

GALACTOOLIGOSACCHARIDE SUPPLEMENTATION REDUCES STRESS-INDUCED
GASTROINTESTINAL DYSFUNCTION AND DAYS OF COLD OR FLU: A
RANDOMIZED, DOUBLE-BLIND, CONTROLLED TRIAL IN HEALTHY UNIVERSITY
STUDENTS

By

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To my husband, my family and all those who helped me along the way

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
DP	Degree of Depolymerization
EFSA	European Food Safety Authority
FOS	Fructooligosaccharides
GI	Gastrointestinal Tract
GOS	Galactooligosaccharides
GSRS	Gastrointestinal Symptom Response Scale
IBS	Irritable Bowel Syndrome
IgA	Immunoglobulin A
ICAM-1	Intercellular Adhesion Molecule-1
IL	Interleukin
ILra-1	Interleukin-1 Receptor Antagonist
NFKB	Nuclear Factor Kappa B
NK	Natural Killer Cells
SCFA	Short Chain Fatty Acids
SEM	Standard Error of the Mean
SI	Symptom Intensity

Abstract of Thesis Presented to the Graduate School
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Acute psychological stress induced by academic exams is associated with dysregulated gastrointestinal and immune function. The purpose of the study was to examine whether supplementation with galactooligosaccharides (GOS) reduced gastrointestinal dysfunction and percentage of days with cold/flu in academically stressed undergraduate students. In a randomized, double-blind study, subjects (n=427) received 0 g, 2.5 g, or 5.0 g GOS for eight weeks around the time of fall final exams.

Level of stress and cold/flu symptoms and intensity (SI, 0=not experiencing to 3=severe) were recorded daily. The SI from nine cold/flu symptoms was summed with a day of cold/flu defined as a sum greater than six. The Gastrointestinal Symptom Response Scale was completed weekly, and associations between the categories of symptoms and GOS were examined. Stress was positively related to diarrhea, indigestion, and reflux syndromes, abdominal pain, average daily cold/flu SI score, and the percentage of days with cold/flu. Gastrointestinal symptom scores for diarrhea ($P=0.0298$), constipation ($P=0.0342$), abdominal pain ($P=0.0058$), and indigestion

(P=0.0003) syndromes were lower with GOS. The cold/flu SI score was affected by GOS and stress (P<0.0001); 2.5 g was associated with a lower SI score across all levels of stress but 5.0 g was protective only at lower levels of stress. The percentage of days with cold/flu was associated with GOS within different BMI categories (P=0.0002) wherein a 40% reduction in the percentage of days with cold/flu was observed in normal-weight (BMI=18.5-24.9) individuals with 5.0 g of GOS. This effect was not observed in overweight/ obese individuals; however 2.5 g was protective in overweight and obese individuals. Acute psychological stress was directly related to symptoms of gastrointestinal dysfunction and cold/flu. GOS supplementation reduced these symptoms and days with cold/flu.

CHAPTER 1

LITERATURE REVIEW

Introductory Notes

It is known that the common cold and influenza are an economic burden, and that they also adversely affect student academic performance. One method to study colds and flu is with the academic stress model, in which a percentage of students undergoing stress get sick and exhibit cold or flu symptoms in a defined period of time. Nutritional interventions could be employed to maintain good health by decreasing cold and flu symptoms. For example, prebiotics, non-digestible carbohydrates fermented in the gastrointestinal tract, have the ability to stimulate the growth of potentially beneficial bacteria which may reduce the immune burden and maintain health. Stressors, such as psychological stress, alter the microbiota and impair health by dysregulating immune function (1). Research is lacking regarding the effect of galactooligosaccharides (GOS), a prebiotic, on the immune outcomes of undergraduate students undergoing stress. This literature review will discuss galactooligosaccharides and the research pertaining to infants, as well as a recent EFSA health claim. Topics will also include the academic stress model, the Gastrointestinal Symptom Rating Scale (2), gastrointestinal microbiota and immune function, cold assessment in research, and other nutrients affecting immunity.

Galactooligosaccharides

The benefits of pre- and probiotics arise from the impact they have on the gastrointestinal microflora. Probiotics are live microorganisms, such as lactobaccili, that act by modifying endogenous bacteria and increasing the proportion of beneficial bacteria. However, the effects of probiotics are difficult to control as the amount of

bacteria reaching the gastrointestinal tract is not known (3). A prebiotic is “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health” (4). A revised definition states that prebiotic is a, “selectively fermented ingredient that allows specific changes, both in the composition and/ or activity in the gastrointestinal tract that confers benefits upon host well-being and health” (5).

Prebiotics are known to be fully fermented in the gastrointestinal tract, producing short chain fatty acids, fuel for the colonocytes (6, 7). By strengthening colonocytes, prebiotics maintain gastrointestinal integrity and promote general good health.

Prebiotics such as GOS facilitate the colonization of gastrointestinal microbiota in the gastrointestinal tract of newborn infants (6). Human breast milk is believed to be the “gold standard” for infant nutrition and is also the most common source of GOS found at a concentration of 1 g/dL (8). The oligosaccharide ratio believed to be most similar to breast milk commonly researched in infant studies is 9:1, GOS: fructooligosaccharide (FOS) (9). GOS is added to various foods in Japan and Europe as a health-promoting ingredient (10).

Galactoliogosaccharides are comprised of a chain of galactose units arising from transgalactosylation reactions with a terminal glucose unit (Figure 1-1). The degree of polymerization can range from 2 to 8 units. In vitro evidence shows they are resistant to salivary and digestive enzymes and are most likely fermented in the gastrointestinal tract (6, 11). Commercial production of GOS occurs through a transgalactosylation reaction (12). The enzymatic treatment of lactose by β -galactosidase produces several oligomers of differing lengths including GOS (5, 10). GOS is a food ingredient that has

GRAS status in the United States and after purification GOS can be added to food products (5).

The GOS employed in the research study was a white powder derived from food grade lactose and β - galactosidase obtained from *B. circulans* LOB 377 which is a non-toxigenic, non-pathogenic organism (Figure 1-2). Synthesis of GOS [Purimmune, GTC Nutrition, Golden, Colorado] starts with a lactose powder which is dissolved in hot water (90 °C) (13). Adjustments are made for pH to make the environment slightly acidic (hydrochloric acid and sodium hydroxide) (13). The solution is stirred until oligosaccharide concentration is greater than 50% w/v then the temperature is raised to inactivate the enzyme. The enzyme is removed; the product is then processed to remove impurities, concentrated, and further purified. The supplement contains 86% GOS, with 21% DP2 and 63% DP3 and above.

The influence of GOS on allergy and eczema development in infants, cholesterol levels in young adults, and calcium absorption in post-menopausal women has been studied (14-18). However, research is lacking regarding the effect of GOS on immune outcomes in a student population.

It is thought that GOS aids gastrointestinal immunity by directly affecting the gastrointestinal epithelium (10). GOS has been shown to prevent the adhesion and invasion of pathogens in the small intestine such as *Escherichia coli* (19). The authors propose that GOS may act as a receptor mimic and may competitively inhibit adhesion and invasion of pathogens into the gastrointestinal epithelium (19).

Another proposed mechanism by which GOS interacts with the immune system involves changing the gastrointestinal microflora by selectively stimulating the growth of

lactobacilli and bifidobacteria (6). These bacteria are considered beneficial. Breastfed infants are believed to have a more beneficial gastrointestinal microflora which produces short chain fatty acids (SCFA), acetate and lactate that lower the pH. These inhibit the growth of potentially detrimental bacteria such as certain Escherichia coli (20). Thus, researchers examined the fecal pH of breastfed infants in comparison to infants on a placebo consisting of maltodextrin (21-24). Ben et al. (n=371) and Bakker-Zierikzee et al. (n=57) (21, 22) found that fecal pH decreased with an increase of lactate, suggesting that conditions are unfavorable for enterobacteria. Moro et al. (2002) also found a decrease in fecal pH with oligosaccharide supplementation versus the placebo (24). The authors also reported a dose dependent effect of oligosaccharides (0.4 g/ dL and 0.8 g/dL) on the stimulation of bifidobacteria and lactobacilli (24). In contrast, Bouhnik et al. examining GOS in adults (n=8) found no significant difference in fecal pH and no difference in fecal concentrations of enterobacteria (23). These findings did show an increase in bifidobacteria and, over time, a decrease in breath hydrogen suggesting that the fermentation of trans-galactooligosaccharide has changed over time possibly more effectively metabolized in the colon (23).

A study by Tiihonen et al. examining GOS and a probiotic mixture in young Dutch men showed no effect on fecal bifidobacteria. However, the authors suggest this could potentially be due to the already high counts of bifidobacteria due to the high fiber content of the typical Dutch diet (3). Another study showed that the bifidobacteria increase was more significant in populations with a low baseline number of bifidobacteria (25). Nonetheless, in part because of the complexity of the gastrointestinal tract, discrepancies exist in the literature (21).

GOS and Infants

Various studies examine the effect of GOS on infants. GOS supplemented in infant formula has been shown to be well tolerated (21). Infants supplemented with GOS have shown similar gastrointestinal microflora as breastfed infants (21). GOS relieves constipated infants by contributing to softer stool formation whilst not increasing stool frequency (26). Infants fed with a formula containing GOS have the same growth and stool characteristics as breast fed infants (27). A study by Ben and colleagues (n=371) found that a low dose of GOS (2.4 g/ L) was shown to be bifidogenic (22). Oligosaccharides in human breast milk are found in a concentration of 8-12 g/ L in mature milk and 25 g/ L in colostrum (28). Researchers preferred the low dose intervention to avoid potentially irritating effects associated with higher doses in infants (22). Scholtens found that infants supplemented with GOS and long-chain FOS (n=215) had an increase in fecal sIgA with an increase in bifidobacteria, suggesting a benefit to gastrointestinal mucosa (29). Oligosaccharides (GOS/FOS) has been shown to decrease stool transit time and increase stool viscosity in premature infants (30). Alternatively, premature formula fed infants tend to have hard stools and delayed gastrointestinal transport (30).

As human milk is rich in oligosaccharides, breast fed infants are shown to have increased bifidobacteria and lactobacilli in their intestines (24). It is this bifidogenic effect that researchers are interested in replicating by performing intervention trials with prebiotics, such as GOS. Moro et al. found that supplementing formula-fed infants with GOS and FOS (either 0.4 g/ dL or 0.8 g/ dL) resulted in a dose dependent effect on bifidobacteria and lactobacilli over the 28 days study period, versus the placebo (24).

Scholtens found that during weaning, infants supplemented with GOS (4.5 g/d) had bifidobacteria counts similar to exclusively breast-fed infants (31).

Early mode of feeding, breastfeeding or formula feeding, and gastrointestinal microbiota are of interest. Kalliomaki et al. hypothesized that early microbiota could be associated with children to becoming obese later in life (32).

EFSA Response to Health Claim

Recently a health claim regarding GOS and its effects on immunity has been investigated for its validity. In response to a health claim application, the European Food Safety Authority (EFSA) examined various studies regarding the effects of GOS/FOS (9:1) (brand name Immunofortis, Danone Baby Nutrition, The Netherlands) on immune function and the incidence of atopic eczema in infants (33). EFSA stated that the evidence demonstrating that Immunofortis had an effect on strengthening the infant's immune system was not adequate (33). One such study sought to examine immune function in infants, and showed a decrease in infection rate and allergic manifestations (15, 16). According to the EFSA, the researchers did not adequately investigate the basis of each diagnosis whether it would be determined from clinical symptoms or even microbiological testing. Mothers reporting allergy symptoms for their infants is not reliable; this is interesting to note because evidence shows that adults self-reporting cold and flu data is reliable. The EFSA also stipulated that a healthy immune system includes an adequate response from the adaptive immune system and that a decrease in infection rate does not show a direct effect on immune function. The EFSA stated that there is lack of evidence showing that an increase in bifidogenic bacteria and a decrease in enterobacteria would be beneficial (33). Also, the board did not find adequate evidence of Immunofortis's decreasing allergic infections or eczema based on

the literature supplied by the researchers (15-17, 34-37). With better design outcome measures the mechanism by which oligosaccharide supplementation impacts immune outcomes may be demonstrated.

Microbiota and Immune Function

The gastrointestinal tract is the largest organ of the immune system and changes in gastrointestinal microbiota could lead to changes in immune health. This section will briefly discuss gastrointestinal microbiota and immune health, the role of stress in gastrointestinal function, the Gastrointestinal Symptom Response Scale, the Bristol Stool Scale, and intervention trials involving GOS's effect on the gastrointestinal microbiota.

The number of bacteria found in the colon and in the feces is 10^{12} cells per gram (38). The gastrointestinal tract is home to a diverse microflora providing beneficial effects to the host. The gastrointestinal microflora has been shown to facilitate excretion of toxins and occupy attachment sites for potentially pathogenic microorganisms (38). It also interacts with immune tissue such as Peyer's Patches and gut-associated lymphoid tissues (38). The most prevalent organisms in the gastrointestinal tract are the Bacteriodes-Prevotella group (gram-negative anaerobe) and Clostridium species (gram-positive anaerobes). Aerobic bacteria are much less prevalent (38). The gastrointestinal microflora differs among people. This begs the question as to the effect of differing microflora on hosts (38). Future research aims to determine which species of bacteria have most significant effects on human health and how nutritional interventions can alter this microbiota.

New evidence suggests that changing microbiota affects human health. Ley has shown that gastrointestinal microbiota differs in lean and obese people and that weight

loss can change the microbiota (39). Ley's data shows less Bacteroidetes in obese individuals and a higher proportion of Firmicutes compared to lean individuals (39). Weight loss of the obese groups can result in an increased proportion of Bacteroidetes, more closely resembling that of lean individuals (39). A weight loss of at least 6% in the fat restricted diet and at least 2% in the carbohydrate restricted diet was necessary to see this effect. This suggests that weight loss, or possibly a change in diet can alter gastrointestinal microflora. Obesity is a disorder characterized by systemic inflammation (40). Neyrick theorizes that prebiotics could be beneficial in the treatment of the inflammation associated with obesity through altering the gastrointestinal microbiota (41).

Stress is known to cause gastrointestinal dysfunction. Sixteen medical and premedical students reported increased abdominal pain during examinations (1). Acute stress has been shown to inhibit gastric emptying and decrease colonic transit time (42). Research is lacking regarding the effect of academic stress on gastrointestinal symptoms.

The GSRS and Bristol Stool Scale

The Gastrointestinal Symptom Rating Scale (GSRS) was developed for irritable bowel syndrome (43). The GSRS has been validated for its ease of use and excellent construct (44-46). The GSRS consists of five syndromes with approximately three symptoms each including: diarrhea syndrome (diarrhea, loose stools, urgent need for defecation), constipation syndrome (constipation, hard stools, and feeling of incomplete evacuation), abdominal pain (abdominal pain, hunger pains, and nausea), indigestion syndrome (rumbling, bloating, burping, and gas) and reflux syndrome (heartburn and acid regurgitation).

The Bristol Stool Form Scale is a seven-point stool consistency scale devised by O'Donnell as an estimate of stool transit time (47). Stools are rated as such: 1 (separate hard lumps like nuts), 2 (sausage shaped but lumpy), 3 (like a sausage or snake with cracks on its surface), 4 (like a sausage or snake, smooth and soft), 5 (soft blobs with clear cut edges), 6 (fluffy pieces with ragged edges, a mushy stool) and 7 (watery, no solid pieces) (47). The scale has been used in research and in clinical practice (48, 49). The Bristol Stool Form Scale helps to clarify whether self-reported stress levels have an impact on gastrointestinal transit time.

GOS and microbiota

There have been few studies describing how GOS changes the gut microbiota and affects immune function and health outcomes. Davis et al. found that doses of 5.0 g/d and 10 g/d produced an increase in bifidobacteria counts and 2.5 g/d did not (50). It is interesting to note that even when a high dose of GOS (10.0 g/d) was administered for three weeks, some participants did not show the bifidogenic effect (50). One study by Bouhnik and colleagues in healthy adults found that GOS was bifidogenic at 2.5 g/d to 10 g/d (n=64) (25). Vulevic et al. is the only study to date examining immune function and GOS in healthy elderly volunteers (51). Participants on GOS (2.64 g/d) had a significant increase in bifidobacteria counts and a decrease in other less desirable bacteria such as some strains of clodistria and enterobacteria. They also found an increase in anti-inflammatory cytokines (IL-10) and a reduction in pro-inflammatory cytokines (IL-6) after peripheral blood mononuclear cell stimulation by lipopolysaccharide. This study shows that immune function can be modulated with the addition of GOS.

GOS, or more specifically trans-galactooligosaccharide, has been examined for its potential effects on irritable bowel syndrome patients(IBS). Silk et al. employed an intervention with either 1.7 g/d, 3.3 g/d or a placebo and asked participants to rate gastrointestinal symptoms on a 7 point Likert scale. Results indicate that 1.7 g of trans-galactooligosaccharide enhanced fecal bifidobacteria numbers and lessened flatulence and bloating (52). The researchers suggest that these results may provide insight into the mechanism of GOS in regards to improving IBS. The researchers state that anti-inflammatory markers in the peripheral blood were normalized with increased bifidobacteria (52).

A study by Marcos examined whether taking a probiotic could alter gastrointestinal microbiota and impact immune response in students (53). One hundred and fifty-five undergraduate students undergoing academic stress consumed a fermented yogurt with *Lactobacillus casei* DN-114001 (53). Marcos and colleagues found that stress causes a dysregulation of the immune system, particularly a decrease in lymphocytes and decreased natural killer cell activity (53). The addition of the fermented yogurt containing *Lactobacillus casei* to the diet of stressed students improved immune function as seen with an increase lymphocyte number as well as CD56 cell activity (53).

Pregliasco et al. found that long term administration (90 days) of a synbiotic improved bowel functions, and also reduced the incidence of the common cold (54). A synbiotic is a prebiotic and a probiotic and acts by, “improving the survival of live microbial dietary supplements in the gastrointestinal tract of the host” (10). Participants were asked to record symptom severity (on a scale of 1-5, with 1= no symptoms and 5=severe symptoms) and whether there were any changes in bowel movements

(increase, decrease or no change) (54). The symbiotic (*Lactobacillus planterum*, *Lactobacillus rhamnosus* and *Bifidobacterium lactis*), lactoferrin and FOS or GOS increased bowel movements and decreased incidence, severity and average duration of colds (54). Immune outcomes have been measured for probiotics, and synbiotics. One study examined this relationship with GOS in aged adults; however no studies have examined whether GOS affects immune outcomes in students undergoing an academic stress.

Academic Stress Model

This section will explore the research regarding the academic stress model and how it relates to the presented research. For students, passing or failing examinations influences a student's future and is classified as an acute stressor (55). Researchers examining health benefits such as decreasing cold and flu symptoms with a particular nutritional intervention have various methods at their disposal to see whether the intervention produces an effect. One way to examine health and colds would be to use a viral-challenge study design by injecting a rhinovirus into participants under stress consuming either the placebo or the compound of interest and to measure what percentage of participants on each treatment exhibit cold symptoms (56). Cohen inoculated 394 healthy participants with viruses (rhinovirus 2, 9 or 14 or respiratory syncytial virus or coronavirus 229E) and examined whether psychological stress impaired immunity (57). The researchers linked higher psychological stress and increased susceptibility to illness (57).

Another way to assess colds and flu would be to monitor study participants for an extended time period, in which participants will almost inevitably get sick (58). The idea behind this method is that the compound of interest could prevent colds symptoms.

Another way of measuring cold symptoms would be by employing a model which shows that a certain percentage of participants would get sick during the time period (i.e., the academic stress model). This model is a method which looks at sickness in undergraduate students undergoing academic stress and the occurrence of cold and flu symptoms in a short time frame (59, 60). The academic stress model can be employed when injection of a virus cannot be justified, or the long time frame of other study designs would deter students from participating. This model has been validated by other studies (59, 60).

Many studies have used the academic stress model to evaluate its effects on immunity. In a naturalistic model, Cobb et al. examined whether psychological style, coping style and family environment had an impact on susceptibility to upper respiratory tract infections (61). The researchers followed 107 participants for one year prior to the study and 15 weeks of the study and found that the risk of illness was higher in those with a higher perceived stress and high life event stress (61). Interestingly, the avoidant coping style was protective against colds in high life stressors. The researchers suggest that avoidance style of coping may prevent people from dwelling on the negative aspects of their lives and thereby shortening their time of vulnerability (61).

Sleep quality and duration are believed to be important predictors of immunity. Cohen at al. performed a study regarding sleep duration and immunity. The researcher found that those with poorer sleep efficiency and sleep duration in the weeks prior to infection exposure had a higher probability to develop a cold (62). The researchers explain that sleep disturbance can be influenced by the regulation of pro-inflammatory cytokines (62).

Physical or physiological stressors can alter immune function thereby potentially increasing susceptibility to infections. Uchakin and colleagues monitored immune responsiveness of first-year medical students during first examinations (63). Blood was collected at either 24 hours or 48 hours after examinations. Peripheral blood mononuclear cells were stimulated with phorbol myristate acetate and lipopolysaccharide for four hours (63). Results indicate that stress can alter immune cells in vitro as seen by a decrease in natural killer cell numbers, and changes in other immune cells (63). A major limitation of this study is that for some parameters there was no adequate power to detect significant changes.

Acute stress increases the activity of the hypothalamus-pituitary adrenal (HPA) axis and raise salivary immunoglobulin A (IgA). A study by Takatsuji et al. examined the effect of examination stress on female nursing students ($n=15$) by salivary IgA and cortisol levels prior to and immediately after an examination. The researchers found an increase in salivary IgA with no significant changes in cortisol levels (55). In contrast Ng et al. studied salivary IgA and cortisol levels in dental undergraduate students prior to and immediately after examination (64). The researchers wanted to link these data with participants' perceived stress and found pre-test stress correlated with an increase in cortisol but no change in IgA (64). An explanation for these divergent results could be accounted for by the fact that cortisol is highest after waking and decreases throughout the day (65). As the researchers did not specify the time of day that salivary cortisol was measured this could account for the differing results.

Kiecolt-Glaser et al. took blood samples from medical students (n=75) one month before examination and on the first day of final exams (66). They found that stress led to a decrease in natural killer cell activity with no changes in salivary IgA (66).

Stress can negatively impact the gastrointestinal tract. Knowles et al. examined whether academic stress impacted salivary cortisol and lactic acid bacteria in the stools of students (65). The results indicate that stress led to a decrease in lactic acid bacteria counts during time of high stress (final examination) (65). The authors suggest that stress causes alterations in the microbiota leading to increased susceptibility to illness (65).

Cold Assessment in Research

Colds and flu have been studied but criteria is lacking regarding what actually constitutes a cold. A weakness in cold research is the lack of well-developed outcome measures. The Jackson scale is typically employed to determine cold symptoms and severity. The index developed in the 1950s assess 8 symptoms (sneezing, nasal obstruction, nasal discharge, sore throat, cough, headache, chilliness and malaise) rating these symptoms on a three or four point scale (67). The scale was shown to have diagnostic validity in Gwaltney's study using the rhinovirus (68).

Another method to assess colds is the Wisconsin Upper Respiratory Symptom Survey (WURSS), developed to assess the quality of life during colds. The WURSS, which was found to be reliable, is a 44-item index that consists of impairments to daily living, breathing, sleeping, working, and interpersonal relationships (69).

Barrett et al. investigated whether there was a relationship between questionnaires (mainly the Jackson scale and the WURSS). The researchers looked at an Echinacea study in which participants were inoculated with the rhinovirus and laboratory

biomarkers including IL-8, nasal neutrophil count, mucus weight and viral titer were examined (70). These biological markers were used to assess whether the participants developed a cold. Modest associations were found and neither questionnaire was a good predictor of infection but both correlate with laboratory- assessed measures (69). The addition of quality of life questions on the WURSS did not strengthen the association with laboratory biomarkers (69). This study suggests that the WURSS, while providing information regarding quality of life, did not provide any additional information pertaining to the actual cold.

Supplements Affecting Immunity

The purpose of this section is to explore supplements affecting immunity. First, a background of the common cold will be discussed including the mechanism of transmission. A literature review pertaining to ascorbic acid, zinc, selenium, garlic, Echinacea, vitamin D, carotenoids, green tea and how these supplements affect immunity will be discussed.

The Common Cold

The economic cost of the common cold is staggering. It is estimated that a working adult loses on average 8.7 work hours when inflicted with a cold and 1.2 work hours when attending to children under the age of 13 inflicted with a cold or flu episode (71). This amounts to 25 billion dollars lost in productivity annually (71). The cost for students is also large. A 6-month study by Nichol et al. revealed that 91% (n=3,249) had a cold (72). Colds and flu accounted for bed days, missed school days and work days, resulting in doing poorly on exams or assignments (72).

A reliable, simple, cost-effective way to reduce the incidence and duration of the common cold is desired. Programs aimed to reduce sickness absence days by

providing physical activity and stress management have been examined. One such study examined whether an intervention on nursing assistants would have a lasting impact, after 3 years follow-up no lasting impact was found (73). It would be beneficial to find nutritional interventions to decrease colds and flu.

The incidence of upper respiratory tract illnesses in adults is from one to six episodes a year (74). The most common causes of upper respiratory tract infections (colds and flu) include the rhinovirus, influenza, parainfluenza, adenovirus, and syncytial virus (74). The peak incidence of colds in temperate regions occurs in the autumn months, for reasons unknown (74). A study performed with young adults (n= 346) found that reverse-transcription polymerase chain reaction for picornavirus and virus isolation for human rhinovirus confirmed 83% of self-reported colds during the autumn months (September thru October) (75). Tyrrell et al. found that after inoculation of picornavirus type 299E or syncytial virus 94% of participant's self-diagnosis agreed with the clinician's diagnosis (76). The study also found that the only difference between the viral strands was the incubation time (76).

The onset of symptoms of upper respiratory tract infection is usually one to two days after the viral infection and the peak of symptoms being two to four days after exposure (76). The illness includes complaints of scratchy throat, sneezing, nasal discharge, sore throat, hoarseness, cough, headache, myalgia, malaise, feverishness, and chilliness (77). In the beginning of a cold, symptoms include nasal obstruction, rhinorrhea, and sneezing with a "scratchy" throat being the first symptom (76). Cough is associated with 30% of colds (78). The cold can last about a week and up to 25% can last 2 weeks (78).

Treatment and patient beliefs

The goals in the management of the common cold include resolving of symptoms, decreasing the person-to-person spread, and preventing complications (79). The common cold is generally associated with low morbidity but complications can include otitis media and sinusitis (79). A survey of pediatricians in 2009 confirmed that 96% reported treating sinusitis with an antibiotic “frequently” or “always” (80). Treatment for colds typically focuses on symptom relief such as cough suppressants, antihistamines and, decongestants (81). Good quality evidence exists that antibiotics should not be used to treat colds as they do not reduce symptom duration or severity and cause gastrointestinal side effects (81).

A telephone survey by Braun (2000) (n= 249 respondents) regarding beliefs related to colds showed that there are erroneous beliefs about the cause and treatment of colds (82). Respondents were called after visit to a primary care physician in which they were seeking care for their children or themselves. Of those respondents 44% believed that antibiotics could help colds, and 42% believed that colds could result from bacteria and viruses. This is astoundingly high as it has been reported that only 5% of colds can be attributed to bacteria, it is therefore a rare occurrence (83). Respondents believed that vitamin C (67%) and inhalation of steam (70%) could reduce cold symptoms. The respondents were well-educated, employed, fully insured and in good health. These results cannot be extrapolated to a population which is less affluent and/or less educated.

Mechanism of transmission of rhinovirus & the immune system

Rhinovirus is delivered to the eye (usually by a finger and travels down the lachrymal duct) or to the nose as an aerosolized particle where it goes to the back of the

throat and the adenoid area (84). This area contains crypts which have lymphoid follicles. These follicles contain intracellular adhesion molecule-1 (ICAM-1) (84). ICAM-1 is the cellular receptor for 90% of rhinovirus serotypes (84).

The peripheral white blood cell count increases in the first 2-3 days of the virus challenge (77). The increase in white blood cells is a result of an increase in circulating polymorphonuclear leukocytes (77). In human studies there is an association between IL-8 and the common cold (77). Increased levels of IL-8 are present in the nasal secretions of participants with the rhinovirus. In experimental rhinovirus infections there is a dose dependent relationship between IL-8 concentrations and the severity of symptoms (77). Virus induced oxidative stress in virus induced NFkB, which in turn activates IL-8 production (77). Studies also show that antioxidants inhibit IL-8 production (77).

Ascorbic Acid

Ascorbic acid, commonly referred to as vitamin C, is a water soluble vitamin found in fruits and vegetables. The most notable source of vitamin C is citrus. Vitamin C is important for iron absorption, wound healing, and collagen formation. Deficiency in vitamin C causes scurvy. The Institute of Medicine recommends that adult males consume 75 mg/d (females 90 mg) and those who smoke, which is an oxidative stress, should consume an additional 35 mg/d.

Research shows that vitamin C cannot prevent a cold but can decrease symptom severity. Baird et al. supplemented (n=362) with orange juice containing 80 mg/d ascorbic acid or a placebo (85). A significant reduction in total cold symptoms was seen with the ascorbic acid (85).

Zinc

Zinc lozenges have an unpalatable taste, may cause mouth irritation, and even taste distortion (86). The effectiveness of zinc lozenges may be related to the amount of charged zinc ions and not total dosage (86).

The literature regarding the effectiveness of zinc on cold and flu prevention is inconclusive. Some literature shows a positive effect of zinc on reduction of cold symptoms but these studies have not been adequately blinded due to adverse effects of the zinc (87). Studies with zinc supplementation showing no significant results on cold and flu and may be attributed to inadequate sample size or inadequate doses (87).

Zinc was shown to inhibit viral growth and in a randomized placebo controlled trial to decrease the length of a cold by seven days (88). Subjects were diagnosed to have a cold by a doctor and given either 23 mg lozenges or a calcium lactate placebo. Subjects were asked to report presence and severity of cold symptoms on a scale of 0 to 3 (with 0 being no symptoms and 3 being severe). Side effects included unpalatable taste and mouth irritation.

A double-blind, randomized, placebo-controlled trial consisting of 50 participants recruited within the first 24 hours of developing a cold, showed that consuming zinc lozenges (13.3 mg zinc acetate) every two to three hours had shorter overall duration of colds, cough, and nasal discharge (89). The authors concluded that the improvement in cold symptoms by the zinc was due to its antioxidant and anti-inflammatory properties, as evidenced by significant differences in soluble interleukin-1 receptor antagonist (IL-1ra) and ICAM-1. Interleukin-1 receptor antagonist is anti-inflammatory and inhibits IL-1 α and IL-1 β . Soluble intercellular adhesion molecule-1 is a major cellular receptor for rhinovirus (89). The authors suggested that zinc acts as an antiviral by decreasing

ICAM-1 or that zinc binds with ICAM-1 thereby preventing the binding of rhinovirus to the cell.

In a study conducted by Eby et al. zinc gluconate, which was administered in a nasal spray, (i.e., avoiding unpleasant taste of zinc lozenges), showed no effectiveness in reduction of cold symptoms (90). In another study, zinc orotate lozenges were used due to their slightly sweet, if not bland taste (90). In this double-blind placebo-controlled trial participants (n=77) rated symptoms on a scale of 0 to 3 (0 being absent and 3 being severe). Participants were told to use the nasal spray two to three times every 15 to 30 minutes while awake to keep the nose tissue moist and to take a lozenge every two to three hours while awake. No significant differences were found (i.e., cold duration was the same for those taking the zinc as those on the placebo). The main side effect of the nasal spray in some participants was long-lasting nasal pain. The nasal pain suggests that 10 mmol of zinc gluconate may be the maximum tolerable amount. The researchers concluded that it is unethical to use zinc or any other metal into the interior of the nose to treat colds (90).

A study conducted by Turner and Cetnarowski with 273 participants with an experimental rhinovirus and 281 participants with naturally-occurring colds took either zinc gluconate, zinc acetate, or a placebo (91). Subjects were asked to rate seven symptoms related to cold and flu on a scale of 0 to 4 (with 0 being absent and 4 being severe). After speaking with study staff daily the total daily symptom score was recorded. For a given participant, the onset of the cold was defined as the start of the study, and the end of the cold was defined when the participant had two consecutive symptom scores of less than or equal to one (91). Evaluation of blinding revealed that

taste alone was not sufficient to determine the identity of the supplement (91). No significant differences in duration or severity of colds were reported.

Selenium

Selenium incorporates into selenoproteins and has an effect on oxidative stress, redox, and critical cellular processes (92). Selenium has been shown to be important for both innate and adaptive immune responses (93). It plays a role in redox reactions, antioxidant function, and membrane integrity and protects against DNA damage (94). A good source of selenium is the Brazil nut, and moderate levels of selenium are found in fish and shellfish (95). In the US, wheat is a good source because of the availability of selenium in the soil, while in Europe wheat is not an adequate source because of low levels found in soil (95).

Keshan's disease, a cardiomyopathy, affects regions of China with selenium-deficient soil. Keshan's disease has been attributed to the coxsackievirus. Selenium elevates antiviral immunity, and prevents genetic adaptations in the viral genomic RNA that lead to virulence and cardiac pathology (96). Selenium deficient mice infected with influenza have higher lavage total cell volumes, suggesting inflammation when compared with selenium adequate mice (96).

Broome's study examined whether otherwise healthy participants (n=22) with suboptimal selenium levels, supplemented with either 50 µg, 100 µg or a placebo for 15 weeks, experienced changes in immune function and rates of clearance of a live attenuated polio virus (97). Participants were vaccinated and re-vaccinated against the polio virus, in order to elicit an immune response characterized by memory B cells and T cells. The results showed selenium supplementation increased plasma selenium concentrations and cytosolic glutathione peroxidase activity (97). Selenium also

increased interferon gamma production, resulted in an earlier peak T cell proliferation, and increased helper T cell production. Additionally participants showed an increased clearance of poliovirus. These factors suggest improved immune function (97). Though selenium has been shown to improve immune function, no evidence exists regarding its effects on cold and flu severity or duration.

Garlic

Published literature regarding the effect of garlic on immune function in clinical studies is sparse. A Cochrane review of the garlic and the common cold revealed just one double blind, placebo controlled article (98). The reviewer concluded that there is insufficient evidence regarding the effects of garlic on the common cold (98).

Josling et al. examined the effect of a garlic supplement on preventing the common cold in 146 participants, matched for age, sex and garlic consumption for 12 weeks (99). The researcher instructed participants to record symptoms daily in a diary (5= well, no problems, 4= quite well with occasional sneeze, not disruptive to normal routine, 3=can feel a cold coming on, 2=feeling low and beginning to exhibit symptoms, and 1=full cold symptoms). A cold was determined by a score of 3 that preceded a score of 1 or 2. Cold duration was determined by the number of days the participant had a score of 1 or 2 (99). The placebo group reported more days with a cold and longer symptom duration.

Ishikawa et al. examined the effect of an aged garlic supplement on quality of life and natural killer (NK) cell activity in participants with inoperable liver cancer, pancreatic cancer, or colon cancer (100). Participants randomized to the aged garlic experienced increases in the number NK and NK cell activity after 6 months; however, they saw no

changes in quality of life (assessed by the Functional Assessment of Cancer Therapy questionnaire) (100).

Echinacea

Echinacea is a flowering plant in the same family as the daisy. Barrett et al. examined the effects of dried Echinacea root (10 g on the first day, then 5 g daily or a placebo) on the common cold (70). Results suggest that Echinacea had no effect on cold duration or symptom severity and there was inadequate power to detect significant results (70). The same group also examined the effects of an unrefined Echinacea at treating colds in a college age population and no significant differences were found (101). *Echinacea purpurea* extract did help in cold prevention with athletes (102). Echinacea has been known to interact with commonly prescribed drugs and caution must be taken when supplementing.

Vitamin D

When skin is exposed to sunlight, more specifically ultraviolet light, a large amount of cholecalciferol is made from 7-dehydrocholesterol. Cholecalciferol is then transported to the liver where it is hydroxylated to calcidiol (25-hydroxyvitamin D). From there calcitriol is made when calcidiol is hydroxylated in the kidneys to 1,25-hydroxyvitamin D. Ergocalciferol (vitamin D2) can be found in cold-water fish, nuts, and egg yolks. Cholecalciferol, or D3, is typically found as the form in supplements. Vitamin D deficiency results in rickets or osteomalacia and is characterized by insufficient bone mineralization. Ginde employing the National Health and Nutrition Examination Survey (NHANES) found an inverse relationship between serum 25-hydroxyvitaminD and the incidence of upper respiratory tract infections (103).

Kriesel et al. found that administering calcitriol (1, 25-dihydroxy vitamin D) to healthy young participants who received the flu vaccine resulted in no significant differences in viral titers when compared with the placebo (104).

Carotenoids

Carotenoids are colored pigments found mainly in plants. The main carotenoids found in the diet are: lycopene (found in tomatoes and processed tomatoes), lutein (peas, dark leafy-green vegetables), β -cryptoxanthin (mandarins, apricots, orange peppers), and beta-carotene (carrots, broccoli, spinach and, apricots) (105).

Carotenoids act as antioxidant agents and quench free radicals. Epidemiological studies have shown a link between a decreased development of cancer and a diet containing carotenoids (105).

Fuller et al. studied whether supplementation with β -carotene (30 mg/d) would protect against long-wave ultraviolet light (UV) exposure in young men as measured by delayed-type hypersensitivity (106). The researchers hypothesized that because UV light decreases immune function, carotenoids can provide photoprotection. Supplementing participants with the antioxidant would protect them against an immune dysfunction (106). The researchers found their hypothesis to be correct that β carotene protects against photosuppression of immune function (106).

Studies have examined the effect of β -carotene on lymphocyte proliferation but results are conflicting. Some reports show increased T helper and natural killer cells with β -carotene supplementation and others do not replicate those positive results (107). Possible reasons for discrepancies include different amounts of β -carotene and different administration lengths used in study protocols (105).

Research is limited regarding other carotenoids effect on immunity. A study by Corridan et al. provided participants with either 13.5 mg lycopene, 8.2 mg β-carotene, or a placebo for 12 weeks to see whether cell-mediated immunity would be changed in free-living adults. The amounts in intervention capsules were based on normal amounts of lycopene and β- carotene obtained from a diet rich in fruits and vegetables. The researchers saw no change in T-cell subsets or lymphocyte proliferation, suggesting that healthy aged adults may not benefit from low doses of carotenoids (108).

Green Tea

Black, green, and oolong tea are manufactured similarly except for the oxidative state of catechins (109). Green tea is manufactured from fresh tea leaves that have been dried at elevated temperatures to avoid destroying antioxidant potential (109). Catechins and polyphenols are the antioxidants found in teas; the latter also contributes to the flavor of the tea. The polyphenol with the most antioxidant activity is epigallocatechin-3-gallate (EGCG). Green tea has been hypothesized to improve immunity, decrease cancer and, prevent inflammation (109). Human randomized clinical trials are lacking regarding the effects of green tea on colds and flu.

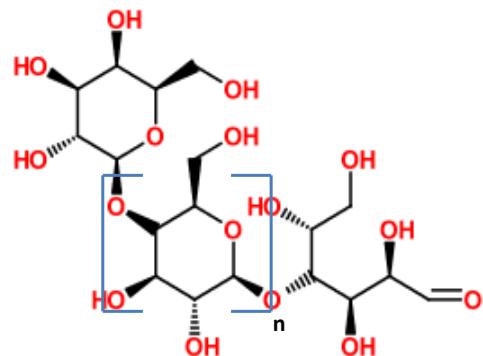


Figure 1-1. The structure of β -(1,4) galactooligosaccharide molecule.

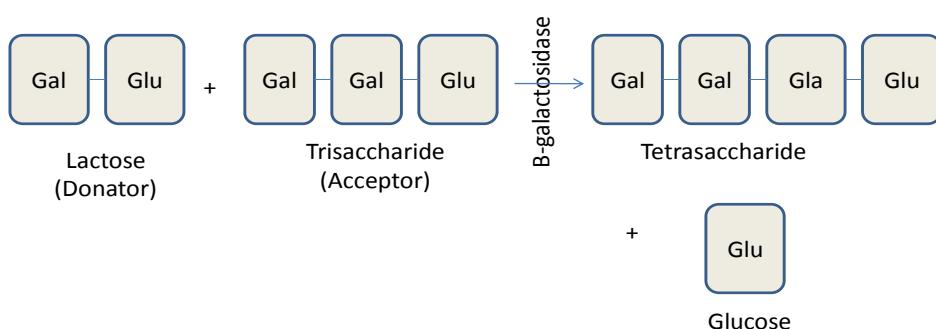
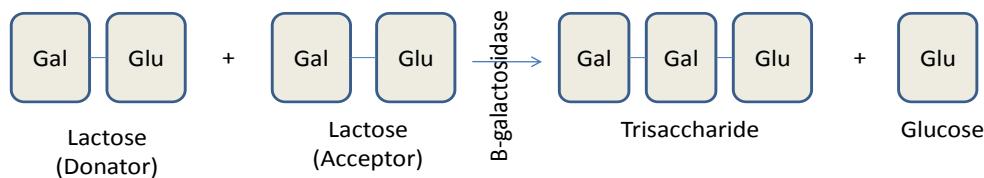


Figure 1-2. The formation of GOS from food grade lactose by β -galactosidase

CHAPTER 2 INTRODUCTION

Acute upper respiratory illnesses are common in college students. Over a six-month period including the winter months, 80% to 90% of students recorded at least one day of cold/flu symptoms, 22% reported at least one healthcare visit, 41% missed at least one day of class, and 28% self-reported that they did poorly on an exam due to upper respiratory illnesses (58, 72).

Acute psychological stress, such as that induced by academic exams, is associated with dysregulated immune and gastrointestinal function (1, 65, 110). Classic studies showed a reduction in cellular immunity in first-year medical students undergoing academic exams (66, 111). Activity of natural killer cells, one of the front-line defenses against viral infections, was reduced during exams, and those students who scored higher for stressful life events had lower natural killer cell activity (66). Cytokine production was also associated with acute psychological stress, i.e., increased (IL)-6 and IL-10 and decreased interferon- γ with increased stress (110). In addition to the changes in immune function, academic stress is associated with increased cortisol production, abdominal pain, and a disruption of the intestinal microbiota resulting in lower viable lactic acid bacteria counts (1, 65).

Psychological stress can modulate the gastrointestinal and immune systems through the hypothalamus-pituitary-adrenal (HPA) axis. Glucocorticoids (e.g., cortisol), which play a key role in the HPA, are likely responsible for the observed changes in natural killer cell function, dysfunction of the gastrointestinal tract, and altered microbiota (65, 112). Recently, the concept of the brain-gut-enteric microbiota axis was introduced suggesting bidirectional communication between the gastrointestinal tract

and brain with input from the enteric microbiota (113). Another model where cortisol may be driving changes in immune function and enteric microbiota is that of immunosenescence (114). Vulevic et al. supplemented the diets of older adults with the prebiotic, GOS and observed increased numbers of beneficial bacteria (i.e., bifidobacteria), decreased production of IL-6, and increased natural killer cell activity (51). With these concepts in mind, it was of interest to determine whether GOS supplementation would reduce gastrointestinal dysfunction and the percentage of days of cold/flu in university students undergoing academic stress. If successful, such an intervention may help reduce personal, academic, and financial costs associated with cold or flu viruses.

CHAPTER 3 METHODS

Subjects

Participants, from the University of Florida, were recruited via listservs, flyers, posters, and announcements in early fall of 2009. Participants were healthy full-time students aged 18 years or older who suffered from at least one cold in the past year. Potential subjects were excluded if they 1) had chronic allergies involving the upper respiratory tract (chronic was defined as taking allergy medicine daily), milk allergy or immunosuppressive illnesses or treatments in the past year; 2) would not have Internet access for the duration of the protocol; 3) did not have at least one scheduled final exam; iv) were a current smoker; 4) received antibiotic therapy during the two months prior to the start of the study; 5) were unwilling to discontinue any fiber or potentially immune-enhancing dietary supplements (e.g., prebiotics, probiotics, Echinacea, fish oil, vitamin E [$>100\%$ of the RDA or >15 mg/day]); or 6) had a cold on the day of enrollment. Subjects gave written informed consent and all study procedures followed were in accordance with the ethical standards of the University of Florida Institutional Review Board (Appendix A).

Experimental Design

Subjects (n=427) were randomly assigned to a supplement group during the first week in November of 2009 and followed for eight weeks including the time of fall final exams. Exams were held over the span of one week during the sixth week of the intervention. The number and scheduled time of exams varied with each subject's academic course schedule. Once students completed their last exam, subjects were on semester break through the remainder of the study.

The study was a prospective, randomized, parallel, double-blind, placebo controlled trial. Subjects were proportionally stratified based on gender (50/50) and randomized via sealed envelopes to receive 0 g, 2.5 g, or 5.0 g GOS (Purimmune™ GTC Nutrition, Golden, CO). The stratification and randomization schemes were generated by the study statistician who did not have direct contact with any subjects.

Fiber Administration Protocol

The GOS supplements were provided in coded packets that were similar in size and shape to commercially-available single serving drink mixes. Bakers' sugar (sucrose) was added to the 0 g and 2.5 g packets so that all packets were the same weight and looked similar. A flow agent (silicon dioxide) was added to all packets to improve emptying of package contents. The supplement contains 86% GOS with 21% DP2 and 65% DP3 and above. The final weights of the packets provided 0 g, 2.5 g, and 5.0 g GOS. The subjects were instructed to pour the contents of the packet into any beverage, mix well, and consume the beverage in its entirety each day for eight weeks. Both the GOS and sucrose had a slight sweet taste. Subjects were unable to distinguish the GOS packets from the placebo. The proportion of subjects who thought they were receiving 0 g, 2.5 g, and 5.0 g of GOS was 44%, 42%, and 14%, respectively with no differences among groups.

Online Study Questionnaires

On the day of randomization subjects met with investigators to have their height and weight measured, be instructed on study procedures, and obtain the supplement packets. Each subject was assigned a study number to use as their login user name for online questionnaires. Subjects provided their own password. User names were not linked to University records; however, paper records retained by the study coordinators

linked subject number to the subjects' identification and contact information. Subjects were instructed on how to complete online baseline, daily, weekly, and final questionnaires. 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.). Questions regarding demographic information were contained on a short questionnaire at baseline. Daily online questionnaires asked about level of stress on a scale from 0 (no stress) to 10 (extremely stressed), consumption of the supplement to the nearest quarter packet, cold and flu symptoms, number and consistency of stools, hours of sleep, and antibiotic use. Subjects were asked to rate symptom intensity (0=none, 1=mild, 2=moderate, 3=severe) for running/congested nose, stiffness or chills, headache, cough, fatigue, fever, sore throat, achiness, and ear discomfort. The cold/flu symptom intensity (SI) score was calculated as the sum of the individual symptom intensities (115). Subjects were asked to score their stool consistency using the Bristol stool score (116). The stool scale, which ranged from 1 (hard to pass stools) to 7 (entirely liquid stools), was shown graphically on the questionnaire. The baseline and weekly questionnaires inquired about gastrointestinal symptoms using the Gastrointestinal Symptom Scale Score (2). On the final questionnaire, subjects were asked whether they received an annual influenza vaccination (2008-2009 and 2009-2010 flu seasons) or the 2009 H1N1 vaccination and to guess the amount of GOS (0 g, 2.5 g, 5.0 g) they received in their daily packets. Each daily questionnaire was to be completed by noon the following day and weekly and final questionnaires were to be completed within three days of online release. Study coordinators monitored subject compliance online on a daily basis and contacted

subjects via email or phone if they missed these time limits. Subjects were discouraged from completing questionnaires that were three days overdue. Questionnaires were no longer available to subjects once completed.

Statistical Analyses

Compliance was assessed by summing the daily reported percentage of the supplement packet consumed and dividing by 56 days. Missing daily questionnaires were considered as an intake of 0% of the supplement packet. We assumed that there would be a lag time between the start of the supplement and effects on study outcomes; therefore, data from the first seven days were not used in analyses of supplement modulated outcomes in the cold/flu symptoms. Differences in demographic data and the average number of days with cold/flu symptoms among groups were analyzed using a one-way ANOVA or the Kruskal-Wallis one-way analysis of variance on ranks. Tests of treatment effects were done using Bonferroni or Dunn's adjustments for multiple comparisons. Categorical data were compared using the chi square statistic.

Variables included in all models for daily cold/flu symptoms and SI were stress; lag stress with a one, two, and three day lag; gender; day of randomization; ethnicity; antibiotic use; vaccination against seasonal influenza for the 2009-2010 and 2008-2009 flu seasons; vaccination against H1N1; number of final exams; BMI; age; and hours of sleep. The two way interactions were also included. Variables and variable interactions that were not significant were eliminated from the model.

To adjust for personal differences in scoring cold/flu SI, subjects were categorized based on the average sum of their SI score from the first week of supplementation. Symptom intensity score categories were an average sum of less than 1 (60% of subjects), 1 to 3 (29% of subjects), and greater than 3 (11% of subjects) over

the first week of supplementation. The average SI sum for the remaining weeks of supplementation was significantly different among categories (<1, 1.2 ± 0.01 ; 1 to 3, 2.0 ± 0.1 ; and >3, 3.4 ± 0.1 ; $P<0.0001$) suggesting that the personal differences in scoring SI continued throughout the study. The daily average SI score from study weeks two through eight was compared among the three groups using a general linear mixed model with an autoregressive correlation structure to account for correlation of daily observation.

Before analyzing data and while still blinded to treatment, the daily cold/flu SI scores for individuals were reviewed to identify a cut point above which a subject was likely to have a cold/flu (i.e., be sick). The cut point was to differentiate cold/flu symptoms from symptoms associated with stress. An SI score above six, which would represent a minimum of three symptoms with at least two of moderate and one of severe intensities, was selected as the SI that reflected a sick day. An indicator variable of being “sick” was compared among groups using a generalized linear mixed model on the response variable with an autoregressive correlation structure to account for the correlation of daily observations. Since, the response variable was a “yes”, the individual had a cold/flu SI score above six, or “no”, they did not, a binomial error distribution was used.

Fifteen gastrointestinal symptoms divided among five categories (diarrhea syndrome, constipation syndrome, abdominal pain, indigestion syndrome, and reflux syndrome) were each rated from 1 = no discomfort to 7 = very severe discomfort. Scores within each category were summed and weekly gastrointestinal symptoms were adjusted for baseline responses. The baseline response was an individual’s response

for the week prior to randomization. A generalized linear mixed model was then used to model gastrointestinal symptoms categories. Because the data were not normally distributed an exponential distribution was specified and an autoregressive correlation structure was used to correct for the correction over time that was caused by individuals being measured repeatedly over the course of the study. The degrees of freedom were corrected using the Kenward-Roger method to control the family-wise error rate. For the weekly gastrointestinal symptoms model, the daily covariates were replaced with their average value for the week. For example, daily stress levels were replaced with average weekly stress. The same process was repeated for hours of sleep. The lag daily stress variables were not used. Unless stated otherwise, data represent least squares means \pm SEM. Analyses were completed using SigmaPlot (version 11.0, 2008, Systat Software, Inc., San Jose, CA) and the generalized linear mixed models were fitted using SAS v9.2 (SAS Institute, Cary, NC).

CHAPTER 4 RESULTS

Four hundred and eighty-one subjects were consented and assessed for eligibility (Figure 3-1). Of this group, 24 did not meet inclusion criteria, eight declined to participate, and 22 did not return for the randomization visit. Of the 427 remaining subjects, 141, 142, and 144 subjects were randomized to 0 g, 2.5 g, and 5.0 g of GOS, respectively. Two subjects dropped out before beginning the supplement. Five subjects completed seven days of records or fewer and were therefore not included in any of the analyses. Data were analyzed on an intent to treat basis from 419 subjects who completed on average 55 out of 56 daily records and consumed an average of more than 93% of the supplement packets each day (Table 3-1).

There were no differences in the number of final exams, vaccination against H1N1 in 2009 or seasonal influenza in 2008-2009, antibiotic use, or subject characteristics among the groups with the exception of age and the number vaccinated against the 2009-2010 influenza (Table 3-1). Subjects consuming 5.0 g of GOS each day were on average six months younger than those subjects who received the placebo. On average, subjects reported one cold/flu symptom per day. Subjects reported fatigue an average of 12 days over the eight weeks, followed by 10 days of running/congested nose, seven days of headache, seven days of cough, six days of sore throat, five days of achiness, stiffness or chills, two days of ear discomfort, and one day of fever. There were no differences among groups in the average number of cold/flu symptoms reported by subjects (data not shown).

Average daily stress was not different among groups (Table 3-1). Peak average stress occurred the week of final exams (5.0 ± 0.2), and the lowest level of stress (1.3 ± 0.2) was reported during semester break (i.e., the final weeks of the study). The average cold/flu SI score was significantly associated with stress and GOS supplementation ($P < 0.0001$). Stress was positively related to SI score and a lower SI score was associated with 2.5 g GOS across all levels of stress (Figure 3-2). At lower levels of daily stress, 5.0 g of GOS was also associated with a lower average SI score. The protective effect of 5.0 g of GOS was not detected at higher levels of stress.

The probability of having a sick day was positively related to level of stress ($P < 0.001$) and significantly associated with GOS supplementation for different categories of BMI (interaction of BMI and GOS, $P = 0.0002$, Figure 3-3). Among individuals with a healthy weight (64% of the study population), those receiving 5.0 g of

GOS reported a 40% reduction in the probability of having a sick day as compared with those receiving 0 g and 2.5 g GOS. This protective effect was not seen in overweight and obese individuals (30% of the study population); however, among these individuals, a lower percentage of sick days was reported in those receiving 2.5 g GOS compared with 0 g and 5.0 g.

Across all treatment groups, stress was positively related to the five gastrointestinal symptom score categories and was highly significant ($P<0.01$) for all but constipation syndrome ($P=0.1017$, data not shown). Across all levels of stress, gastrointestinal symptom scores were significantly lower with GOS supplementation for all symptom categories (e.g., diarrhea syndrome [treatment group effect, $P=0.0298$], constipation syndrome [$P=0.0342$], abdominal pain [$P=0.0058$], and indigestion syndrome [$P=0.0003$]) except reflux syndrome ($P=0.2073$, Table 2). There were no differences in the number of stools per day; however, daily stool consistency was softer with 2.5 g of GOS (Table 3-2).

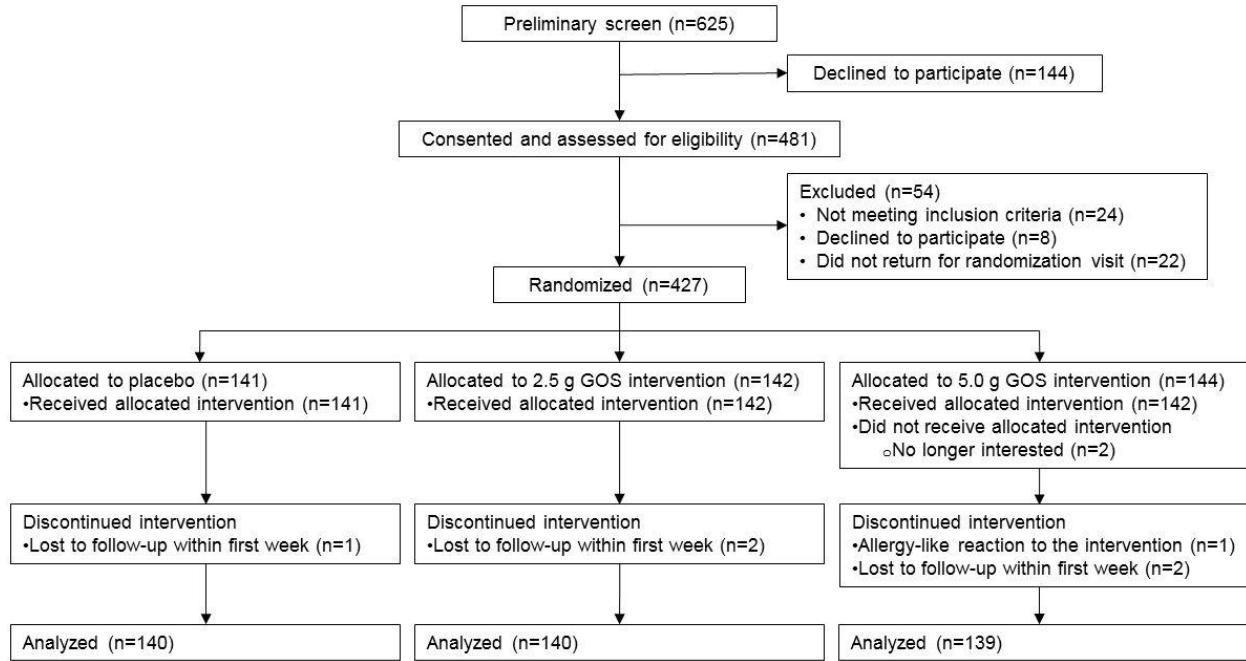


Figure 3-1. Flow chart of subject recruitment, allocation and analysis.

Table 3-1. Subject characteristics and compliance

	0 g GOS¹ (n=140)	2.5 g GOS (n=140)	5.0 g GOS (n=139)
Gender (M/F)	69/71	70/70	68/71
Race/Ethnicity [n,(%)] ²			
White	83 (59%)	72 (51%)	76 (55%)
Hispanic	21 (15%)	28 (20%)	25 (18%)
Asian	18 (13%)	23 (16%)	21 (15%)
Black	18 (13%)	17 (12%)	16 (12%)
Age (y)	20.1 \pm 0.1	19.9 \pm 0.1	19.6 \pm 0.1 ³
BMI (kg/m ²)	23.8 \pm 0.3	23.9 \pm 0.3	23.4 \pm 0.3
Final exams (n)	2.9 \pm 0.1	2.8 \pm 0.1	2.9 \pm 0.1
Daily stress level (0=no stress, 10=extreme stress)	3.1 \pm 0.1	3.1 \pm 0.1	3.1 \pm 0.1
Hours of sleep each night	7.2 \pm 0.1	7.2 \pm 0.1	7.2 \pm 0.1
Average d on antibiotic over study period	0.018 \pm 0.006	0.015 \pm 0.005	0.016 \pm 0.004
Vaccinated against the 2008-2009 seasonal influenza [n,(%)]	27 (20%)	23 (17%)	26 (24%)
Vaccinated against the 2009-2010 seasonal influenza [n,(%)] ⁴	13 (9%)	23 (16%)	31 (22%)
Vaccinated against 2009 H1N1 [n,(%)]	24 (17%)	12 (9%)	16 (12%)
Daily questionnaires completed (out of 56 d)	54.7 \pm 0.4	54.8 \pm 0.4	54.8 \pm 0.3
Percentage of supplement packet consumed per d	95% \pm 1%	93% \pm 1%	94% \pm 1%

¹GOS, galactooligosaccharides. Differences among groups were calculated using a one-way ANOVA or Kruskal-Wallis one-way analysis of variance on ranks. Tests of treatment effects were done using Bonferroni or Dunn's adjustments for multiple comparisons. Categorical data were compared using the chi-square statistic. Data are reported as mean \pm SEM unless stated otherwise.

²One subject in the 5.0 g GOS group did not report his race/ethnicity.

³P=0.002 vs. 0 g GOS.

⁴P=0.013. The observed proportions among groups were significantly different than expected (chi-square test).

Table 3-2. Weekly gastrointestinal symptoms and daily stool characteristics in control subjects and subjects supplemented with galactooligosaccharides.

	0 g GOS¹ (n=140)	2.5 g GOS (n=140)	5.0 g GOS (n=139)
GSRS ²			
Diarrhea syndrome ³	4.24 ± 0.20 ^a	4.06 ± 0.17 ^{a,b}	3.97 ± 0.15 ^b
Constipation syndrome ⁴	4.51 ± 0.10 ^a	4.41 ± 0.09 ^{a,b}	4.20 ± 0.08 ^b
Abdominal pain ⁵	4.65 ± 0.08 ^a	4.32 ± 0.07 ^b	4.54 ± 0.08 ^a
Indigestion syndrome ⁶	6.88 ± 0.13 ^a	6.23 ± 0.10 ^b	6.53 ± 0.11 ^c
Reflux syndrome ⁷	2.27 ± 0.03 ^a	2.20 ± 0.03 ^a	2.25 ± 0.03 ^a
Number of stools/d	1.50 ± 0.03 ^a	1.47 ± 0.03 ^a	1.51 ± 0.03 ^a
Daily stool consistency ⁸	3.29 ± 0.03 ^a	3.42 ± 0.03 ^b	3.25 ± 0.03 ^a

¹GOS, galactooligosaccharides.

²Gastrointestinal Symptom Response Scale (GSRS). Gastrointestinal symptoms were scored at baseline and weekly during the eight weeks of supplementation with 1=no discomfort to 7=severe discomfort. Individual scores were summed within each category. Symptom scores from baseline questionnaires were used to control for personal variation in responses to the subsequent eight treatment questionnaires. A generalized linear mixed model was used to model gastrointestinal symptom categories. Data for symptoms are back-transformed least squares means and approximate SEMs for the back transformed least squares means. Data represent the least squares means ± SEM unless stated otherwise. Means with different superscript letters within each row are significantly different at

$P < 0.05$.

³Diarrhea syndrome included questions regarding diarrhea, loose stools, urgent need for defecation.

⁴Constipation syndrome included questions regarding constipation, hard stools, and feeling of incomplete evacuation.

⁵Abdominal pain included questions regarding abdominal pain, hunger pains, and nausea.

⁶Indigestion syndrome included questions regarding rumbling, bloating, burping, and gas.

⁷Reflux syndrome included questions regarding heartburn and acid regurgitation.

⁸Stool consistency represents the average daily Bristol stool score with a score of 1 denoting hard to pass stools and a score of 7 denoting an entirely liquid stool.

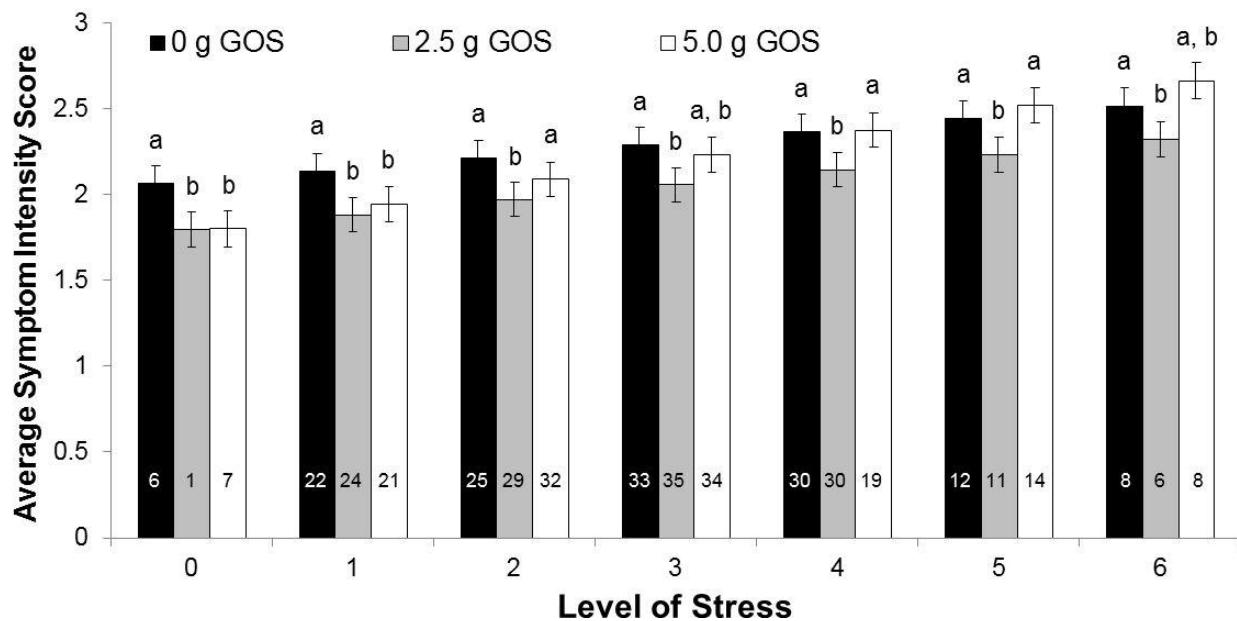


Figure 3-2. Cold/flu symptom intensity (SI) score by level of stress in individuals receiving 0 g, 2.5 g, 5.0 g galactooligosaccharides (GOS). The SI for nine cold/flu symptoms was scored for intensity from 0 (not experiencing) to 3 (severe) and summed to reflect the SI score. Level of stress was scored from 0 (no stress) to 10 (extremely stressed). The average SI score from the first week of supplementation was used to control for personal differences in scoring symptoms. Few subjects ($n=17$) had an average daily level of stress above 6. The daily average SI score was compared among groups using a general linear mixed model. At each level of stress, bars with different letters are significantly different at $P<0.05$. The number on each bar denotes the number of subjects within each supplement group at each level of stress. Data represent the least squares means \pm SEM.

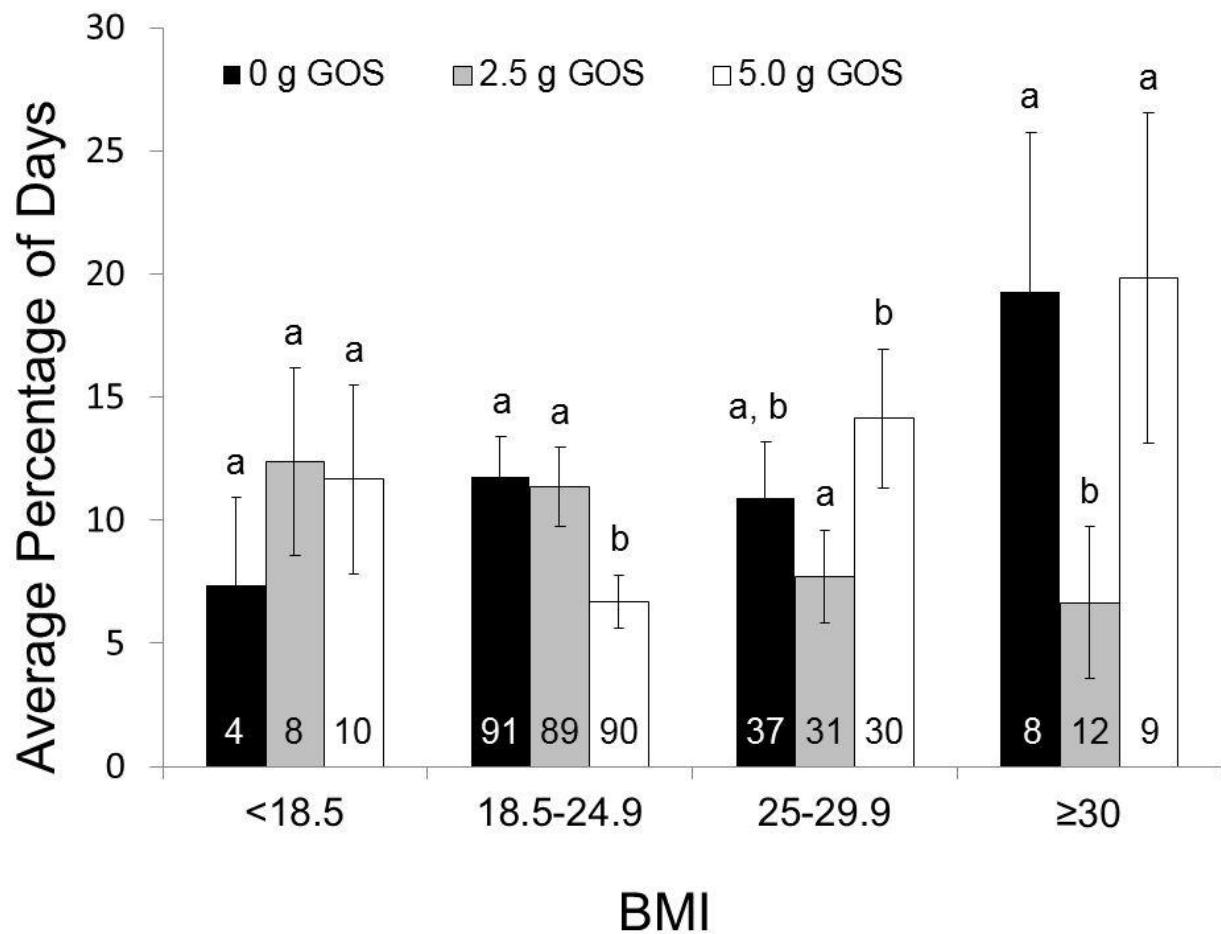


Figure 3-3. Average percentage of days with a cold/flu symptom intensity (SI) score greater than 6 by BMI in individuals receiving 0 g, 2.5 g, 5.0 g galactooligosaccharides (GOS; interaction of BMI and GOS, $P=0.0002$). An SI score above six would represent a minimum of three symptoms with at least two of moderate and one of severe intensities. The average percentage of days from study weeks two through eight was compared among supplement groups using a generalized linear mixed model. Within each BMI category, bars with different letters are significantly different at $P<0.05$. Data represent least squares means + SEM.

CHAPTER 5 DISCUSSION AND CONCLUSION

This study provides new information on the benefit of GOS on gastrointestinal and immune health outcomes in apparently healthy young adults undergoing an academic stress. As anticipated, in the eight weeks before, during, and after fall final exams, university undergraduates self-reported daily stress that was positively related to gastrointestinal symptoms, cold/flu symptom intensity, and the percentage of days with cold/flu. Galactooligosaccharide supplementation modulated all of these symptoms. The dose of 2.5 g GOS was more effective than the 5.0 g dose at reducing symptoms associated with abdominal pain (i.e., abdominal pain, hunger pains, and nausea) and indigestion syndrome (i.e., rumbling, bloating, burping and gas). This is of interest to note considering that GOS is fermented by the intestinal microbiota, which results in gas production (16). The higher dose of GOS (5.0 g) was better than the placebo in regards to indigestion syndrome (Table 2). Although 2.5 g of GOS was associated with slightly improved stool consistency, 5.0 g of GOS was associated with the decreased likelihood of constipation syndrome.

To our knowledge this is the first study to demonstrate a benefit of GOS, a prebiotic, on modulating stress-induced gastrointestinal dysfunction in adults. Previously, Diop and colleagues demonstrated a protective effect of a probiotic mix containing *Lactobacillus acidophilus* and *Bifidobacterium longum* on stress-induced gastrointestinal symptoms (116). In that three month study, adults aged 18 to 60 years who were affected daily by symptoms of stress reported significantly less nausea and vomiting and abdominal pain with the probiotic compared with the placebo. The authors suggested that effects on the residual intestinal microbiota, intestinal barrier, and

immune system may have modulated these symptoms (116). In our study, GOS may have worked through similar mechanisms or through the HPA axis (65, 112, 113). GOS intake has previously been shown to increase numbers of bifidobacteria and lactobacilli (25, 51) as well as fecal short-chain fatty acids, which have been associated with decreased epithelial permeability(117, 118).

It is possible that gastrointestinal symptoms actually contributed to the reported level of stress. If this were the case, it may help to explain why 5.0 g of GOS lost its effectiveness at increased levels of stress (Figure 3-2). At lower levels of stress 5.0 g of GOS was associated with lower average cold/flu symptom intensity, but this protective effect was lost at higher levels of stress. The 5.0 g dose of GOS was not as effective as 2.5 g at reducing abdominal pain (Table 3-2). It could be that abdominal pain contributed to overall stress and ultimately risk of cold/flu symptoms.

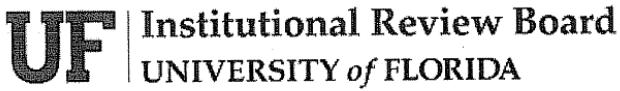
In healthy weight individuals, supplementation with 5 g of GOS reduced the average percentage of days of cold/flu (Figure 3-3). This protective effect was not seen in underweight, overweight, and obese individuals. It should be noted that because 64% of the subjects fell within the healthy weight category, we are most certain about these data. Additionally, emerging evidence points to differing microbiota including a reduction in bifidobacteria in obese versus lean individuals (39, 119, 120). If GOS improved gastrointestinal and immune function by changing the microbiota, then it is possible that we would observe different effects in individuals within different BMI categories.

This study is one of the first to demonstrate a protective effect of GOS in relation to the percentage of days with cold/flu; however, there are a number of limitations that need to be addressed. Academic stress was used as a model of acute psychological

stress; though students only reported an average daily stress of three on a 10-point scale with a high of five during exam week. Populations with higher levels of stress may experience more severe gastrointestinal and immune dysregulation. With a greater degree of daily stress, the GOS-associated health benefits may have been observed with a smaller sample size or alternately, not observed to the same degree. Another limitation is in how a day of cold/flu was defined as an SI sum of greater than six, which would represent a minimum of three different symptoms of more than minimal intensity. Although this is a fairly conservative definition in comparison to that used in other studies (72, 121-123), the presence of cold/flu-associated pathogens was not confirmed. It was advantageous to report the percentage of days with cold/flu rather than incidence and duration because it is difficult to determine when one cold ends and a new cold begins. Another potential limitation is the use of Internet-based questionnaires in place of traditional paper questionnaires. Although student responses to the Internet version of the questionnaires were not compared to that of the traditional paper version, these comparisons have been done with other questionnaires. Ritter and colleagues tested the reliability of 16 different health-related instruments administered online compared to the traditional mailed paper questionnaires (124). In every case, the Internet-based instrument appeared to be reliable and the Internet participation in the study was as good if not better than that of the traditional method (124). For the undergraduate students in our study, the convenience of completing questionnaires online and the ability of the investigators to follow subject compliance in real time likely contributed to the high compliance rate.

In summary, acute psychological stress, such as that associated with academic exams, was directly related to symptoms of gastrointestinal dysfunction and cold/flu. GOS supplementation attenuated these symptoms. Future studies should determine the mechanisms by which GOS improves health outcomes within the brain-gut-enteric microbiota axis as these findings may have wide applicability beyond academic stress.

APPENDIX A IRB APPROVAL LETTER



Health Center Institutional Review Board

FWA00005790

PO Box 100173
Gainesville, Florida 32610-0173
Tel: (352) 846-1494
Fax: (352) 846-1497

MEMORANDUM

DATE: July 14, 2009

TO: Bobbi Langkamp-Henken, Ph.D., RD
Box 110370

FROM: Patricia Shearer, M.D.
Vice Chairman, IRB - 01 *Patricia Shearer*

EXPIRES: Thursday, July 01, 2010

SUBJECT: EXPEDITED IRB #307-2009

TITLE: EXPEDITED: PROTOCOL 1: GALACTOOLIGOSACCHARIDE, IMMUNE STRENGTH,
AND DIGESTIVE HEALTH IN ACADEMICALLY STRESSED UNIVERSITY STUDENTS

You have received IRB approval to conduct the above-listed research study. Approval of this study was granted on July 1, 2009. Enclosed is the dated, IRB-approved Informed Consent Form that must be used for enrolling subjects into this project from July 1, 2009 through June 30, 2010. This study is approved as expedited as it poses minimal risk and is approved under the following expedited category/categories:

Expedited #3: Prospective collection of biological specimens for research purposes by noninvasive means. Examples: (a) hair and nail clippings, if collected in a non-disfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth, if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax or by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane before or during labor; (h) supra- and sub-gingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization.

Expedited #7: Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. Note: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR § 46.101(b) (2) and (b)(3). This listing refers only to research that is not exempt.

You are responsible for applying for renewal of this study prior to the expiration date. Re-approval of this study must be granted before the expiration date, or the study will automatically be suspended. If suspended, new subject accrual must stop. Research interventions must also stop unless there is a concern for the safety or well-being of the subjects. You MUST respond to the Continuing Review questions within 90 days or your study will be referred to the Board for termination.

The IRB has approved exactly what was submitted. Any change in the research, no matter how minor, may not be initiated without IRB review and approval, except where necessary to eliminate hazards to human subjects. If a change is required due to a potential hazard, that change must be promptly reported to the IRB.

If applicable, only a qualified clinician may be responsible for study-related healthcare decisions.

An Equal Opportunity Institution

Bobbi Langkamp-Henken, Ph.D., RD July 14, 2009

Page 2

Any severe and unanticipated side effects or problems and all deviations from federal, state, university, or IRB regulations must be reported, in writing, within 5 working days.

Upon completion of the study, you are REQUIRED to submit a summary of the study and a Study Closure report to the IRB office.

Research records must be retained for 3 years after completion of the research; if the study involves medical treatment, it is recommended that the records be retained for 8 years.

If VAMC patients will be included in this study, or if the study is to be conducted in part on VA premises or performed by a VA employee during VA-compensated time, review by the VA Subcommittee for Clinical Investigations is required.

You are responsible for notifying all parties about the approval of this study, including your co-Investigators and Department Chair. If you have any questions, please telephone the IRB-01 office at (352) 846-1494.

cc: IRB file / VA Research Center / Clinical Research Center

An Equal Opportunity Institution

APPENDIX B IRB INFORMED CONSENT



Institutional Review Board
UNIVERSITY of FLORIDA

INFORMED CONSENT FORM
to Participate in Research, and
AUTHORIZATION
*to Collect, Use, and Disclose Protected
Health Information (PHI)*

University of Florida
Health Center
Institutional Review Board
APPROVED FOR USE
From 9/12/09 Through 6/30/2010 gm

INTRODUCTION

Name of person seeking your consent: _____

Place of employment & position: _____

This is a research study of fiber on immune strength and digestive health.

Could participating in this study offer any direct benefits to you? Yes, as described on page 5.

Could participating cause you any discomforts or are there any risks to you? Yes, as described on page 4.

Please read this form which describes the study in some detail. I or one of my co-workers will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. If you choose to participate you can change your mind at any time and withdraw from the study. You will not be penalized in any way or lose any benefits to which you would otherwise be entitled if you choose not to participate in this study or to withdraw. If you have questions about your rights as a research subject, please call the University of Florida Institutional Review Board (IRB) office at (352) 846-1494. If you decide to take part in this study, please sign this form on page 11.

GENERAL INFORMATION ABOUT THIS STUDY

1. Name of Participant ("Study Subject")

IRB Project #: 307-2009 – Main Study
IRB Version: 04/15/2009
PI Version: 08/28/2009

Page 1 of 10



2. What is the Title of this research study?

Protocol 1: galactooligosaccharide, immune strength, and digestive health in academically stressed university students

3. Who do you call if you have questions about this research study?

Bobbi Langkamp-Henken, PhD, RD Principal Investigator Work: 352-392-1991 ext. 205 Cell: 352-642-3669 Email: henken@ufl.edu	Wendy Dahl, PhD, RD Co-Investigator Work: 352-392-1991 ext. 224 Cell: 352-226-1773 Email: wdahl@ufl.edu
--	--

Christine Hughes, Study Coordinator
Work: 352-682-4883
Cell: 352-275-8961
Email: nutrition-study@ufl.edu

Contact information for emergencies after hours or on weekends: For emergencies call 911 or contact Student Health Care Services.

4. Who is paying for this research study?

The sponsor of this study is GTC Nutrition.

5. Why is this research study being done?

The purpose of this research study is to determine whether a fiber, galactooligosaccharide, can help maintain immune strength and digestive health in undergraduate students who are undergoing an academic stress (i.e., final exams).

You are being asked to be in this research study because you are an undergraduate student at the University of Florida who will be taking fall exams.

WHAT CAN YOU EXPECT IF YOU PARTICIPATE IN THIS STUDY?

6. What will be done as part of your normal clinical care (even if you did not participate in this research study)?

This study is not related to your normal clinical care. Your physician will not be informed of your immune or gastrointestinal health.



7. What will be done only because you are in this research study?

If you choose to participate in this study, you will be given an appointment time to begin the study. If you still meet the inclusion criteria at that time, we will obtain your height, weight, and body mass index. You will then be randomly assigned, much like a roll of the dice to receive 2.5 grams or 5 grams of galactooligosaccharide (i.e., fiber) or placebo. This means:

- A placebo is a substance that looks like and is given in the same way as an experimental treatment but contains no fiber, for example, a sugar pill.
- A placebo is used in research studies to show what effect a treatment has compared with taking nothing at all. If you are assigned to receive placebo, you will not receive the benefits or be exposed to the risks of the fiber, if there are any (any risks or benefits are described below).
- You will have a 2 out of 3 chance of receiving the fiber and a 1 in 3 chance of receiving placebo. In the remainder of the Consent Form, both the fiber and the placebo will be called "fiber".
- You and the persons doing the study will not know whether you are receiving placebo or the fiber, but that information is available if it is needed.

After you have been randomized we will:

- assign you a study number and password for confidentially completing online questionnaires through the U.F. E-Learning or equivalent system.
- ask you to complete UF paperwork to receive your study payment. This form will ask for your social security number.
- ask you to complete two online questionnaires, which should take less than 5 minutes to complete. These questionnaires ask about your age, ethnicity gastrointestinal symptoms and physical activity.
- give you your fiber packets containing approximately one tablespoon of fiber to mix into a beverage each day. **Please do not share these packets with anyone and return any unused packets at the end of the study.**

You will then be asked to complete an online questionnaire each day that asks you about your health (i.e., cold symptoms, antibiotic use), level of stress, hours of sleep, well being, and bowel habits and whether or not you consumed your fiber supplement. This questionnaire should as few as three minutes to complete. Each week we will ask you to complete the same questionnaire you answered regarding your gastrointestinal symptoms and physical activity.



You will most likely complete this study while you are on winter break from the University of Florida. Once you return to UF for spring semester, we will set up an appointment for you to return your unused fiber packets and complete a short questionnaire regarding the fiber. If you are not planning on returning to Gainesville for the spring semester, arrangements can be made to complete all of the last study procedures by phone and mail.

Study coordinators may contact you via phone or email to remind you to complete your online questionnaires or of study appointments.

If you have any questions now or at any time during the study, please contact the investigators, Bobbi Langkamp-Henken, PhD, RD, or Wendy Dahl, PhD, RD, or the study coordinator, Christine Hughes in question 3 of this form.

8. How long will you be in this research study?

If you choose to participate, you will meet with a study coordinator for approximately 30 minutes on the first day of the study in the Food Science and Human Nutrition Department. You will then consume the fiber and complete online questionnaires daily for eight weeks. This should take you less than five minutes to complete. At the conclusion of the eight weeks you will meet with the study coordinator in the Food Science and Human Nutrition Department for approximately five minutes to return any remaining fiber packets and answer a few questions regarding the fiber.

9. How many people are expected to take part in this research study?

420 students will be participating in this study.

**WHAT ARE THE RISKS AND BENEFITS OF THIS STUDY AND
WHAT ARE YOUR OPTIONS?**

10. What are the possible discomforts and risks from taking part in this research study?

In some people, fiber intake may cause gas, bloating, or increased frequency of stools. This study provides at most 5 grams of fiber. The recommended adequate intake for fiber for men and women 19 to 30 years of age is 38 grams and 25 grams, respectively.

Some people may feel uncomfortable when body weight and height are measured.

Some people may feel uncomfortable when questions regarding bowel habits are answered.



Other possible risks to you may include: N/A
This study may include risks that are unknown at this time.

Participation in more than one research study or project may further increase the risks to you. If you are already enrolled in another research study, please inform Bobbi Langkamp-Henken, PhD, RD or Wendy Dahl, PhD, RD (listed in question 3 of this consent form) or the person reviewing this consent with you before enrolling in this or any other research study or project.

Throughout the study, the researchers will notify you of new information that may become available and might affect your decision to remain in the study.

If you wish to discuss the information above or any discomforts you may experience, please ask questions now or call the PI or contact person listed on the front page of this form.

11a. What are the potential benefits to you for taking part in this research study?

You may or may not benefit from participating in this research study. You may experience improved bowel habits and softer stools. The fiber may also stimulate the growth and activity of beneficial bacteria resulting in improved overall health.

11b. How could others possibly benefit from this study?

If this fiber is associated with improved immune strength and digestive health, it could be added to commonly consumed foods to keep others healthy.

11c. How could the researchers benefit from this study?

In general, presenting research results helps the career of a scientist. Therefore, Bobbi Langkamp-Henken, PhD, RD and Wendy Dahl, PhD, RD may benefit if the results of this study are presented at scientific meetings or in scientific journals.

The researchers declare no conflict of interest.

12. What other choices do you have if you do not want to be in this study?

The alternate option to taking part in this study is to do nothing. If you do not want to take part in this study, tell the Principal Investigators and do not sign this informed consent form.

You have been invited to participate in this research project because you are a healthy full-time student aged 18 years or older, who had at least one cold in the past year, and because you will be taking fall semester final exams. The investigators associated with this project may or may not teach in your college or be associated with courses for which you are enrolled or might be expected to register in the future.



Your participation in this study is voluntary and any decision to take part or not to participate will in no way affect your grade or class standing.

If you believe that your participation in this study or your decision to withdraw from or to not participate in this study has improperly affected your grade(s), you should discuss this with the dean of your college or you may contact the IRB office.

13a. Can you withdraw from this study?

You are free to withdraw your consent and to stop participating in this study at any time. If you do withdraw your consent, you will not be penalized in any way and you will not lose any benefits to which you are entitled.

If you decide to withdraw your consent to participate in this study for any reason, please contact Bobbi Langkamp-Henken, PhD, RD at 392-1991 ext. 205 or Wendy Dahl, PhD, RD at 352-392-1991 ext. 224. They will tell you how to stop your participation safely.

If you have any questions regarding your rights as a research subject, please call the Institutional Review Board (IRB) office at (352) 846-1494.

13b. If you withdraw, can information about you still be used and/or collected?

If you withdraw, we will use the information you have provided up until the time that you withdraw, unless you request otherwise.

13c. Can the Principal Investigator withdraw you from this study?

You may be withdrawn from the study without your consent for the following reasons: you do not meet the inclusion/exclusion criteria, the number of required subjects has been met, or you do not follow the instructions given to you by the investigators.

WHAT ARE THE FINANCIAL ISSUES IF YOU PARTICIPATE?

14. If you choose to take part in this research study, will it cost you anything?

The fiber will be provided at no cost to you while you are participating in this study.

15. Will you be paid for taking part in this study?

Yes, you will be paid \$7.50 per week for a total of \$60 for completing the study. If you are not employed by the University of Florida, you will be paid when you return from winter break. During your initial study visit you will be given an appointment time and day to receive the payment. If you are employed by the University of Florida, the



payment will be added to your regular pay check at least one pay cycle after the study is complete.

If you are paid for taking part in this study, your name and social security number will be reported to the appropriate University employees for the purposes of making and recording the payment. You are responsible for paying income taxes on any payments provided by the study.

16. What if you are injured because of the study?

If you are injured as a direct result of your participation in this study, only professional consultative care that you receive at the University of Florida Health Science Center will be provided without charge. However, hospital expenses will be billed to you or your insurance provider. You will be responsible for any deductible, co-insurance, or co-payments. Some insurance companies may not cover costs associated with research studies. Please contact your insurance company for additional information.

No additional compensation is offered. The Principal Investigator and others involved in this study may be University of Florida employees. As employees of the University, they are protected under state law, which limits financial recovery for negligence.

Please contact the Investigators listed in question 3 of this form if you experience an injury or have questions about any discomforts that you experience while participating in this study.

17. How will your health information be collected, used and shared?

If you agree to participate in this study, the Principal Investigator will create, collect, and use private information about you and your health. This information is called protected health information or PHI. In order to do this, the Principal Investigator needs your authorization. The following section describes what PHI will be collected, used and shared, how it will be collected, used, and shared, who will collect, use or share it, who will have access to it, how it will be secured, and what your rights are to revoke this authorization.

Your protected health information may be collected, used, and shared with others to determine if you can participate in the study, and then as part of your participation in the study. This information can be gathered from you or your past, current or future health records, from procedures such as physical examinations, x-rays, blood or urine tests or from other procedures or tests. This information will be created by receiving study treatments or participating in study procedures, or from your study visits and telephone calls. More specifically, the following information may be collected, used, and shared with others:

- history of allergy
- known illnesses or conditions that may impact perceived health such as HIV/AIDS, diabetes, renal or gastrointestinal diseases



- history of chemotherapy or other immune suppressing therapy within the last year or antibiotic therapy in the past two months
- height, weight, and body mass index
- gender, age
- questionnaires regarding level of stress, bowel habits, well being, and gastrointestinal and cold symptoms
- your social security number for compensation purposes

This information will be stored in locked filing cabinets or on computer servers with secure passwords, or encrypted electronic storage devices.

Some of the information collected could be included in a "limited data set" to be used for other research purposes. If so, the limited data set will only include information that does not directly identify you. For example, the limited data set cannot include your name, address, telephone number, social security number, photographs, or other codes that link you to the information in the limited data set. If limited data sets are created and used, agreements between the parties creating and receiving the limited data set are required in order to protect your identity and confidentiality and privacy.

18. For what study-related purposes will your protected health information be collected, used, and shared with others?

Your PHI may be collected, used, and shared with others to make sure you can participate in the research, through your participation in the research, and to evaluate the results of the research study. More specifically, your PHI may be collected, used, and shared with others for the following study-related purpose(s):

- to determine eligibility for this study
- to determine the effectiveness of fiber on maintaining immune strength and digestive health

Once this information is collected, it becomes part of the research record for this study.

19. Who will be allowed to collect, use, and share your protected health information?

Only certain people have the legal right to collect, use and share your research records, and they will protect the privacy and security of these records to the extent the law allows. These people include the:

- The study investigators, Bobbi Langkamp-Henken, PhD, RD and Wendy Dahl, PhD, RD and research scientists, statisticians, and staff associated with this project.
- other professionals at the University of Florida or Shands Hospital that provide study-related treatment or procedures



- the University of Florida Institutional Review Board (IRB; an IRB is a group of people who are responsible for looking after the rights and welfare of people taking part in research).

20. Once collected or used, who may your protected health information be shared with?

Your PHI may be shared with:

- the study sponsor, GTC Nutrition.
- United States and foreign governmental agencies who are responsible for overseeing research, such as the Food and Drug Administration, the Department of Health and Human Services, and the Office of Human Research Protections
- Government agencies who are responsible for overseeing public health concerns such as the Centers for Disease Control and federal, state and local health departments

Otherwise, your research records will not be released without your permission unless required by law or a court order. It is possible that once this information is shared with authorized persons, it could be shared by the persons or agencies who receive it and it would no longer be protected by the federal medical privacy law.

21. If you agree to take part in this research study, how long will your protected health information be used and shared with others?

Your PHI will be used and shared with others for two years following the completion of the study. After that time information that directly links you (e.g., name, address, telephone number, email, social security number) to your study records will be deleted from the database and deidentified on the paper copies. If you withdraw your permission for the use and sharing of your PHI, then your information will be removed from the database.

You are not required to sign this consent and authorization or allow researchers to collect, use and share your PHI. Your refusal to sign will not affect your treatment, payment, enrollment, or eligibility for any benefits outside this research study. However, you cannot participate in this research unless you allow the collection, use and sharing of your protected health information by signing this consent and authorization.

You have the right to review and copy your protected health information. However, we can make this available only after the study is finished.

You can revoke your authorization at any time before, during, or after your participation in this study. If you revoke it, no new information will be collected about you. However, information that was already collected may still be used and shared with others if the researchers have relied on it to complete the research. You can revoke your authorization by giving a written request with your signature on it to the Principal Investigator.



SIGNATURES

As an investigator or the investigator's representative, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternative to being in the study; and how the participant's protected health information will be collected, used, and shared with others:

Signature of Person Obtaining Consent and
Authorization

Date

You have been informed about this study's purpose, procedures, possible benefits, and risks; the alternatives to being in the study; and how your protected health information will be collected, used and shared with others. You have received a copy of this Form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your protected health information as described in sections 17-21 above. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing

Date

APPENDIX C
RECRUITMENT MATERIALS AND QUESTIONNAIRES



Date: _____ Time: _____ Subject Number: _____

Inclusion criteria after obtaining consent.

- YES No Are you a full time undergraduate student (taking \geq 12 credit hours)?
- YES No 18 years of age or older?
- YES No willing to complete a daily assessment form via computer?
- YES No willing 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)?
- YES No willing to take the fiber for 8 weeks?
- Yes/No No willing to provide a social security number to receive study payment? *Note: the student can still participate if unwilling to provide SS#, but no financial reimbursement can be provided.*
- YES No Have you had a cold in the last 12 months?
- YES No Do you have at least 1 final during the Fall 2009 exam week, between Saturday, December 12 and Friday, December 18, 2009?
- YES No daily access to a computer with Internet access for the entire 8-wk study?

- NO Yes Are you a current smoker?
- NO Yes Do you have chronic allergies involving the upper respiratory tract? (*Chronic = taking allergy medicine daily*)
- NO Yes an allergy to milk (note lactose intolerance should not be a problem because 1 fiber packet contains only 0.3 g of lactose whereas 1 cup of milk contains 12.8 g of lactose)?
- NO Yes known illnesses or conditions that may impact perceived health such as HIV/AIDS, diabetes, renal or gastrointestinal diseases?
- NO Yes Have you received chemotherapy or other immune suppressing therapy within the last year?
- NO Yes antibiotic therapy in the past two months?
- NO Yes Do you have a cold or cold symptoms (on day of randomization)?

Qualifies? (Yes, if all boxes in first column marked)	<input type="checkbox"/> yes	<input type="checkbox"/> no
Gender	<input type="checkbox"/> male	<input type="checkbox"/> female
Willing to provide 4 stool samples during the study? (If yes, complete the second consent.)	<input type="checkbox"/> yes	<input type="checkbox"/> no

Discard this notice after 12-1-09.

IRB
APPROVED
On 9/12/09 gm

Research Study

Examining Immune and Digestive Health

- Are you an undergraduate student?
- Will you be taking at least 1 final exam during the fall semester?
- Are you in overall good health?

If you said yes to these questions, you may be eligible to participate in this study.

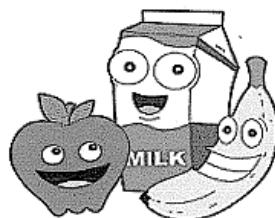
If you participate, you will receive dissolvable, flavorless fiber to add to a beverage on a daily basis for 8 consecutive weeks. Each day you consume the fiber you will answer questions online regarding your health. You could earn up to \$60 for completing the study and a smaller group could earn up to \$120.

If Interested Please Contact:
Christine Hughes, Study Coordinator
Food Science & Human Nutrition Department
FSHN Building Rm. 309
(352) 682-4883
nutrition-study@ufl.edu

Research Study (352) 682-4883								
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Discard this notice after 12-1-09.

Nutrition Fiber Study



IRB
APPROVED
On 9/12/09 gm

- Are you an undergraduate student?
- Will you be taking at least 1 final exam during the fall semester?
- Are you in overall good health?

If you said yes to these questions, you may be eligible to participate in this study.

If you participate, you will receive dissolvable, flavorless fiber to add to a beverage on a daily basis for 8 consecutive weeks. Each day you consume the fiber you will answer questions online regarding your health. You could earn up to \$60 for completing the study and a smaller group could earn up to \$120.

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Template for the verbal telephone screening script:



Hello! My name is _____. With whom am I speaking? ___ (record name on telephone inclusion criteria form) ___ I would like to thank you for responding to the flyers regarding this study. I am a study coordinator/principal investigator for this research study in the Food Science and Human Nutrition Department here at the University of Florida. Do you have a few minutes right now for me to tell you about the study or would you like me to call back at a more convenient time? (If yes continue, if no get contact information and good time to call.)

First I will tell you about the study. If you think you may be interested in participating I will then list the inclusion/exclusion criteria so you can determine if you qualify for this study. If you think you qualify, then I will set up your first study visit.

The purpose of the study is to evaluate the effect of a naturally occurring fiber on maintaining digestive health and immune strength. We are looking for healthy full-time students who will be undergoing an academic stress (such as final exams) during cold and flu season. We want to know if students who consume this fiber each day for 8 weeks before and during fall final exams will have more days where they feel healthy than those who do not receive the fiber.

If you decide to participate, we will obtain your height and weight and ask you to complete short daily and weekly questionnaires on E-Learning. These questions will ask about your level of stress, health status, gastrointestinal symptoms, and bowel habits. You will be asked to mix a packet of tasteless fiber into a beverage and consume it each day of the study.

For completing all 8 weeks you will receive financial compensation of \$60. If you would like to provide stool samples, you could earn an additional \$60 (so \$120 total), but you don't have to do that to participate in the study. Do you think you might be interested in participating in the study?

If response is “no”: say, “thank you very much for responding feel free to call me back at 352-682-4883 if you change your mind”.

If response is “yes”:

Great, now I will list the inclusion / exclusion criteria so you can determine whether you may be eligible to participate in the study.

Complete the Telephone Script - Inclusion Criteria form.



Daily Questionnaire (completed online)

1. How much of the packet of fiber did you consume today (select the closest estimate)?

100% 75% 50% 25% 0

2. Rate your overall level of stress from 0 to 10 (0 = no stress; 10 = extremely stressed).

0 1 2 3 4 5 6 7 8 9 10

3. Cold symptoms and Intensity. For each symptom please check "0" if you did NOT experience the symptom today. If you did experience the symptom check 1 to 3 for the intensity with 3 equal to very intense or severe. If the symptom can be attributed to an allergy or an allergic response and not a cold, check "0 (not experiencing)".

Cold symptom	Intensity 0=none and 3=very intense or severe			
Running/congested nose	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Stiffness or chills	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Headache	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Cough	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Fatigue	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Fever	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Sore throat	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Achiness	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)
Ear discomfort	<input type="checkbox"/> 0 (not experiencing)	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3 (severe)

(Total number of symptoms and the sum of the intensity score needed for outcomes analyses)

4. How many bowel movements did you have today?

0 1 2 3 4 5 6 7 8 9 10 >10

5. Please rate the stool consistency (select one). Note: – we will try to include clip art/drawings/pictures for each see below)

Type 1: separate hard lumps, like nuts (hard to pass)

Type 2: sausage-shaped but lumpy

Type 3: like a sausage but with cracks on its surface

Type 4: like a sausage or snake, smooth and soft

Type 5: soft blobs with clear-cut edges (passed easily)

Type 6: fluffy pieces with ragged edges, a mushy stool

Type 7: watery, no solid pieces (ENTIRELY LIQUID)



Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. Entirely Liquid

6. How many hours of sleep did you get last night?

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 >14

7. Did you take an antibiotic today?

no yes

Final Questionnaire (completed online)

1. How many final exams did you take during exam week?

1 2 3 4 5 6 7 8 >8

2. Did you receive the influenza vaccination this season (2009-2010)?

yes no

3. Did you receive the influenza vaccination last season (2008-2009)?

yes no



Final Questionnaire (completed online)

1. How many final exams did you take during exam week?

1 2 3 4 5 6 7 8 >8

2. Did you receive the influenza vaccination this season (2009-2010)?

yes no

3. Did you receive the influenza vaccination last season (2008-2009)?

yes no

4. Did you receive the swine (H1N1) flu vaccination this season?

yes no

If yes, did you receive one or two swine (H1N1) flu shots?

1 2

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BIOGRAPHICAL SKETCH

Christine Hughes received a bachelor's degree in food science and human nutrition with an emphasis in dietetics from the University of Florida in 2009 and a master's in nutritional sciences in 2011. She has worked for Dr. Bobbi Langkamp-Henken for 4 years on 6 clinical nutrition studies. In the fall of 2011, she plans to become a registered dietitian by completing her dietetic internship. Christine is married to a wonderful man and has a fabulous Yorkshire terrier named Sophie. Christine has high hopes that one day galactooligosaccharides will be taken routinely by academically stressed undergraduate students.