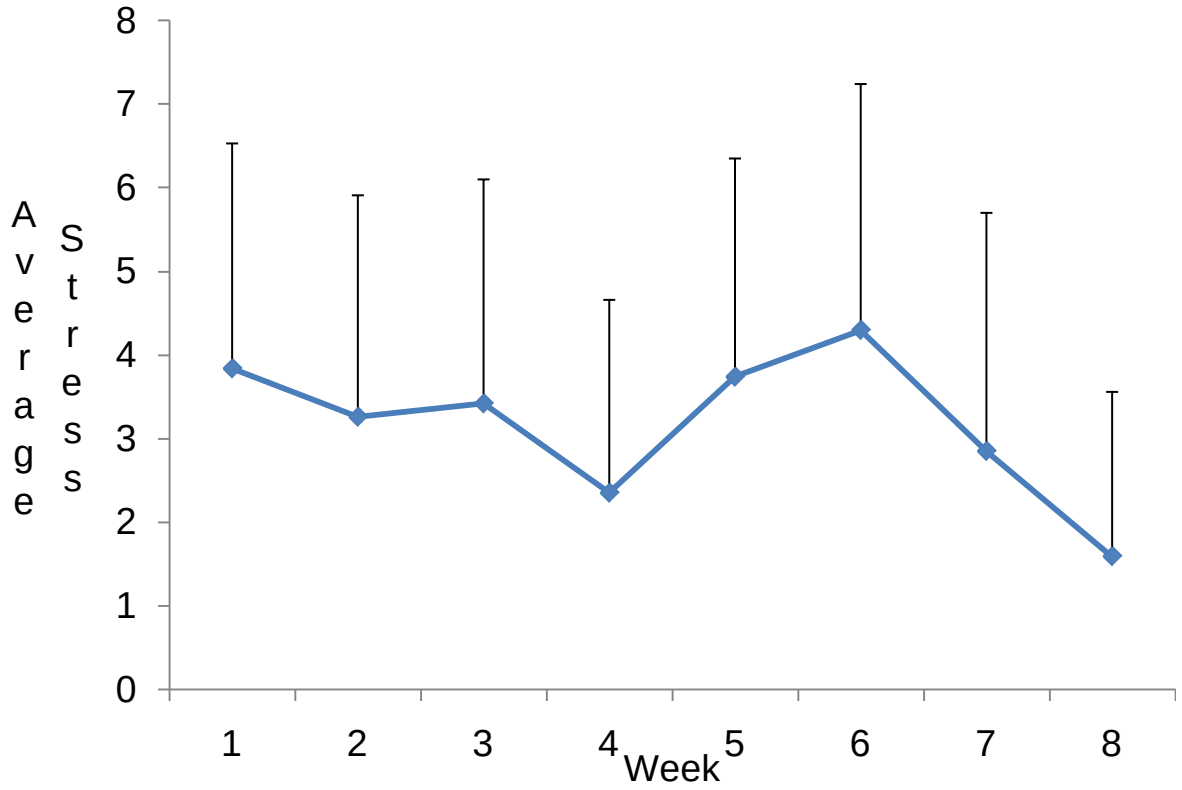

Figure 3-2. The average level of stress for the subjects is plotted in the graph above. Week 6 is showing the highest level of stress; their final exam week and week eight exhibiting the lowest week of stress; their beginning to their winter break. Mean + SD.

Table 3-1. Demographic information.

	Fiber Group		
	A (n=141)	B (n=142)	C (n=144)
Sex (Male:Female)	70:71	71:71	71:73
Age (average)	20.1±1.6[a]	19.9±1.4	19.6±1.7[b]
BMI[c]	23.8±3.5	24.0±4.2	23.4±4.0
Ethnicity			
Asian	18 (12.8%)	20 (14.1%)	21 (14.7%)
Black	18 (12.8%)	18 (12.7%)	18 (12.6%)
Hispanic	22 (15.6%)	29 (20.4%)	25 (17.5%)
White	83 (58.8%)	72 (50.7%)	78 (54.5%)
American Indian/Alaskan	0	3 (2.1%)	0
Native Hawaiian/Pacific Island	0	0	1 (0.7%)

[a]Data represent the mean ± SD
[b] P≤0.05 vs. groups A and B,
[c]Body Mass Index (BMI): Kg/m^2,

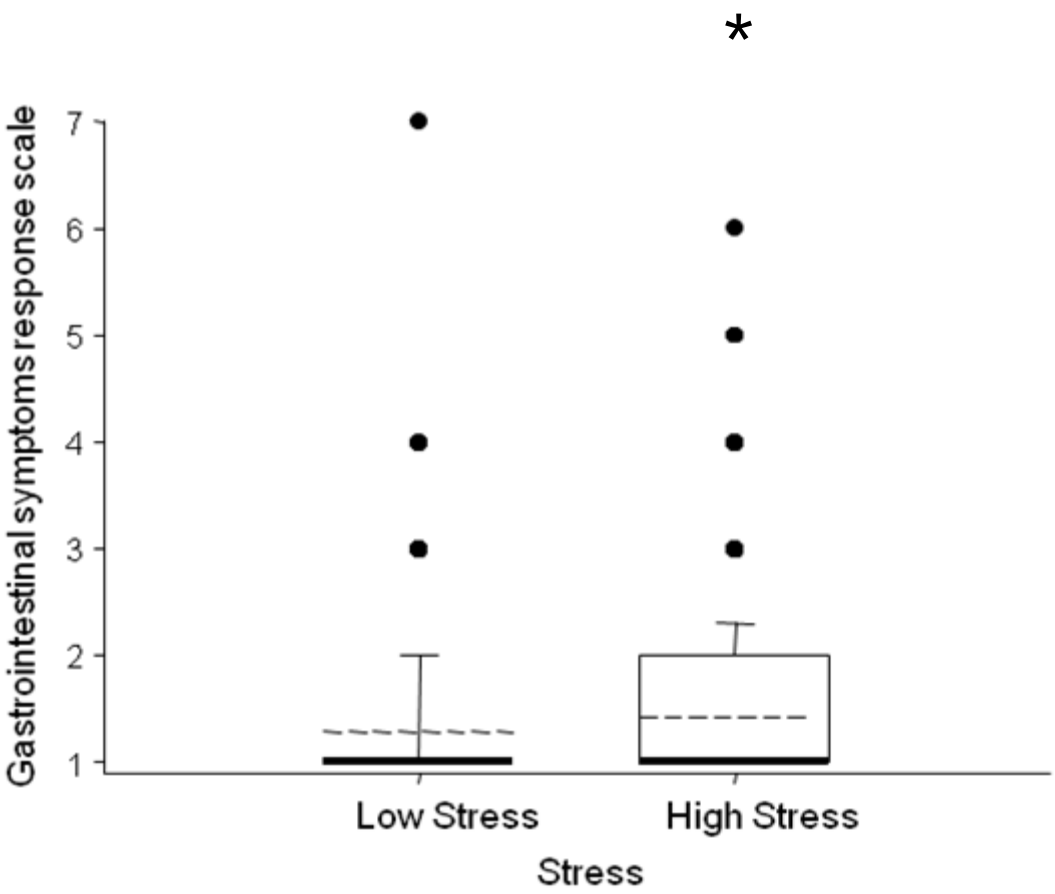

Figure 3-3. Gastrointestinal Symptoms Response Scale score for loose stools showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=406) and high stress weeks (n=406) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P = 0.007 vs. low stress.

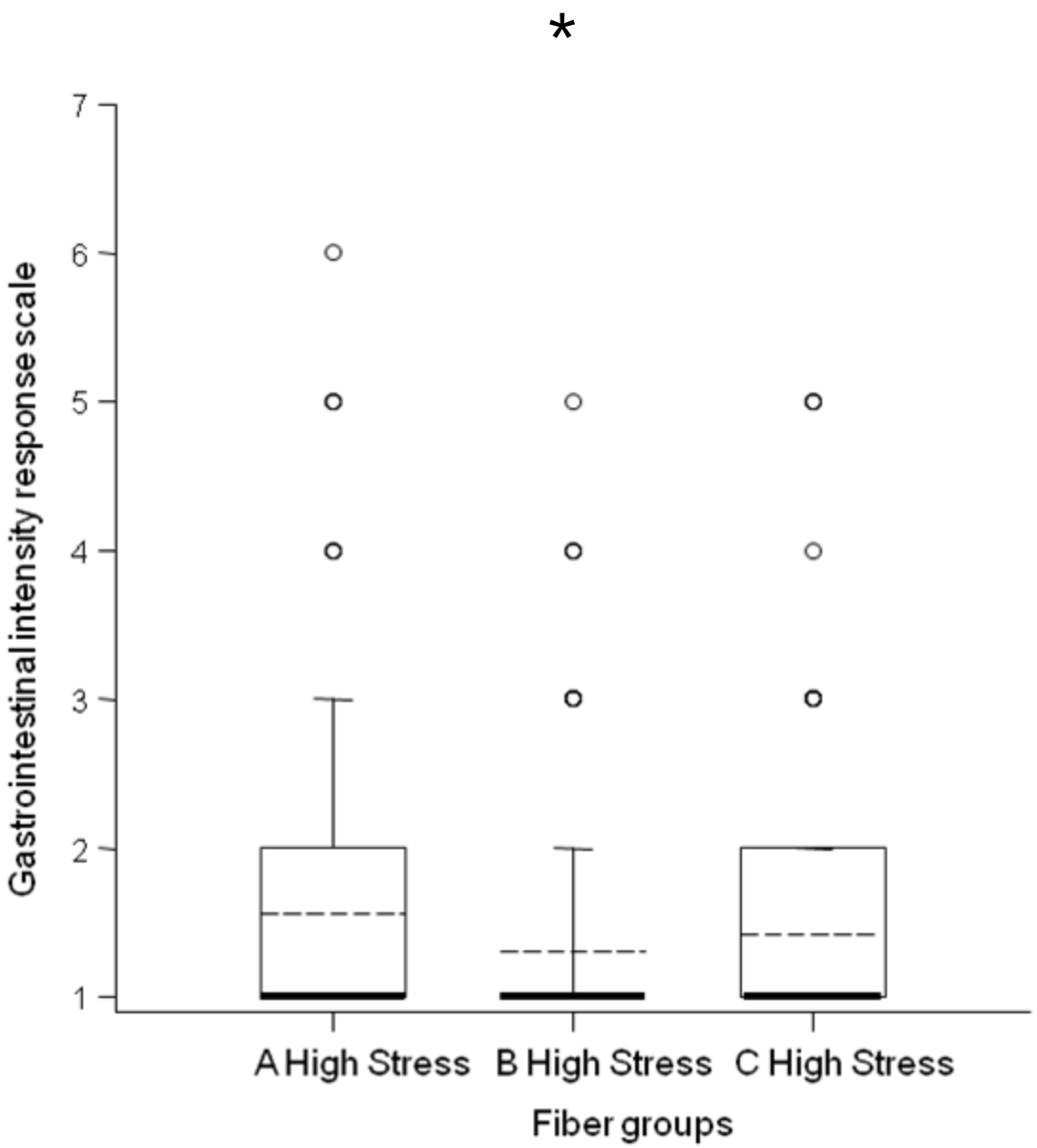

Figure 3-4. Gastrointestinal Symptoms Response Scale score for loose stools showing the effect of fiber during the week of high stress in individuals consuming fiber supplement A (n=135), B (n=137) or C (n=134). The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.045 vs. group A high stress.

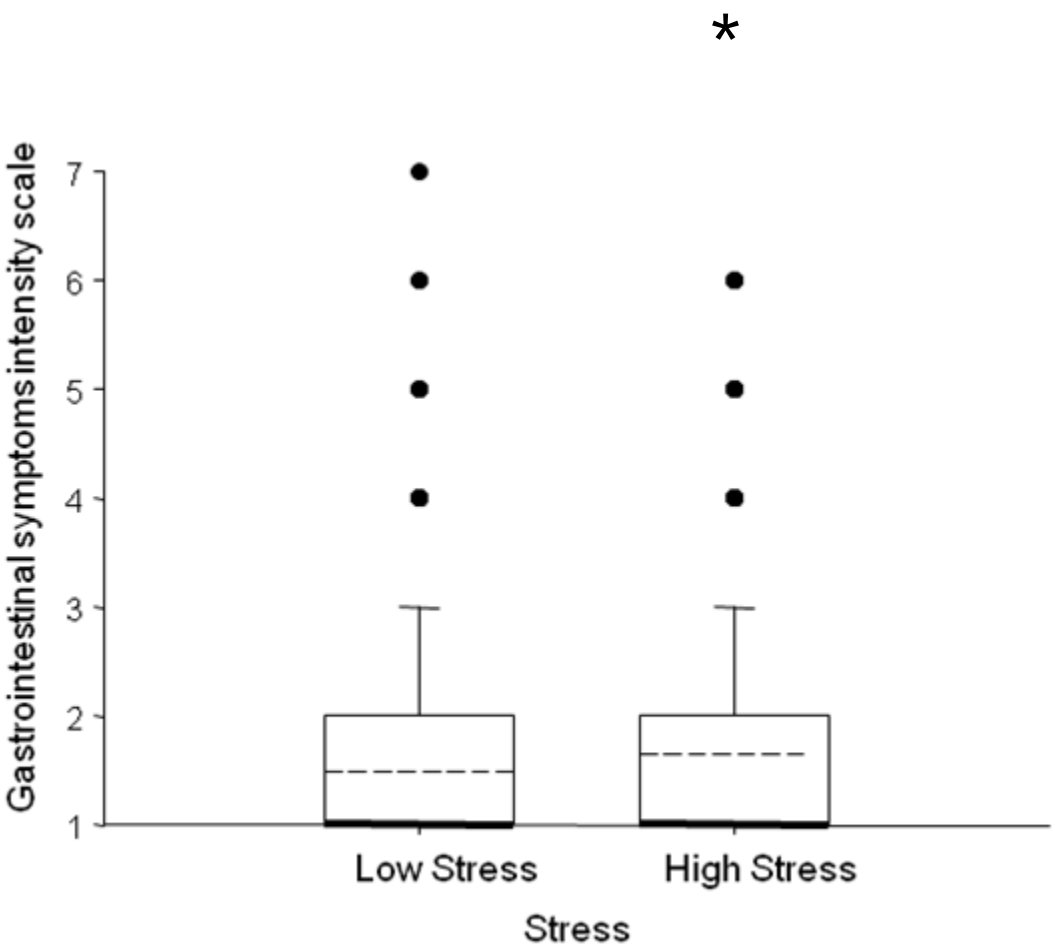

Figure 3-5. Gastrointestinal Symptoms Response Scale score for urgent bowel movement showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=406) and high stress (n=406) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.001 vs. low stress.

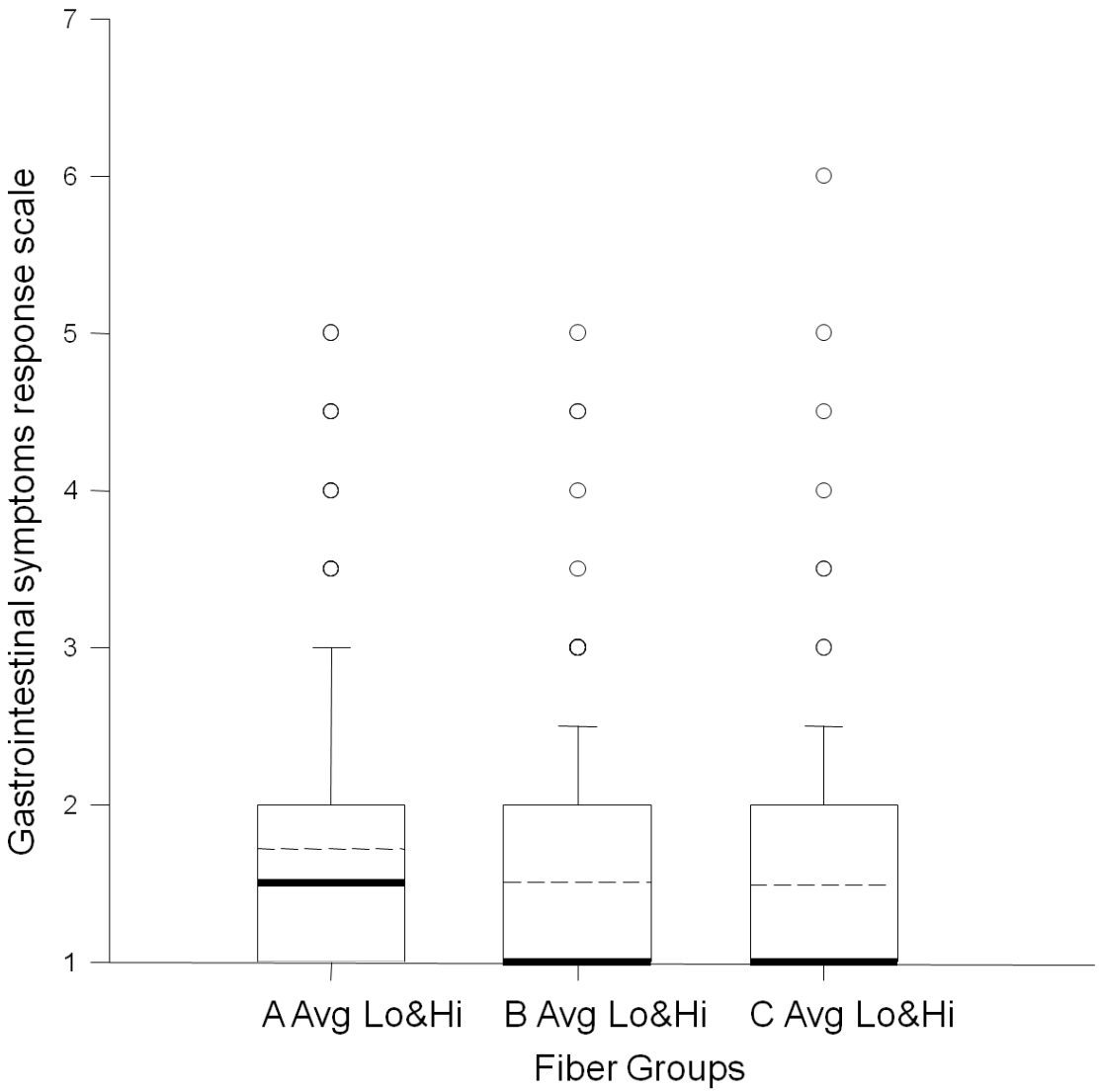

Figure 3-6. Gastrointestinal Symptoms Response Scale score for urgent bowel movement showing the effect of fiber during the average of high and low stress in individuals consuming fiber supplement A (n=138), B (n=138) or C (n=137). Response scores from the low and high stress weeks were averaged within each fiber group. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line within each box. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. The effect of fiber was significant (P= 0.041) across weeks of stress.

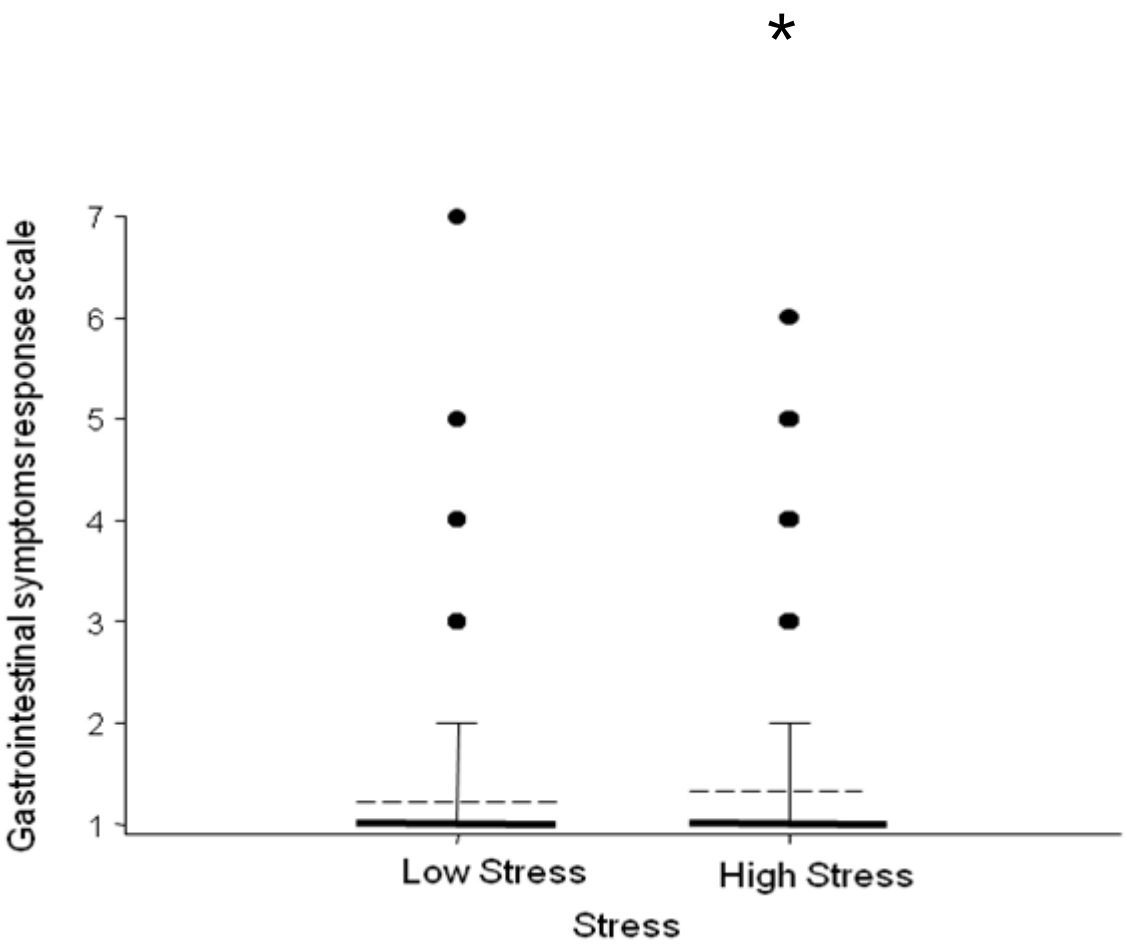

Figure 3-7. Gastrointestinal Symptoms Response Scale score for diarrhea showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=408) and high stress (n=406) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.013 vs. low stress.

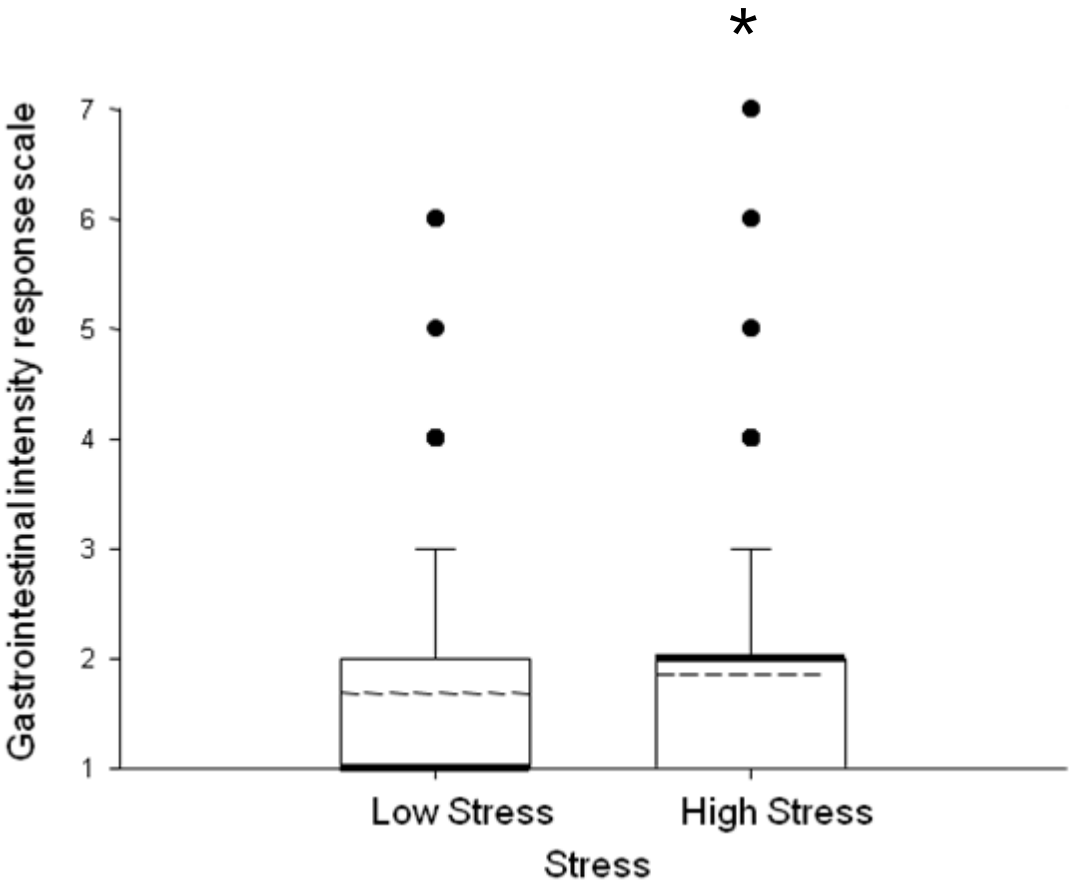

Figure 3-8. Gastrointestinal Symptoms Response Scale score for rumbling showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=407) and high stress (n=407) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.001 vs. low stress.

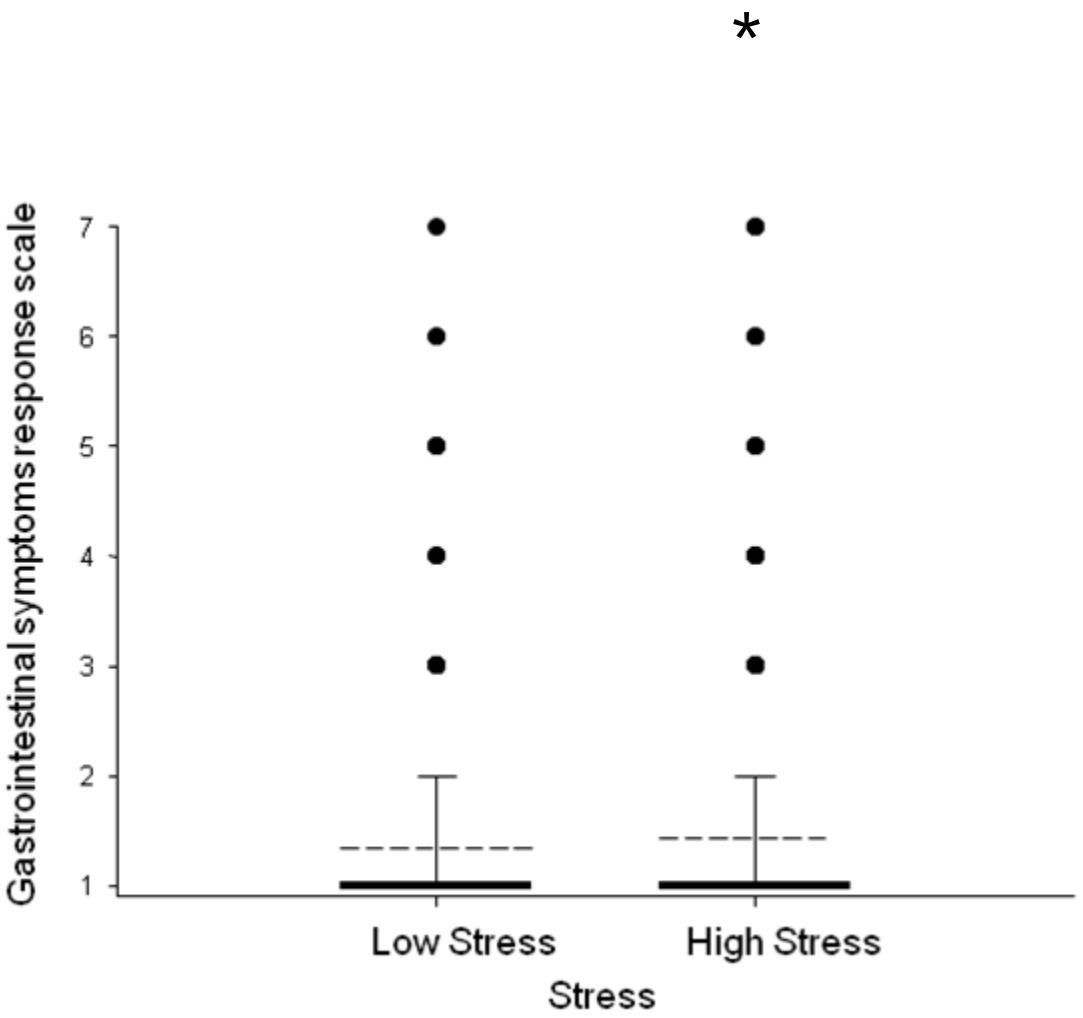

Figure 3-9. Gastrointestinal Symptoms Response Scale score for nausea showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=408) and high stress (n=407) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.047 vs. low stress.

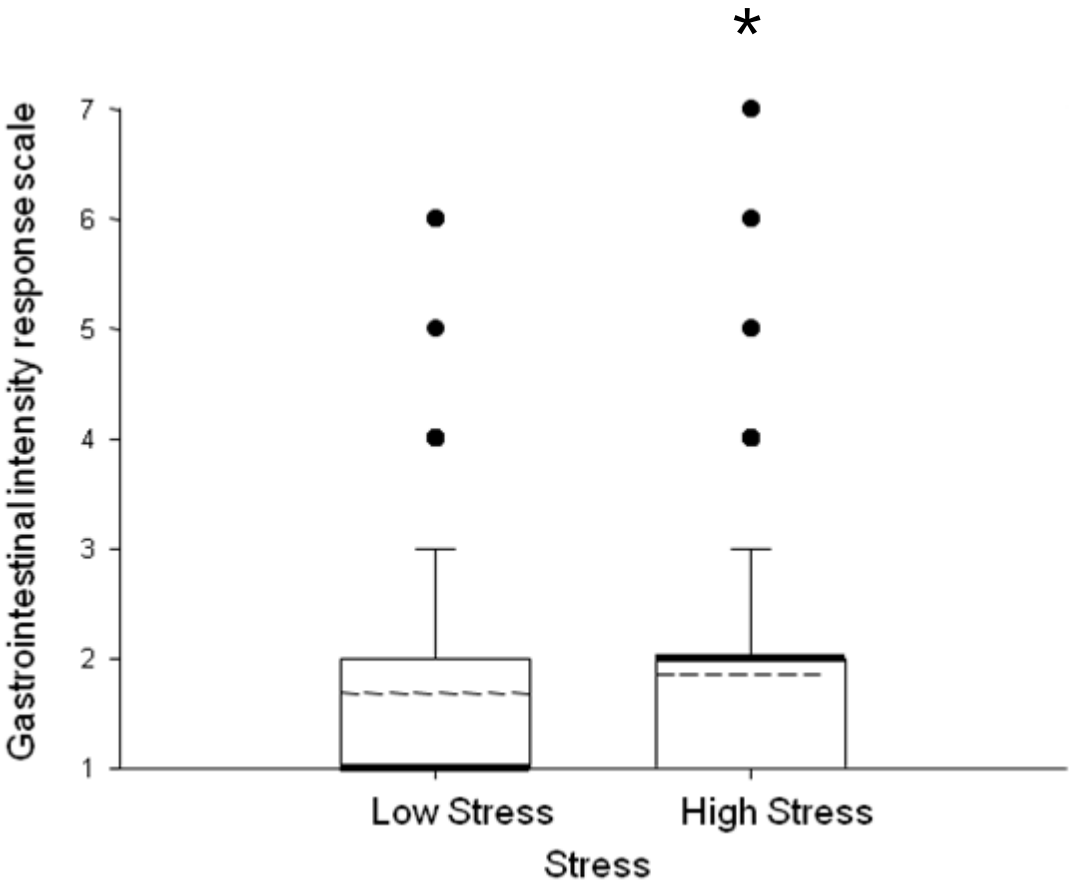

Figure 3-10. Gastrointestinal Symptoms Response Scale score for hunger pains showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=407) and high stress (n=406) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.001 vs. low stress.

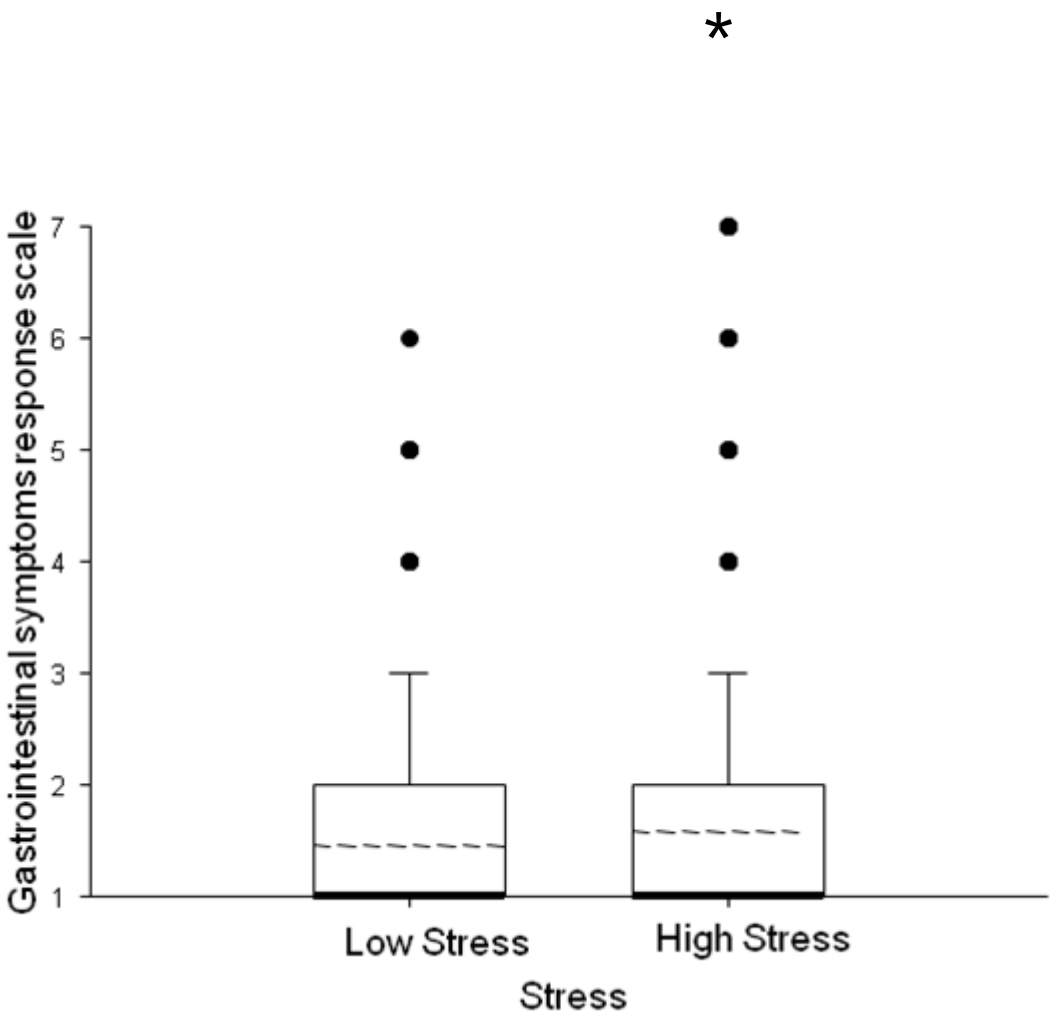

Figure 3-11. Gastrointestinal Symptoms Response Scale score for stomach ache or pain showing the effect of stress during weeks of low and high stress in individuals consuming galactooligosaccharide (0 g, 2.5 g, or 5 g). Data from low (n=407) and high stress (n=407) represent pooled responses from all three fiber groups. The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.02 vs. low stress.

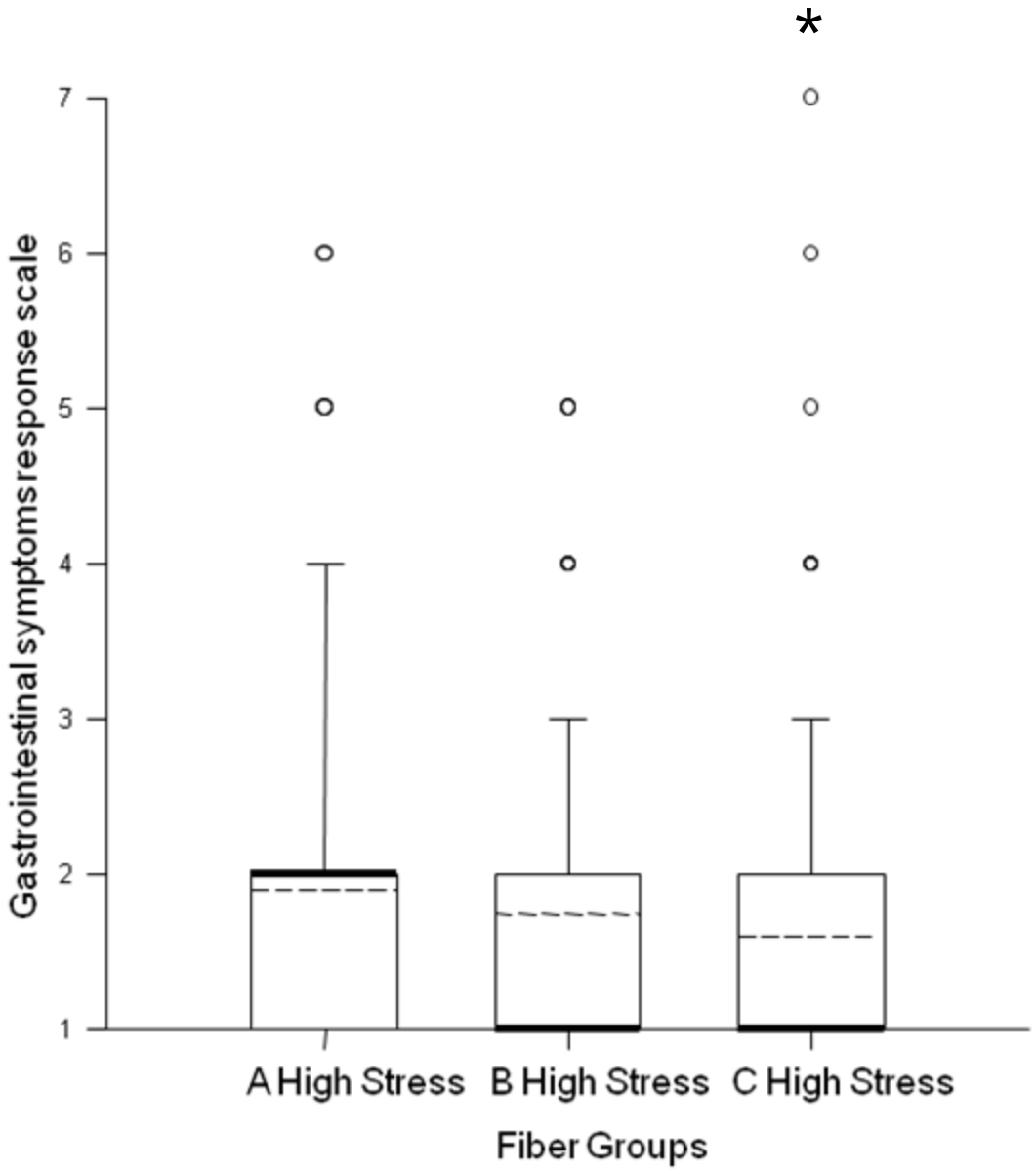

Figure 3-12. Gastrointestinal Symptoms Response Scale score for not emptying showing the effect of fiber during the week of high stress in individuals consuming fiber supplement A (n=135), B (n=137) or C (n=134). The top and bottom edges of the box plot represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th percentile and outlying points are represented by points above the box. The median values are represented by the dark black line. The mean is represented by the black dashed line. On the gastrointestinal scale 1 represents no discomfort and 7 represents severe discomfort. *P= 0.025 vs group A high stress.

CHAPTER 4
DISCUSSION

Prebiotics offer extensive benefits to the consumer. However they have been known to lead to unfavorable side effects such as gas and bloating. The benefits that fiber can offer to the overall gastrointestinal health of the individual outweigh the symptoms greatly; however it would be great to take advantage of all the benefits of fiber without having to experience the unfavorable symptoms.

Glactoologosaccharide is a novel prebiotic and the prebiotic potential has been assessed in previous research [21]. However the effect of this prebiotic on the various gastrointestinal symptoms using the GSRS has not been investigated nor has its interaction with stress. The purpose of the proposed study was to demonstrate that galactooligosaccharides supplemented in the diet will lead to better gastrointestinal health in academically stressed undergraduate students. Four hundred and twenty seven subjects were randomized to one of three fiber groups, they were then asked to take one packet of fiber each day for eight weeks and to record the various gastrointestinal symptoms on the GSRS. Due to the complex design and the severely skewed responses the interaction of stress and fiber could not be statistically analyzed however the effects of fiber and stress could be assessed separately. Fiber seemed to have an effect on only three of the 15 symptoms on the scale, and stress had an effect on seven of the fifteen symptoms on the scale.

During the week of high stress there was a significant difference among the different groups of fiber with loose stools. Because the study is not yet un-blinded, it is not possible to state whether this difference was due to low or high intake of GOS. Due to previous research that has been done it is reasonable to assume that the higher

levels of fiber could be linked to loose stools because in infant studies it has been shown that formula supplemented with GOS led to softer stool in comparison to unsupplemented group [22-23]. In the current study there was a significant difference among the three fiber groups in urgent bowel movement, again which group elicited what effect is unknown. In this case, however the effect was seen to be significant when the average of the low and high stress weeks were taken. This suggests that the effect is solely attributed to the fiber.

Stress has been linked to depletion or lowering of bacterial count of bifidobacteria and certain gram negative bacteria that are beneficial to the intestinal mucosa [20]. Due to the reduction of the beneficial bacteria such as bifidobacteria and lactobacilli it is reasonable to assume that the reduction of these bacterial populations in the colon will lead to unfavorable gastrointestinal symptoms as was observed in this research. An increase in stress was associated with higher levels of discomfort with loose stools, urgent bowel movement, diarrhea, rumbling, nausea, hunger pains and stomach ache. There was no significant effect from either fiber or stress on appearance of hard stools, constipation, passing gas, burping, bloating, acid reflux or heartburn. Other researchers have also reported on decreased production of gas with GOS compared to other prebiotics [9]. Glactooligosaccharide has also been shown to decrease traveler's diarrhea and thus may be the reason why the subjects experience no diarrhea [16] .

There were several limitations with this research. Subjects could have gone back to record their symptoms over the previous week, 36 hours or more after they were asked to complete the questionnaire. Doing so would have lowered confidence in their recall of memory. This takes into question how well they really remembered their

symptoms. Also, there were no resources to validate whether the online questionnaire method was better than the old traditional paper record; however this online approach insured that the subjects complete the questionnaires in a timely manner as completion of study records by subjects could be followed on a minute to minute bases. Another limitation that existed was how the subjects rated their stress, and the different perceptions of stress that exist. The associated weeks of low and high stress seemed reasonable because the week of high stress was associated with the week where the students had final exams and the week of low stress was associated with the week after final exams when the students were on semester break.

Another limitation related to the purpose of the study, which was to see the effect of GOS on gastrointestinal health in academically stressed undergraduate students. The interaction between stress and fiber was not analyzed due to the complexity of the design and skewness of the data, but it was observed that stress does have an effect on various self observed gastrointestinal symptoms and the different levels of fiber did have an effect on some of the gastrointestinal symptoms. There are many beneficial applications for glactrooligosaccharide. There are many products that contain GOS in the field of pediatrics [24]. It has been added to infant formula and cereal based foods in Europe and Japan, and is considered “foods for special medical uses” by the Japanese government [24].

Another great aspect to consider is whether consumers would be willing to use GOS when looking at the results that were obtained. When looking at the graphs in Chapter 3, while the results may have had statistical significance there seems not to be significant difference among the various treatment groups to the naked eye. So the

question that must be raised is whether consumers will be willing to take on the extra financial expense and the hassle of incorporating the product into their daily diet?

Future studies could include the use of this prebiotic with a different group of subjects that include all age groups and not limited to just students and without the use of the academic stress model. It could also be possible to supplement a certain food product with the fiber and then use that product in the research, for example supplementing coffee creamer with GOS, because of its similar texture and slightly sweet taste. Further analyses will be done with stools collected from this group of subjects, however that information is not available at this time.

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BIOGRAPHICAL SKETCH

Yalda was born in Shiraz, Iran. She moved to Scotland at the age of seven, where she attended primary school and then high school. She then moved to the United States, where she finished high school and later attended the University of Florida where she received her bachelor's in food science and a master's in nutritional sciences. She will be attending NOVA Southeastern University in the fall of 2010 to pursue a career in dentistry.