

Effects of a novel fiber blend on human gut function

A DISSERTATION
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

KATIE JANETTE KOECHER

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

JOANNE L. SLAVIN, PHD, RD, ADVISER

SEPTEMBER 2013

© KATIE JANETTE KOECHER 2013

Acknowledgements

The following work would not be possible without the extraordinary support of Dr. Joanne L. Slavin. Not only did she encourage me to take on a unique degree/project combination, but she weathered all of the ups and downs that came with it. I will forever appreciate her continued patience, guidance, flexibility and support she extended to me. Thank you.

Many thanks to Dr. Joellen Feirtag, Dr. David Smith, Dr. Michael Sadowsky and Dr. William Thomas for their support and contribution to this project. I sincerely appreciate your guidance in achieving my educational goals. Special thanks to Dr. Will Thomas for his guidance and patience for the statistical aspects of this research. He is the one of the most intelligent and helpful people that I know. I would also like to thank Dr. Gary Reineccius for encouraging me to pursue a graduate education, and for his flexibility in allowing me to take classes while working.

Thank you to everyone who worked in the “Slavin Lab”- in particular Abby, Jackie, Derek, and Renee. Your patience with my constant need to discuss ideas, to have someone double check my work, and with my SAS issues will always be appreciated. A special thanks to Abby, Jackie, and Derek for graciously sharing their *in vitro* data so that I could attempt to relate it to the human study!

I would also like to thank the men and women who participated in the fiber study as subjects. Their patience and good humor were crucial to the success of the study. I will never forget our many “visits”.

Last but not least, I would like to thank my wonderful family and friends. I thank them for their positive energy and for always listening to me when I needed to vent. I especially need to thank my mom and dad for always supporting me, but never hesitating to remind me that I shouldn't take work or school too seriously.

Abstract

Enteral nutrition (EN) or enteral tube feeding is an effective means of nutritional support for individuals who do not or cannot eat adequately. Patients restricted to EN frequently suffer from abnormal bowel function which affects their intestinal bacteria and impacts quality of life. Dietary fibers have a variety of physiological benefits; fibers that provide fecal bulk promote regular bowel movements while fermentable fibers are utilized by gut bacteria to produce short chain fatty acids (SCFA) and gas in the process. SCFAs positively influence gut health, gut transit time, and fecal moisture while gas may reduce the tolerance of fibers. Fortification of EN with a blend of fibers with various physicochemical properties more closely represents a normal diet, and may maximize physiological benefits. Moreover, using a blend of fibers with different rates of fermentation may minimize gas and bloating commonly associated with highly fermentable fibers.

Batch *in vitro* systems allow fiber fermentation modeling without absorption and may help to estimate potential health benefits and gastrointestinal tolerance of fiber *in vivo*. Well controlled, blinded and randomized intervention human studies are the “gold standard” for human nutrition research. The primary aim of this project was to conduct a human clinical trial utilizing a fiber blend fortified EN product. The secondary aim was to relate the human study findings to the *in vitro* fermentation profiles of the fiber blend and its individual components.

The objective of the human study was to compare the effects of a fiber blend fortified enteral formula (FB, 15 g/L), a fiber-free formula (FF) and habitual diet on bowel function, fecal bacteria and quality of life. The fiber blend consisted of a 50:50 insoluble:soluble ratio of fructo-oligosaccharides (FOS), inulin, gum acacia and outer pea hull fiber. In a randomized, double-blind, crossover design, 20 healthy subjects consumed both FF and FB for 14 days with a 4 week washout. Fecal samples were collected the last 5 days of each period and assessed for fecal output, whole gut transit time (WGTT), and major bacterial groups. Subject gastrointestinal quality of life index (GIQLI) and tolerance were also measured. On formula diets, 5-day fecal output decreased by more than 55% from habitual diet, but was 38% higher on FB than FF ($p=0.0321$). WGTT was approximately 1.5 times longer on formula diets than habitual diet ($p<0.0004$). Total bacteria declined from habitual diet on FF ($p<0.004$), but not on FB. Numbers of bifidobacteria and lactobacilli declined from habitual diet on both formula diets, but bifidobacteria was higher on FB compared to FF ($p<0.0001$). Bacteroides and clostridia numbers did not change between diets. GIQLI and incidence of gas symptoms did not differ between formulas.

The objectives of the second project were to compare the *in vitro* fermentation profiles of FOS, inulin, gum acacia, and pea fiber alone or blended using a 24 h batch model and relate these finding to the human study results. For the *in vivo* measurements, stool samples were collected to measure pH and SCFA. Tolerance was also measured. The *in vitro* fermentation of the fiber blend resulted in a delayed pH decrease and gas and SCFA

production compared to the FOS and inulin. Human samples had higher total SCFA on the fiber formula compared to the fiber free formula ($p=0.029$), and both formulas yielded lower SCFA than habitual diet (both $p<0.0001$). Mean fecal pH for both formulas was 7.5; higher than habitual diet pH 6.5 ($p<0.0001$). No differences in gas/bloating were found between any diet. By blending fibers, a slower fermentation was observed *in vitro* and was well tolerated in human subjects. Fiber addition to enteral formula increases fecal short chain fatty acids which may reflect increased fermentation.

Table of Contents

Acknowledgements.....	i
Abstract.....	iii
List of Tables.....	ix
List of Figures.....	x
CHAPTER ONE: LITERATURE REVIEW	
Definition of fiber.....	1
Physicochemical properties of fiber.....	2
Prebiotic fibers – definition, index, and requirements.....	4
Enteral nutrition – definition, usage, formula types and complications.....	9
Fiber in enteral nutrition.....	11
Effects of dietary fiber on gut function.....	13
Stool weight and whole gut transit time.....	14
Stool form and fecal moisture.....	19
Short chain fatty acids and pH.....	22
Subjective tolerance.....	27
Gut microbiology.....	30
Specific types of fiber.....	38
Fructans (fructo-oligosaccharides and inulin).....	38
Gum acacia.....	43
Outer pea hull fiber.....	47
Rationale for a fiber blend.....	51

CHAPTER TWO: HEALTHY SUBJECTS EXPERIENCE BOWEL CHANGES ON ENTERAL DIETS: ADDITION OF A FIBER BLEND ATTENUATES STOOL WEIGHT AND GUT BACTERIA DECREASES WITHOUT CHANGES IN GAS

Executive Summary.....	53
Introduction.....	54
Materials and methods.....	55
Results.....	62
Discussion.....	64
Conclusion.....	67

CHAPTER THREE: COUPLING FERMENTATION RESULTS FROM A 24 HOUR BATCH *IN VITRO* SYSTEM WITH FECAL MEASUREMENTS AND SUBJECTIVE TOLERANCE FROM A HUMAN INTERVENTION FEEDING STUDY USING FRUCTO-OLIGOSACCHARIDES, INULIN, GUM ACACIA AND PEA FIBER

Executive Summary.....	73
Introduction.....	74
Materials and methods.....	75
Results.....	81
Discussion.....	85

Conclusion.....	88
References.....	97
Appendices.....	113

List of Tables

Table 1-1. Effects of fructans on gut function and tolerance in human subjects.....	41
Table 1-2. Effects of gum acacia on gut function and tolerance in human subjects.....	46
Table 1-3. Effects of outer pea hull fiber on gut function and tolerance in human subjects.....	50
Table 2-1. Subject baseline demographics and diet characteristics of 20 subjects consuming fiber free formula, fiber blend formula and habitual diet.....	69
Table 2-2. Fecal output, stool characteristics and mean transit time of 20 subjects consuming fiber free formula, fiber blend formula and habitual diet.....	70
Table 2-3. Gastrointestinal quality of life index and incidence of gastrointestinal symptoms of 20 subjects consuming fiber free formula, fiber blend formula and habitual diet.....	71
Table 2-4. Log ₁₀ fecal bacteria populations and pH of 20 subjects consuming fiber free formula, fiber blend formula and habitual diet.....	72
Table 3-1. Gas volume produced during 24 h <i>in vitro</i> fermentation of fibers.....	92
Table 3-2. pH during 24 h batch <i>in vitro</i> fermentation of fibers.....	93
Table 3-3. Total short chain fatty acids produced during 24 h <i>in vitro</i> fermentation of fibers.....	94
Table 3-4. Energy, fiber and protein intake of 20 human subjects consuming enteral formula and habitual diet.....	95
Table 3-5. Fecal short chain fatty acids and pH of 20 human subjects consuming fiber free formula, fiber blend formula and habitual diet.....	96

List of Figures

Figure 1-1. Basic GFn type fructan structure.....	38
Figure 1-2. Generic polysaccharide structure of arabinogalactan portion of gum acacia.....	43
Figure 1-3. Proposed arabinogalactan-protein fraction of gum acacia.....	44
Figure 1-4. Generic structures of cellulose and pectin found in outer pea hull fiber.....	48
Figure 3-1. Gas volume produced during 24 h batch <i>in vitro</i> fermentation of fibers.....	90
Figure 3-2. Total short chain fatty acids produced during 24 h batch <i>in vitro</i> fermentation of fiber.....	91

CHAPTER ONE

Literature Review

Definition of dietary fiber

Determining a single definition of the diverse compounds considered to be “fiber” has been a debate since the early 1950s (1) and still differs among organizations. The American Association of Cereal Chemists defines dietary fiber as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.

Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation” (2). Of clinical relevance, the Food and Nutrition Board of the Institute of Medicine proposed that fiber be split into categories: *dietary fiber* is the non-digestible carbohydrates and lignin occurring intrinsically and intact in plants; *functional fiber* is the isolated, non-digestible carbohydrates which exert beneficial physiological effects in humans; and *total fiber* is the sum of dietary and functional fiber (3). Most recently, the Codex Alimentarius Commission of the United Nations Food and Agriculture Organization/World Health Organization Food Standards Program, attempted to standardize the use of the term and proposed that “dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: 1) edible carbohydrate polymers naturally occurring in the food as consumed; 2) carbohydrate polymers, which have been

obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; 3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities”(4). The definition further states “when from a plant origin, dietary fibre may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls and that the decision on whether to include carbohydrates from 3-9 monomeric units should be left to national authorities” (4).

In the United States, the Nutrition Facts panel on food labels report amounts of fiber as determined/defined by chemical methods approved by the Association of Analytical Chemists (AOAC) (5). Methods exist for determining total fiber, insoluble fiber, and soluble fiber although due to the diversity of compounds defined as fiber, additional methods are being used and developed to quantify specific components (e.g. inulin). The amount of total fiber must be included on the food label while amounts of insoluble and soluble fiber are voluntary unless a specific health claim relating to fiber is made. Classification of fiber solubility is based on its ability to dissolve in aqueous solutions (6).

Physicochemical properties of fiber

By definition, fibers are non-digestible to the human but include a wide range of compounds with various physicochemical properties. The chemical composition and physical structure of fiber largely dictates the physiological effects of in the body. The most important properties of fiber believed to influence its physiological and nutritional effects include solubility and hydration properties, viscosity, and fermentability (7).

The carbohydrate constituents (chemical composition), chain length and degree of carbohydrate branching (physical structure) of a fiber influences its solubility. For example, linear polymers of β (1,4) linked glucose (cellulose) that are packed together into an ordered assembly may resist hydration and neither absorb water or dissolve (8). Other neutral polymers may be able to swell and absorb some water (water binding capacity) while others such as fructose polymers may be able to totally dissolve and exist in solution. Other fibers such as pectin may be ionic and pH/ionic environment of the gut would influence solubility (9). The integrity of the cell wall in a fiber particulate may also influence solubility and water binding capacity. Extensive packing of polysaccharide chains into ordered structures in cell walls may be further stabilized by lignifications and resist hydration (10).

Viscosity, or the measure of resistance to flow, is influenced by the fiber's physicochemical properties in addition to the concentration of fiber in the gastrointestinal tract (10). Polymeric fibers able to dissolve and entangle increase the viscosity of the gut contents, with longer chain polymers creating higher viscosities (9). Insoluble fibers may

also contribute to viscosity as particulates increase viscosity, but because of the compact physical form, make a smaller contribution to digesta viscosity than dissolved polysaccharides (10).

Fermentation is the incomplete oxidation of substances in the absence of oxygen, and fiber fermentability refers to the ability of the gut bacteria to breakdown and metabolize fiber (11). Soluble fibers are more accessible to gut microbe enzymes due to their ability to swell and/or dissolve which influences fermentability (10). Since the fermentation of insoluble fibers may be limited, the physiological effects are more related to the fiber's physical properties of water holding capacity and fecal bulking (12).

Prebiotic fibers- definition, requirements and index

In 1995, the term prebiotics was introduced as 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 bacterial species already resident in the colon, and thus attempts to improve host health” (13). By 2004, a refined definition was published. The updated definition stated that “a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confers benefits upon host well-being and health” (14). Classification of a food component as a prebiotic was contingent upon meeting these criteria; “1) non-digestible i.e. resistant to gastric acidity, and hydrolysis by mammalian enzymes and gastrointestinal absorption, 2) fermentation by intestinal microflora, 3) selective

stimulation of the growth and/or activity of intestinal bacterial associated with health and well-being” (14). This definition is generally used in literature and is referenced in a number of reviews, book chapters and proceedings (15-20). By definition, prebiotics are dietary fibers, but not all dietary fibers are prebiotic.

To be considered a prebiotic, a food component must be non-digestible, fermented by the intestinal microflora, and selectively stimulate the growth and/or activity of the intestinal bacteria associated with host health. Ideally, potential prebiotics would be tested to fulfill each criterion using protocols that are validated to measure and/or model *in vivo* conditions (14). To determine digestibility, substances must be resistant to endogenous gastric acid and enzymatic digestion, and although complete resistance to human digestion is not necessary, a “significant amount” should be available for fermentation (21). Fermentation by the intestinal microflora is commonly measured using *in vitro* systems that utilize fecal slurries or rats that have been inoculated with human fecal flora although fermentation can also be indirectly measured via breath hydrogen or fecal recovery (22, 23). Measuring selective growth and activity is the most complex; the beneficial species must be known and distinguishable from other bacteria and current estimates approximate the human gut microbiome to include over 400-800 cultivatable and non-cultivable species (17). Although which bacteria are beneficial and pathogenic is not currently agreed upon or known in entirety, certain traits show promise. Beneficial or health-promoting effects of microbes include the inhibition of growth of harmful bacteria (nutrient/site competition or antimicrobial production), improved digestion of

nutrients, decreased gas, improved bowel function, absence of toxin production, immunity benefits and modification of phytochemicals/synthesis of vitamins (13, 24, 25). Negative effects include diarrhea, intestinal putrefaction, infections, and potential carcinogen production (13, 25). Generally, saccharolytic (carbohydrate) fermentation results in the production of short chain fatty acids and gas whereas proteolytic/putrefactive fermentation results in branched-chain fatty acids (BCFA) and potentially toxic ammonia, amines, phenols, indoles, cresols and sulphides (25). Selective stimulation of saccharolytic fermentation is suggested to be more beneficial than one that stimulates the metabolism of both carbohydrates and protein (24). The current body of research suggests that the main saccharolytic species in the colonic microflora belong to the genera *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus* and *Clostridium* (24). The main proteolytic species belong to the genera *Bacteroides* and *Clostridium* (24, 25).

Traditionally recognized “beneficial” groups have included bifidobacteria and lactobacilli (18), but research is suggesting *Eubacterium*, *Faecalibacterium* and *Roseburia* species may also be included (24). It is likely that additional bacterial groups related to positive or negative physiological functions in humans will be determined as more research is published using high throughput functional genetic testing. Regardless, current research suggests that bifidobacteria and lactobacilli are beneficial and are the base of many probiotic supplements (26). Bifidobacteria are saccharolytic organisms which produce lactate and acetate during heterofermentation of carbohydrates (via the “bifidus

pathway”) (27). Although the products of fermentation from lactobacilli vary (homofermentation or heterofermentation) they are also considered lactic acid bacteria and primarily produce lactate or lactate and acetate (27). These acids are thought to lower the pH of the fermentation area and potentially inhibit the growth of potential pathogens (13). In addition to pH effects, some bifidobacteria produce antimicrobial substances (28).

Species that belong to groups such as clostridia and *Bacteroides* have been recognized as potentially harmful (24). Clostridia are of particular importance in this study, as *Clostridium difficile* is a frequent problem associated with occurrence of diarrhea in patients on enteral nutrition (29). When disruption of the normal flora occurs, *C. difficile* is able to overgrow and produce enterotoxins and diarrhea can result (26). Some clostridia, however, are also able to further breakdown lactate produced in the colon to acetate, propionate and butyrate and is considered to be one of the major butyrate formers in the human intestine (30). *Bacteroides* is a numerically dominant group of bacteria in the gut and represents approximately 30% of culturable fecal isolates (31). Although the genus *Bacteroides* includes enterotoxigenic *B. fragilis* that is associated with diarrhea, as a whole the bacteria have diverse metabolic capabilities which contribute to more complete food digestion (32). Conversely, decreased *Bacteroides* has been observed in patients with antibiotic associated diarrhea due to *C. difficile* (33).

Beyond determining which bacteria are associated with health, selective stimulation is difficult to measure since the growth of a particular group of bacteria is influenced by the initial numbers of bacteria present (34), dose, type of prebiotic and the complex interactions between the various intestinal microflora which occur in different regions in the gastrointestinal tract (15).

In an attempt to examine a prebiotic model, quantitative data from 17 published and unpublished human intervention feeding studies which fed inulin-type fructans was meta-analyzed to determine correlations between fiber, dose, and bifidobacteria increases (21). Although singular, the author targeted a bifidobacteria model since bifidobacteria exert a variety of health effects and comprise one of the dominant bacterial populations in the human large intestine (34). From the data, daily dose of prebiotic did not correlate with the bacterial colony forming units per gram (CFU/g) differences, but the initial numbers of bifidobacteria did inversely correlate with the crude increases (21). Other studies have also reported the inverse correlation between initial bifidobacteria numbers and “crude” increase (34-36). Prebiotic index was introduced as “the increase in bifidobacteria expressed as the absolute number of ‘new’ CFU/g in feces divided by the daily dose (in grams) of prebiotic ingested” (21). The prebiotic index proposed, therefore, is a bifidogenic index which reports the increase of fecal bifidobacteria in relation to the daily dose of prebiotic. Although it was an overly simplistic model of prebiotic responses, the manuscript elucidated the complicated factors that influence how a fiber may be considered prebiotic. For the bifidobacteria model, the initial size of the population

within the intestinal tract was inversely proportional to the response of the prebiotic i.e. those with small initial populations have a strong dose response while those with high populations (measured at 10^8 CFU/g) did not increase their numbers further with increased fructan consumption (18).

Enteral nutrition- definition, usage, formula types and complications

Enteral nutrition (EN) typically refers to nutritional support that is delivered to the gastrointestinal tract through a tube, but can also be used to refer to “dietary foods for special medical purposes” that are administered via tube or oral supplements (37, 38).

Enteral tube feeding is an effective means of offering nutritional support to those who are or are at risk of being malnourished in both hospital and community settings (29). EN may be chosen for patients who do not or cannot eat adequately for a variety of medical reasons ranging from acute and chronic illnesses to those with swallowing disorders (39).

Enteral feeding differs from parenteral feeding in that parental nutrition is the provision of nutrients intravenously (38).

EN can be delivered via nasoenteric or percutaneous endoscopic gastrostomy or jejunostomy depending on the patient’s condition and needs (40). Nasogastric feeding is preferred since it allows for normal digestive and hormonal processes (in the stomach and further) to occur (38). EN is delivered to the patient via bolus, intermittent drip, continuous drip or a combination depending on the patient’s gastrointestinal function, tolerance to feeding and quality of life issues (40).

According to the latest annual statistics from the American Society for Parenteral and Enteral Nutrition, approximately 245,000 patients received EN in the hospital and around 31,000 people received it in the home setting (41, 42). With a large and diverse population receiving EN, a wide range of enteral products are available. Formulas may be classified into nutritional categories, and choosing the correct product for a patient is based on the functional status of the patient's gastrointestinal tract, the energy and nutrient needs of the patient, cost and other logistic considerations for the patient (29, 40). Intended to meet the nutritional needs of the general patient population, standard formulas are lactose free, contain intact proteins, carbohydrates and lipids, supply 1 kcal/ml and are designed to meet the dietary reference intakes for vitamins and minerals of healthy adults (with volumes administered) (38). Specialized formulas that are energy concentrated, high nitrogen, pre-hydrolyzed or chemically defined are also available for patients with specific dietary needs. During EN, patient hydration should be considered since without an additional source of fluid, tube-fed patients may not receive enough fluids to meet their needs especially when energy concentrated formulas (1.5-2 kcal/ml) are administered (although fluid restricted patients differ).

Complications of EN can vary from aspiration of formula to gastrointestinal disturbances to skin lesions if a percutaneous tube insertion is used. The most common gastrointestinal complications include diarrhea, constipation, bloating and nausea and the occurrence rates of each vary depending on patient situation (length of treatment, etc.)

(39, 43). In hospitals up to two thirds of patients on EN have been reported to have diarrhea, but the lack of a standard diarrhea definition makes it unclear (39).

Constipation is also an issue, but appears to be more associated with long term home EN (44). Nausea also occurs, but is thought to be associated with abnormal gastric emptying (29). EN associated gastrointestinal intolerance is a complex issue, and may be the result of multiple factors; feed delivery site/rate, feed type, concurrent laxative or antibiotic/drug therapies, small bowel bacterial overgrowth, *Clostridium difficile* infection, enzyme deficiencies (for example, lactase) and nutrient malabsorption (29). There is a potential interaction between some of these factors, dietary fiber and the colonic microflora.

Fiber in enteral nutrition

Fibers with various physicochemical properties have different physiological effects.

Generally, less fermentable and/or insoluble fibers increase fecal bulk and/or hold water which facilitates colonic motor activity and promotes normal laxation (10). Fermentable fibers are metabolized in the distal colon to produce SCFAs which through their absorption promote colonic fluid absorption and may prevent or ameliorate diarrhea-like symptoms (45). SCFAs also decrease colonic pH which may favor the growth of non-pathogenic bacteria (46). Furthermore, fibers are a source of energy for gut bacteria and maintaining a normal (in health) flora provides competitive exclusion for potentially pathogenic organisms (e.g. *C. difficile*) and promotes bowel health (17, 47). Despite the positive effects of fiber, contraindications do exist and some fibers may cause gas,

bloating and flatulence in individuals (48, 49). Excessive doses of fiber may also cause diarrhea and reduced nutrient absorption (49). Furthermore, when added to enteral formulas, fiber may cause blockages in small-bore feeding tubes and is typically the most problematic with highly viscous fibers (29, 49). Sedimentation of fibers in the formula is also an issue (39).

If the contraindications are minimized, a fiber enriched enteral formula may provide advantages over a fiber free formula. Fiber supplementation to EN is not new, and in a 2008 meta-analysis of fiber containing enteral formula studies that included 51 studies (43 randomized-controlled trials), fiber supplementation significantly reduced the incidence of diarrhea and was generally well tolerated (39). Fiber supplementation to formula also moderated bowel function; fiber inclusion reduced bowel frequency when baseline was high and increased it when it was low (in both patients and healthy subjects) (39). Furthermore, a fiber consensus panel recommended the inclusion of fiber in the diets of all patients if no contraindication exists, based on the benefits of diarrhea, constipation and feeding tolerance (50).

Currently, there is no agreement on the best fibers to add to enteral products (39), and no recommendations exist for fiber intake in several disease states or in patients in long term care facilities (49). Recommendations from the European Society of Parenteral and Enteral Nutrition suggest adding fiber to EN at a rate of 10-15g/L (37). Other enteral guidelines suggest 15-30 g/day for patients on EN if no contraindications exist (37, 39).

For reference, in the United States, the Institute of Medicine recommends that adults (50 years or younger) consume 38 g (men) and 25 g (women) (3). For those over 50, the recommendations are 30 and 21 g, respectively, due to a decreased energy need. The average usual intake of an American is 14-15 g per day (51).

Effects of dietary fiber on gut function

Brief overview of foodstuffs through the gastrointestinal system

Fiber travels through the gastrointestinal (GI) system intact in foods or as an isolated source. Substances enter the GI system through the oral cavity, are mixed with saliva and enzymes and quickly travel to the stomach. In the stomach, foodstuffs are exposed to a low pH, enzymes, and come in contact with a relatively low load of microbes (in health) with 10^2 CFUs per milliliter (ml) of contents (52). Within minutes, the contents of the stomach are propelled to the small intestine where it is mixed with pancreatic and biliary secretions, the pH increases and the microbial loads increase. In the duodenum (first section of the small intestine), the microbial load rises to 10^2 - 10^4 CFU/ml contents (53) to the ileum where 10^6 - 10^8 CFU/ml contents have been found (54). The small intestine is the main site of nutrient and water absorption with transit time through the stomach and small intestine taking an average of 4-6 hours in health (47). The undigested foodstuffs, digestive substances, and sloughed off cells are then propelled to the large intestine. The large intestine, or large bowel, is the main site of microbial colonization in the gut and harbors approximately 10^{11} - 10^{12} bacteria per gram of intestinal contents (47, 55).

Metabolism of fiber by microbes does not exclusively occur in the large intestine, but is the primary site of utilization. Bacterial metabolism includes fermentation of carbohydrates and proteins, and a variety of compounds including (but not limited to) flavonoids, heterocyclic amines, and bile acids (56). The large intestine is hallmarked by long residence times (up to 60 hours), a more neutral pH and anaerobic environment (47). Endogenously, the primary function of the large intestine is to store and concentrate undigested matter through the absorption of salt and water. Water absorption in the GI tract is passive and although the small intestine absorbs the majority of water, the colon has a large absorption capacity as well. If exceeded, or without sufficient time to absorb, watery diarrhea may occur (57). Upon completion of digestion and absorption, feces are eliminated. Fecal matter is complex and is composed of approximately 75% water, and varying amounts of bacteria, unfermented fiber residue, inorganic substances, bacterial fermentation products and undigested protein, cells and bile pigment (with estimates that bacteria contain 7% of the dry weight) (10, 47).

Stool weight and whole gut transit time

Stool weight is currently the most direct biomarker of total fiber intake. Stool weight is influenced by the presence of fiber, by the water the fiber holds, increases in bacterial biomass if the fiber is fermented, gut transit time and abnormalities in fecal moisture (49, 58). Laxation studies have also found significant differences in stool weight between genders and personality types, suggesting that other factors play a role in stool weight (59-61). In general, there is a positive correlation between fiber and stool weight,

although fibers that differ in fermentation characteristics and/or particle size affect stool weight to different degrees (58, 61-63). Smaller stool weight increases have been observed with highly fermentable fibers such as pectin or mixed fruit and vegetable fibers compared to wheat bran (less fermentable) despite a similar dose, although the fibers also differed in particle size (64). A similar trend was observed when feeding wheat bran and a mixture of pea fiber, soy polysaccharide and pectin in a liquid diet base; wheat bran increased wet stool weight more than the vegetable fiber, although increases were seen with the vegetable fiber compared to the fiber free control (65). Studies suggest the smaller stool weight increases seen in more fermentable fibers is due to the loss of structure as the polysaccharide is digested which affects its water holding capacity/viscosity (61, 66). Stool weight increases observed in fermentable fibers are through increased bacterial mass that occurs with growth (58, 62). Fiber particle size also influences stool weight, and human feeding studies conducted with wheat bran observed a larger stool weight increase with larger particle sizes (6, 67).

Gender differences have been observed in stool weight; women were found to have smaller stool output than men and have a smaller stool weight increase as compared to men (61, 64).

The time required for substances to travel from ingestion to defecation is generally referred to as transit time, gastrointestinal transit time or whole gut transit time (WGTT) (68, 69). Since most of the WGTT reflects passage through the colon, WGTT

measurement techniques are an approximation of colonic transit time (70). Fecal weight is negatively correlated with transit time, although dietary fiber intake has a more inconsistent correlation, which may reflect the varied physicochemical properties of fiber (63, 67, 71). Fecal bulk is thought to increase colonic motility, while short chain fatty acids produced through fiber fermentation are also thought to play a role in increased colonic motility (decreased transit time) (72, 73). Whole gut transit time influences bacterial growth and water absorption; the longer feces remains in the large intestine, the more water may be absorbed and the fewer bacteria that are excreted (71). Similar to fecal weight, there is a large variability in gut transit time between individuals and intra-individually (61, 62, 64).

Constipation and diarrhea are two extremes of bowel function that are related to stool weight, transit time, and fecal moisture. Etiology of these conditions is vast and neither have a consistent definition in the literature. Constipation has been defined as three or fewer spontaneous bowel movements per week (74), stool weights of less than 100 g/day (75), and/or hard stool that retains its shape, with difficulty in defecation or patients requiring laxatives/enemas” (39). Constipation may occur when fecal matter remains in the large intestine for an extended period of time and becomes hard, dry and difficult to pass (76). Diarrhea is typically characterized by large, frequent, watery stools and can result from decreased fluid absorption, increased fluid secretion, or both (76). Another definition includes elevated stool output (>200 to 250 g/day) that is watery and difficult to control with more than three bowel movements per day (77). Normal transit time has

been defined as producing between three stools per day to three per week with an absence of diarrhea and constipation (78, 79).

Fecal weight measurement techniques vary based on the length of time stool samples are collected, and common periods are 1-10 days (62, 80, 81). Ideally, a fecal sampling procedure collects multiple replicates, allows for tandem gut measurements if necessary, and has the least possible subject burden. Longer sampling protocols allow for a more representative sample (versus 1 sample) of the individual's stool pattern since daily stool weight intra-individual variation has been observed to be as much as 100% or more (63). Furthermore, stool weight, when combined with fecal moisture and other subjective measures, may indicate diarrhea or constipation (39).

Various measurement techniques have been utilized to measure whole gut transit time. Free dyes may be consumed and the color appearance tracked in the fecal sample; dye recovery can be affected by fiber content, however, and may not reflect gut movement when fiber is low (82). Fiber mordants may be synthesized, but mordants are not possible with all fibers (67). Radio labeled substances that mirror the transit of fiber may be used to track transit through each region of the GI, but are expensive and require high subject burden (70). Similarly, wireless devices track digest transit through the entire GI and additionally measure pressure, pH, and temperature (70). Such devices are costly and measurement is influenced by the timing of the capsule and test meal administration (70, 83). Although it does not provide regional gastrointestinal information, measuring

whole gut transit time with radio-opaque markers provides a relatively inexpensive and low participant burden method (70, 84). Markers may be taken continuously or as a bolus, and fecal samples are then recovered and pellet content is determined by x-ray (84, 85). Previous recovery studies have shown that normal individuals retain less than 80% of pellets of a 20 pellet dose within 5 days (86). Using a bolus dose, whole gut transit time may be calculated based on the time to excrete 80% or as mean transit time (a weighted average of pellets over time) (82, 84). Comparing the 80% transit time and mean transit time calculation techniques, both have similar variability and yield comparable results (82).

Several studies administering a polymeric oral enteral formula to healthy subjects (both fiber free and fiber blend) observed an approximate 50% decrease in daily stool weight compared to a self selected diet (48, 79, 87). This was observed despite a higher daily fiber intake on the fiber containing liquid diets than the self selected diets. In one study, addition of 60 g of soy fiber to the liquid base diet was required to increase stool weight to similar self-selected diets (79). In regard to formula comparison, a blend of soy fiber, cellulose, gum acacia, inulin, oligofructose, and resistant starch added to a fiber formula (daily intake 30 g/day), did not increase stool weight compared to a fiber free formula (87). Daily average stool weights were 95 g on the fiber formula and 97 g on the fiber free (79). Another study fortifying with a blend of fructo-oligosaccharides and pea fiber (average 16.5 g/day), measured stool weights at 44 g/day on fiber free formula and 73 g/day on fiber supplemented formula (although the differences were not significant) (48).

As measured by radio-opaque pellets, Slavin et al found that transit time on fiber free enteral formula was 72.4 h and approximately 48 h during both the soy fiber supplemented enteral and self-selected diets (79). Another study using a blend of soy fiber, cellulose, gum acacia, inulin, oligofructose, and resistant starch in the fiber formula measured a shortened gut transit time compared to fiber free formula (76 h fiber free and 50 h fiber blend), but both were longer than the habitual diet transit time of 46 h (87). Although not directly transit time, addition of fructo-oligosaccharides and pea fiber to an enteral formula increased fecal frequency (number of stools/day) compared to the fiber free formula. Mean fecal frequency of this study was 1 per day on habitual diet, 0.6 per day on standard fiber free formula and 0.9 per day on fiber formula (48).

Stool form and fecal moisture

Stool form is a visual estimation of stool consistency which is generally “measured” by comparison to a chart that includes pictorial and verbal descriptors (88). Some stool form charts also include information for estimating stool weight (89). A more objective measurement of stool consistency may be achieved with fecal moisture or rheological assessments (90). Stool formed-ness has been correlated with whole gut transit time and can be used to monitor changes in colonic function (91). While whole gut transit time may affect fecal moisture, fecal consistency is related to both water content of the stool and the water-holding capacity of the fecal insoluble solids (90). Fiber affects stool consistency and moisture through its physiological effects on transit time and its

physicochemical properties and particle size which affect water holding capacity. Fecal moistures of healthy subjects consuming normal diets without constipation or diarrhea are approximately 70-80% moisture (wet basis) (63, 65, 67). In a stool form validation study, water content of fecal samples correlated with a four category scale of formed-ness; hard and formed-62% moisture, soft and formed-72% moisture, loose and unformed-79% moisture, liquid-87% moisture and all were significantly different (88). Furthermore, the ratio of fecal water to fecal solids increased only slightly in stool weights of healthy subjects despite stool weights ranging from 39 to 235 g/day (90). The apparent consistent water/solid ratio in fecal samples may be due to a considerable amount of fecal water contained within fecal bacteria that is unavailable for absorption (only free fluid is available for absorption) (57, 90). In patients with chronic diarrhea that have high moisture stools, but normal stool weight, it was observed that low or low to normal fecal insoluble solids were present (90).

Several manuscripts describe categorizing fecal samples into consistency categories which are typically author defined and vary in number of categories from two to up to twelve (65, 89, 91, 92). Two common stool charts which have been used extensively include the King's stool and Bristol stool chart (89, 91). Both scales have been validated in various studies (88, 89, 93-95). The King's chart organizes stool into 12 categories using pictorial and verbal descriptors to determine consistency and stool weight (89). The Bristol stool chart was developed to assess stool form in relation to changes in transit time in healthy adults and organizes stool into seven types using pictorial and verbal

descriptors to determine consistency. Each consistency is assigned a numerical value ranging from 1 (most formed) to 7 (least formed) (91).

Moisture determination methods vary widely, and several methods are suitable for determining fecal moisture. Drying methods utilize ovens or infrared to heat the sample, evaporate the water, and measure weight loss (assumed to be all water) to determine moisture content. Heat drying methods are subject to bias as volatile components (such as short chain fatty acids) are lost, carbohydrates decompose at the temperature and time utilized and incomplete moisture removal may occur due to form of the water present (free, adsorbed, water of hydration) (96). Drying methods are simple, allow for simultaneous analysis of large number of samples, and specialized equipment is not required; some laboratories utilize this method for fecal moisture (97, 98). Freeze drying methods are also applicable. Samples are frozen, placed into a freeze dryer where the pressure is reduced to allow the frozen water to sublimate. Freeze drying requires similar (or more) time as drying methods, requires specialized equipment and sample throughput is not as high volume. It has also been used in determining fecal moisture (99). Infrared spectroscopy (IR) has also been used to determine moisture in fecal samples (100). IR is based on the selective absorption of electromagnetic radiation of functional groups (such as –OH in water) since each has a characteristic vibration frequency (96). IR requires specialized equipment. Overall, a forced draft oven is an inexpensive and non-labor intensive method that doesn't require solvents or specialized equipment.

In enteral studies examining the effects of fiber addition on fecal consistency and moisture in healthy subjects, the results have been mixed. Enteral formula supplemented with hydrolyzed guar gum (101), soy fiber (79) or pectin (102) resulted in a more normally formed stool than either a hard/dry or liquid stool in the fiber free formulas. In other studies using guar gum (103) or soy polysaccharide (104), no consistency differences were found. As far as those studies that also measured fecal moisture, Lampe et al observed that fecal moisture on a self selected diet was the same as the enteral diet supplemented with soy fiber, but higher than both fiber free and guar fortified formula (104). Another group examining dose of soy fiber in enteral formula did not see statistically significantly different fecal moistures, but the values appear to slightly increase as fiber increased; self selected-72%, no fiber-75%, 30g-75% and 80% for 60 g (79). Finally, adding a blend of fructo-oligosaccharides and pea fiber to enteral formula did not affect fecal moisture – no differences were found between habitual diet, fiber free formula or fiber supplemented formula (48).

Short chain fatty acids and pH

The production of SCFA in the gut is dependent on the type of substrate available, the amount of substrate, the transit time (retention time of substrate), the composition of the bacteria, and the characteristics of the bacterial groups present (enzymes, optimal growth pH, etc.)(105). Gut bacteria ferment non-digestible carbohydrates and proteins arriving from the small intestine although protein fermentation is dependent on the amount of carbohydrate available (105, 106). If sufficient carbohydrates are available, protein

fermentation is reduced while protein fermentation is favored if carbohydrates are insufficient (107). Carbohydrates for fermentation are mostly derived from the diet, while protein is from both dietary and endogenous sources (enzymes, mucus and exfoliated cells) (106, 108). Carbohydrate fermentation results in lactate, ethanol, pyruvate and succinate production in some species, but as a whole gut, these can be subsequently used by other bacteria and the major end products are the short chain fatty acids acetate, propionate and butyrate (56). Protein fermentation yields branched chain fatty acids, ammonia, amines, phenolic and volatile sulfur compounds (56). Other fermentation products such as caproate and valerate also occur, but in lower amounts (24). SCFA are thought to be readily absorbed in a normal colon via both nonionic diffusion and ionic exchange and up to 95% of SCFA are thought to be absorbed from colonic contents (45, 109).

As a whole, SCFAs reduce fecal pH, affect colonic moisture, inhibit pathogen growth, provide energy to the host, and may affect gut transit time (24, 46, 72, 110). SCFAs may also have roles in inflammation, mineral absorption, and improvement of barrier function which may prevent the translocation of bacteria and toxins from gut to system (24, 39). SCFA absorption by colonic epithelial cells stimulates sodium dependent fluid absorption, which affects fecal moisture and benefits those with diarrhea (45, 111). SCFAs are also thought to affect gut transit time. Using a rat model, physiological concentrations of SCFA increased motility and accelerated colonic transit which was suggested to be due to the release of serotonin in response to the SCFA (72, 73).

Individually, butyrate is the preferred form of fuel for colonocytes and most is oxidized in the colonic mucosa during absorption (109, 112). Butyrate also plays a role in epithelial intestinal cell differentiation and proliferation (113). Propionate is absorbed, partially utilized by the colonocytes depending on the availability of butyrate, and then utilized by the liver (112, 114). Propionate has also been proposed to influence cholesterol synthesis (115). Acetate is absorbed and utilized by the liver and/or enters the systemic circulation for use by muscle and adipose depending on the metabolic state (116). Acetate is the major acid entering systemic circulation (115, 117).

The pH of the colonic lumen is in part determined by host secretions and in part by the acidic fermentation products of gut microorganisms. Using telemetry it has been observed that the proximal colon generally has a lower pH than the contents of the distal colon and feces (118). The pH spectrum in the colon is thought to be due to the fermentation of dietary substances (producing acidic compounds) as they travel through the colon (119). Intake of fermentable carbohydrates has been observed to decrease fecal pH, and it is hypothesized that colonic pH fluctuates depending upon dietary intake (118). Colonic pH influences the solubility of bile acids, ammonia and minerals as well as affecting gut bacteria growth and activity (46, 110, 118). Each type of bacteria has an optimal pH and reductions in pH may increase or decrease bacteria (depending on the type), which in turn affects the profile of SCFA produced (46, 118, 120). In an *in vitro* system utilizing human fecal donors, growth of *Bacteroides* and pathogenic *E. coli* was

inhibited by a pH of 5.5 and *E. coli* was further inhibited when SCFAs were added to the growth medium (118). Conversely, butyrate producing *Eubacterium* represented 50% of the microbes present at pH 5.5, but was not detected at pH 6.7 when *Bacteroides* dominated at 86% of microbes present (118). These results were corroborated with another *in vitro* study measuring increased butyrate at pH 5.5. versus 6.5 (46).

In humans, SCFAs may be measured from colonic contents, fecal samples or in the blood: due to the subject burden (in healthy subjects) and ethical issues, most are collected from feces or blood (105). SCFA in fecal samples represent residual amounts after production and absorption in the gut which is thought to be approximately 5-10% of SCFA that is produced. Since SCFA production varies depending on the fermentability of the substrate, amount and retention time of the substrate, bacterial populations present, and absorption may be affected by concentration, fecal SCFA provide little specific information about each of the influences (105). Blood SCFA, however, have similar drawbacks. SCFA are produced, absorbed and utilized by the colonocytes in varying amounts, utilized by the liver in varying amounts and finally enter the bloodstream (112). Timing of blood samples is also important as fatty acids are cleared from circulation (112).

Beyond the challenges of collecting a representative samples containing SCFA, sample preparation and analysis are not consistent in the literature. Methods include fecal water separation and analysis by liquid chromatography (121) or gas chromatography (99),

distillation of fecal samples and analysis by gas chromatography (122), and fecal extraction via solvent and analysis by gas chromatography (123). Regardless of method, it is important to note that SCFA are volatile and weak acids with pKa's approximately 4.8 making nearly all SCFA found in stool samples present as anions rather than free acids (112). Solubility due to chain length differences has also been found (124). Overall, measurement of SCFA in human samples via feces or blood reflects residual values after production and absorption. Finally, although ratios of the various SCFA is relevant when studying the effects of chemical and structural features of fiber on the production of SCFA, the absorption processes of individual acids may confound the measurement and values obtained may not reflect production levels within the gut.

Fecal pH is measured using a calibrated probe (48, 125). Since fecal samples are not homogenous, samples should be mixed before measurement.

In healthy subjects consuming enteral nutrition with and without fiber, the SCFA and pH results have been mixed. Total SCFA in fecal samples significantly decreased when consuming enteral formula compared to habitual diet in two studies (48, 102), while another study found no statistically significant difference between normal diet and enteral diets (although the values were lower) (104). Between fiber free or fiber containing formulas, a fructo-oligosaccharide and pea fiber containing formula had higher total fecal SCFA than fiber free (48), a pectin fortified formula had higher total SCFA than fiber free (but was not statistically significant) (102), and a soy or guar gum fortified formula

was not different than fiber free (104). Another study measured SCFA in the plasma and found no differences between a fiber free formula and one containing hydrolyzed guar gum (101). Generalized comparison between enteral studies for SCFA production is difficult due to various fiber sources used (different fermentabilities), and differing sample techniques and analysis methods.

There is more agreement in measuring pH. Three studies found normal diet to have significantly lower pH (all approximately a pH unit lower) than when consuming enteral formula (48, 102, 104). Subjects consuming pectin, guar gum, or soy fiber fortified formula did not have a different fecal pH than when on fiber free formula (102, 104). Consumption of a formula fortified with blend of fructo-oligosaccharides and pea fiber, however, led to a significantly lower fecal pH than the fiber free formula (48).

Subjective tolerance

A common side effect of fiber ingestion is “gas”. Gas, however, may be from swallowed air, production within the gut, or diffusion from the blood (126) and is composed of nitrogen, oxygen, carbon dioxide, hydrogen, and methane (127). Gas production in the gut may be produced via acid reaction with bicarbonate to liberate carbon dioxide, or from fiber fermentation by bacteria in the gut (producing hydrogen, carbon dioxide, methane and hydrogen sulfide gases) (115, 127). An endogenous source of acid is gastric acid, while acids are also produced by the gut bacteria during fiber fermentation. If gas is produced in sufficient amounts, it may cause bloating and flatulence in individuals (128).

Gas may be eliminated via diffusion into the blood, metabolism by gut bacteria and/or passage via the rectum (126) . Symptoms commonly attributed to “gas” are belching, bloating, abdominal pain, and excessive flatulence (127).

In addition to tolerance of fiber side effects in the diet, exclusion consumption of enteral nutrition (in patients) has been associated with several gastrointestinal side effects; diarrhea, constipation, nausea and selective bacterial overgrowth (29). Furthermore, in studies examining healthy subjects converting to a liquid diet, subjects experienced changes in bowel habits (48, 79) which may influence quality of life (129).

Various fiber feeding studies monitor gastrointestinal side effects, although many do not cite previously published methods to do so. A validated gastrointestinal symptom scale is The Gastrointestinal Symptom Rating Scale (130). Many tools exist to monitor generic quality of life as well – Nottingham Health Profile and the Centers for Disease Control and Prevention’s Health Related Quality of Life to state a few (131). One index, the Gastrointestinal Quality of Life (GIQLI) measures both quality of life and gastrointestinal symptoms (132). Using 36 questions, the GIQLI measures overall quality of life related to gastrointestinal wellness and inquires about side effects related to digestion and defecation as well as physical and mental well-being. In particular, the questionnaire assesses the frequency of gas/bloating, abdominal pain, reflux, diarrhea/urgent bowel movements, constipation, nausea, and reflux. The GIQLI is a validated questionnaire that

has been used in previous enteral studies (129) and in the original version was administered to normal individuals to assess validity.

Although widely monitored, tolerance of exclusive enteral formula administration (via oral consumption) in healthy humans has been mixed. Slavin et al found no differences in symptoms of nausea, cramping, diarrhea, constipation and diarrhea between habitual diet, fiber free enteral formula and enteral formulas containing up to 60 g soy polysaccharide (79). Lampe et al found that subjects had increased reporting of water stools, anal burning during defecation and gas with the fiber free formula and modified guar formula, but not with soy supplemented formula. Objective measurements of fecal moisture in the same study, however, did not corroborate the subjective reported side effects (104). Examining fructo-oligosaccharide and pea fiber added to formula, Whelan et al found that gastrointestinal symptoms were not increased on either enteral formula compared to habitual diet and flatulence decreased on fiber free formula compared to habitual diet. Between the formulas, nausea incidence and severity was increased on fiber formula while flatulence incidence and severity was increased on the fiber formula (48). Silk et al found no adverse gastrointestinal effects while on a normal diet but between formulas, the fiber formula containing soy polysaccharides, cellulose, gum acacia, inulin, oligofructose and resistant starch had less nausea and bloating than the fiber free formula. Additionally, all but 2 subjects developed headaches when first transitioning to the enteral diet (regardless of fiber or fiber free) although the symptoms subsided within a few days and did not reoccur during the second enteral diet (87).

Finally, two subjects on fiber free reported watery stools and two subjects on the fiber free formula reported feeling constipated. No diarrhea or constipation was found during the fiber enriched enteral formula (87).

Gut microbiology

Eukaryotes, Bacteria, and Archea may be found in the up to 10^{12} microorganisms per milliliter of human large intestine contents (47, 55). Of these domains, Bacteria predominate (133). The overall population and constituent groups of the bacteria are important for competitive exclusion of pathogens, short chain fatty acid production (i.e. complete food digestion and energy production), and enhanced immune function (not addressed in this study) (134, 135). Although the human colonic bacterial community differs between individuals (136-138), it is represented by four main phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (17, 133, 139). In general, Firmicutes and Bacteroidetes make up the dominant portions of the colonic bacteria (140). Recently, personal “enterotypes”, or proportions of bacteria as determined by 16S rDNA sequencing have been found to be relatively stable in individuals with consistent long-term dietary habits (136). Earlier studies utilizing temperature gradient gel electrophoresis of 16S rRNA on fecal samples have also observed stability of dominant groups of bacteria over the course of 6 months (141).

The gut bacterial population is affected by the type and amount of fermentable substrates (diet), exogenous bacteria, concentrations of antimicrobial substances, ionic and pH

conditions, and gut transit time (13, 72, 118, 136). Those with long term protein and animal fat based diets are associated with the *Bacteroides* (Bacteroidetes) enterotype and carbohydrate diets with *Prevotella* (Bacteroidetes) (136). Prebiotic fibers such as fructans are known to increase *Bifidobacteria* (142-144). Exogenous bacteria consumed in fermented foods such as yogurt also modify the gut bacteria and fecal bifidobacteria levels were increased in subjects consuming fermented dairy products within a few days (145). Additional host factors found to influence gut microbe composition are many. Body mass index has been observed to affect the gut microbes as the gut bacteria of obese adults have fewer Bacteroidetes and more Firmicutes than lean counterparts as demonstrated by a human study (146). Additionally, a cross-sectional study utilizing fluorescence in situ hybridization on 230 subjects observed that *Enterobacteria* was 1.7 times higher in elderly (>60 yr.) compared to younger adults suggesting that age may be a factor in gut ecology. This same study also found a significant difference by gender with males having higher levels of *Bacteroides-Prevotella* group than women (147). Data is limited on menstrual status of females and gut microbiota although a study observed that post-menopausal women had higher fecal clostridia levels than fertile women (148). Individual genotype also influences gut bacterial populations; monozygotic twins had fecal bacteria profiles (determined by denaturing gradient gel electrophoresis) that were significantly more similar than those for unrelated individuals. The same study observed that marital partners living in the same environment with comparable feeding habits had low similarity (149). Antibiotic usage is also known to affect gut microbes (150) as well as host diseases (151).

Further complicating the large and diverse population in the gut, the bacteria are not evenly distributed and micro-habitats include the lumen, mucous layer, crypts and surface of the epithelial cells (152). Although many sampling techniques are based on fecal samples, biopsies may also be performed to observe mucosal associated bacteria (151).

At present, methods exist to identify bacteria present, enumerate specific groups or profile the microbial diversity in samples and all have advantages and disadvantages. Until recently, our knowledge of the human gut microbiota was based on conventional microbiological culturing techniques, which are currently estimated to measure 15-54% of the microbial diversity in a fecal sample due to the inability to culture the bacteria (unknown growth requirements/restriction factors), microbe death and microbe viability (17). Cultivation methods have also been used to characterize and/or identify bacterial colonies based on morphological and biochemical traits, but these can be unreliable and time consuming (153).

Although originally used in conjunction with bacterial cultivation, molecular approaches based on the bacterial ribosomal RNA gene are extensively used (139). The 16S rRNA gene is universally distributed in bacteria, has a slow rate of evolutionary change, and contains various regions with different degrees of conservation (153). A highly conserved genetic region of the rRNA may be targeted in experiments (using “universal” primers) to measure or probe for all bacteria. Other variable or hypervariable regions of

the rRNA may be targeted to yield information about genus or species of bacteria (18). In some instances, the rRNA sequence may be highly conserved within a genus and the use of the internal transcribed spacer region between the 16S and 23S rRNA genes or other specific genes may be used (154).

The most basic culture independent method is a direct microscopic analysis of all bacteria present. Bacteria from fecal samples are heat fixed onto a surface, stained (typically with DNA staining fluorescent 4',6-diamidino-2-phenylindole-stain "DAPI") and enumerated to give total cell counts (138). With this technique, cell detachment may occur, and cells may differ in stain uptake (154). Other culture independent methods can be divided into molecular probing without polymerase chain reaction (PCR) and PCR based strategies.

As an aside, indirect analysis of gut microbes may also be considered a culture independent method. Functional measurements, such as short chain fatty acids (fermentation products) or enzyme analysis provide information on the metabolic activities of the microbial community, but used alone yield vague information regarding the groups of bacteria present (154).

Molecular probing methods that do not utilize PCR may be used to detect, enumerate, or assess activity of bacterial groups present in gastrointestinal samples (139, 155).

Molecular probing techniques involve the hybridization of a specific sequence of oligonucleotides ("probe") with a target sequence of bacterial DNA or RNA that is able

to be detected when hybridized to the target. Examples include dot blot assays and in situ hybridizations. In dot blot assays total RNA is isolated from a sample, immobilized onto membranes and then hybridized with universal and specific probes. The assay is able to detect bacteria and provide a relative concentration (abundance) (155). The numbers obtained are relative quantification since cells of different species have different ribosome contents and the ribosome content also varies with growth rate (156).

Whole cell in-situ hybridization is commonly coupled with fluorescently labeled probes and appropriated called fluorescent in-situ hybridization (FISH). With FISH, bacterial cells are permeabilised and probed with labeled 16S (or 23S) rRNA targeted oligonucleotides. If the cell's rRNA contains a complementary sequence to the probe, a hybrid is formed and the cell will be visualized. Each active cell typically contains several thousand ribosomes, and the fluorescence can be visualized and enumerated by fluorescent microscopy or flow cytometry (153, 155). When coupled with fluorescent microscopy, whole bacterial cells are fixed onto a slide, and method detection limits are estimated at 10^4 - 10^6 bacteria per gram stool sample (17, 157). Using flow cytometry, whole cell hybridization techniques may have a limit of detection around 10^4 CFU/ml (17). Additional limits of FISH include differences in cell wall permeability and subjectivity in low fluorescent signals (139, 157, 158).

PCR is a molecular technique used to amplify a targeted DNA sequence in an exponential manner. Because PCR exponentially increases DNA sequences, methods are able to

detect sequences in low concentrations (155). PCR, however may be biased from incomplete nucleic acid extraction, variable gene copy numbers, amplification cycle numbers, cell viability, and indiscriminate detection of genes (139, 155, 159). For gut microbiology, PCR based strategies have been developed to profile bacterial communities and detect, identify and/or enumerate bacteria present (139, 153, 155). Using PCR, 16S rRNA genes may be amplified and the resulting amplicon separated based on melting temperature (temperature gradient gel electrophoresis-TGGE) or chemical stability (denaturing gradient gel electrophoresis-DGGE) to generate bacterial community fingerprints (158). PCR-DGGE or TGGE yields a profile of bacterial groups present and although one band is supposed to correspond to one species, co-migration may occur (158). Bands may also be excised and sequenced to identify species (139). Another type of community profiling is terminal restriction fragment length polymorphism (TRFLP). Again, the 16S amplicon is generated but is then digested and the fragments are separated to visualize the community fingerprint (158). Profiles obtained in these methods are representative of the primer used: a universal primer will produce a broad view of the community, while a more specific primer (such as one that targets bifidobacteria) results in a specific community profile. In addition to the biases of PCR, TRFLP encounters additional bias of the restriction enzyme specificity (158). Besides profiling, 16S amplicons may be cloned and sequenced to identify the bacterial community present. Identification using clones and 16S sequencing is laborious and expensive –especially for monitoring a community (139).

By using quantitative PCR (real-time or competitive), quantification of target bacterial DNA or RNA can be achieved. In real-time, a fluorescent system is used to monitor and quantify the accumulation of amplicons during PCR. In theory, the fluorescence signal is proportional to the accumulation of amplicons, and by relating the fluorescent intensity with that of known amounts of target DNA, an extrapolation of the initial quantity of DNA (or RNA in the case of reverse transcriptase) is possible (158). In addition to PCR biases, the fluorescent dye may be non-specific and different methods of standard curve generation may affect the outcomes (158).

To correct for sample-to-sample variation resulting from PCR inhibitors or extensive background DNA, competitive qPCR utilizes a co-amplified internal standard to calculate the ratios of target amplicons to competitor amplicons (155, 158).

Other PCR based methods include microarray or membrane array techniques. For this, multiple 16S rDNA probes targeting a panel of fecal bacteria species (or specific genes) are pre-immobilized on a surface. The microbial genetic material of the sample to be tested is amplified, added to the surface to hybridize with the probes present, and then visualized to detect and/or estimate the relative abundance of bacteria present (139).

Most recently, techniques have been developed to examine the gut environment via “meta-omics” approaches. In a metagenomic approach, the genetic material of all bacteria present in a fecal sample is extracted and either cloned and sequenced or directly

sequenced to characterize and quantify the bacterial taxa present (158, 160, 161). Instead of determining the microbial genes present, meta-transcriptomics inventories the genes that are being expressed by isolating the transcripts (RNA) of a sample, synthesizing the complimentary DNA and then proceeding as in the metagenomic approach (158).

Transcriptomics provides information about actively expressed genes to determine functional properties of the gut population (162). Both methods are limited by nucleic acid extraction techniques, cloning and/or sequencing and the difficulty of linking genetic sequences to microbes (158).

Few studies on healthy subjects consuming enteral nutrition have measured gut bacteria with any method. In a study on 10 healthy subjects, total fecal bacteria (measured via FISH) was significantly decreased on both enteral formulas (fiber fortified or fiber free) compared to habitual diet (48). Furthermore, between enteral formulas, total bacteria were increased on the fructo-oligosaccharide and pea fiber containing formula compared to the no fiber formula. Bifidobacteria was significantly increased on fiber formula compared to both fiber free formula and habitual diet. Clostridia were decreased on fiber formula compared to habitual diet, but not fiber free formula. No differences were found between any diet for populations of *Bacteroides* (48).

Another study comparing patients on enteral nutrition to healthy controls, bifidobacteria levels were 10 fold lower as measured by quantitative PCR in patients on enteral nutrition

regardless of receiving fiber free or fiber enriched (oat, soy, gum acacia, carboxymethylcellulose and fructo-oligosaccharides) formula (129).

Fructo-oligosaccharides and inulin

Fructo-oligosaccharides (FOS) and inulin are “inulin type fructans” (ITF). Fructans can also be levan or graminan types which are primarily found in microbes or grasses, respectively. The various fructans are distinguished based on the glycosidic linkages present between the fructose residues and ITF are predominately linear molecules with β -(2-1) fructosyl-fructose glycosidic bonds (163). ITF can be extracted from plants (the predominant commercial inulin originates from chicory root) or synthesized from sucrose. Inulin extracted from chicory root yields fructans with varying chain lengths; fructans range in degree of polymerization (DP) of 2-60 with an average of 12. Chicory root fructans are generally a terminal glucose molecule linked to a fructose with “n” number of fructose units (“GF_n type”-see Figure 1-1) (24).

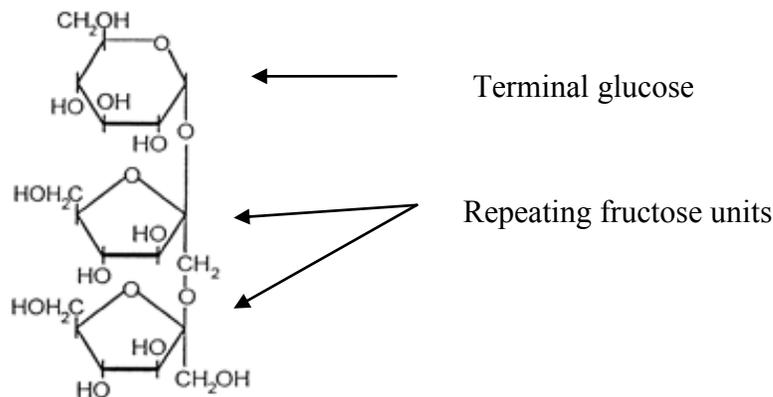


Figure 1-1. Basic GF_n type fructan structure (163)

Chicory-inulin extracts can undergo physical separation and/or enzymatic treatments to yield a variety of products (13, 24). High molecular weight inulin can refer to physically purified inulin extracts that contain polysaccharides with a DP 10-60 and an average of 25 (24). Partial enzymatic hydrolysis of inulin can yield lower molecular weight versions, generally termed oligofructose (OF) with a DP 2-8 and an average of 4 (24). Enzymatic hydrolysis products of inulin can be composed of homopolymers of fructose with “m” number of fructose units (“Fm” type) or GF_n (163). ITF synthesized from sucrose are generally termed fructo-oligosaccharides (FOS) and have a DP 2-8 with an average of 3-6 (24). ITF synthesized from sucrose can also be referred to as “neosugar” (2). Sucrose derived FOSs are largely composed of a mixture of three oligosaccharides of the GF_n type, i.e., 1-kestose (GF₂), 1-nystose (GF₃) and 1^F-fructofuranosyl nystose (GF₄) (164). Because of the low molecular weight of the sucrose derived products, they are also frequently referred to as short-chain FOS or sc-FOS (164). Since there is no official definition for ITF compounds, nomenclature is not consistent in the literature and some consider OF and FOS synonymous since both have a DP<10.

Oligofructose and inulin are both highly soluble in water, although inulin is less soluble relative to oligofructose due to chain length. Comparing a “standard” inulin (average DP of 12) to an enzymatic hydrolysis of inulin oligofructose (average DP of 4), the solubility of inulin was 120 g/L and oligofructose was >750 g/L (165). Using a 5% w/w fiber in water solution, standard inulin had a viscosity of 1.6 mPa/s while oligofructose was <1.0

mPa/s (165). Overall, both FOS and inulin are non-viscous, non-digestible (to humans) and do not contribute an objectionable flavor to food systems (163). As mentioned previously, they are primarily obtained from chicory root but also occur naturally (in small amounts) in leeks, asparagus, Jerusalem artichoke, garlic, onion, wheat, banana and oats (164). Inulin and shorter chain oligofructoses are well known to be highly fermentable at a variety of dosages in a variety of models: *in vitro* systems, animal models, and humans (14, 163, 166, 167) with this list representing a small sample of the studies performed. As a whole, fructans generally do not affect stool weight, transit time, fecal SCFA/pH or fecal moisture. Human studies have observed selective stimulation of bifidobacteria when consuming fructans, but increased incidence of gas and bloating is a common side effect. Table 1-1 contains a summary of the effects of fructans on gut function and tolerance in human subjects.

Table 1-1. Effects of fructans on gut function and tolerance in human subjects.

Fiber	Stool weight	Transit time or stool frequency	Fecal short chain fatty acid	Fecal pH	Fecal moisture or consistency	Fecal bacteria	Tolerance
<p>FOS, Inulin</p> <p>-DP_{av} listed if reported</p> <p>-Excludes DP_{av} >12 if defined</p>	<p><i>No increase when supplemented to controlled diet</i></p> <p>-15 g/day inulin (DP_{av} 10) and 15 g/day FOS (DP_{av} 2-6) (168)</p> <p>-15g/day inulin and 15g/day FOS (169)</p> <p>-20g/day inulin, (DP_{av} 9)(170)</p>	<p><i>No difference in transit time compared to controlled diet</i></p> <p>-15 g/day inulin (DP_{av} 10) and 15 g/day FOS (DP_{av} 2-6) (168)</p> <p>-15g/day inulin and 15g/day FOS (169)</p> <p>-20g/day inulin, (DP_{av} 9)(170)</p>	<p><i>No difference in total SCFA compared to controlled diet</i></p> <p>-15g/day inulin and 15g/day FOS (169)</p> <p>-20g/day inulin (DP_{av} 9) (170)</p> <p>-22-34 g/day inulin (DP_{av} 9) (142)</p> <p><i>Difference in individual SCFA compared to control</i></p> <p>-20g/day inulin (DP_{av} 9), higher acetate:propionate ratio (170)</p> <p>-15g/day inulin higher acetic acid (169)</p>	<p><i>No difference compared to habitual diet</i></p> <p>-Controlled diets: 15g/day inulin and 15g/day FOS (169)</p> <p>-Uncontrolled habitual diet: 20 g/day FOS (143)</p>	<p><i>No difference in fecal moisture compared to controlled diet</i></p> <p>-15 g/day inulin (DP_{av} 10) and 15 g/day FOS (DP_{av} 2-6) (168)</p>	<p><i>No difference in total bacteria compared to habitual diet</i></p> <p>-Controlled diets: 22-34 g/day inulin (DP_{av} 9). FISH. (142)</p> <p>-15 g/day inulin (DP_{av} 10) and 15 g/day FOS (DP_{av} 2-6). Culture. (168)</p> <p>-Uncontrolled diet: 20 g/day FOS. Culture. (143)</p> <p><i>Increase in total anaerobes compared to controlled habitual diet</i></p> <p>-20g/day inulin (DP_{av} 9). Culture. (170)</p>	<p><i>Increased gastrointestinal symptoms compared to habitual diet</i></p> <p>-Controlled diets: Bloating and flatulence, 22-34 g/day inulin (DP_{av} 9) (142)</p> <p>Flatulence, 20g/day inulin (DP_{av} 9) (170)</p> <p>Flatulence and abdominal pain (12.5% participants report) flatulence and abdominal pain 15 g/day FOS (DP_{av} 2-6) (168)</p> <p>-Uncontrolled diets:</p> <p>Increased flatulence 20</p>

						<p><i>Differences in groups of bacteria</i></p> <p>-1.2 log₁₀ increase in bifidobacteria, 22-34 g/day inulin (DP_{av} 9). FISH. (142)</p> <p>-Increased lactobacilli, no differences in bifidobacteria, clostridia, Enterobacteriaceae, 20g/day inulin (DP_{av} 9). Culture. (170)</p> <p>-Increased bifidobacteria at 5, 10, 20 g/day FOS. Culture. (143)</p> <p>-Increased bifidobacteria and decreased <i>Bacteroides</i>, clostridia and fusobacteria 15 g/day FOS (DP_{av} 2-6) (168)</p>	<p>g/day FOS (143)</p> <p>Increased gastrointestinal discomfort during 7.8 g/day inulin period (no increase with 5 g) (171)</p>
--	--	--	--	--	--	---	---

Gum acacia

Gum acacia, or gum Arabic, is the “dried exudation obtained from the stems of *A. Senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae)” (172). The gummy exudate is a highly heterogeneous material containing complex branched-chain polysaccharides found as a mixed calcium, magnesium and potassium salt of arabinic acid with small amounts of protein (173, 174). Using hydrophobic affinity chromatography, gum acacia has been separated into three major fractions: 88.4% arabinogalactan (AG) with a low protein content (0.35%) and a molecular mass of 3.8×10^5 Da; 10.4% arabinogalactan-protein complex (AGP) containing 11.8% protein and molecular mass of 1.45×10^6 Da; and 1.2% as a low molecular weight glycoprotein and a molecular mass of 2.5×10^5 Da (175). In general, the polysaccharide backbone is composed of 1,3-linked β -D-galactopyranosyl units with branches of similar galactopyranose units linked to the main chain via 1,6 linkages. Refer to Figure 1-2 for the generic polysaccharide structure of the arabinogalactan portion.

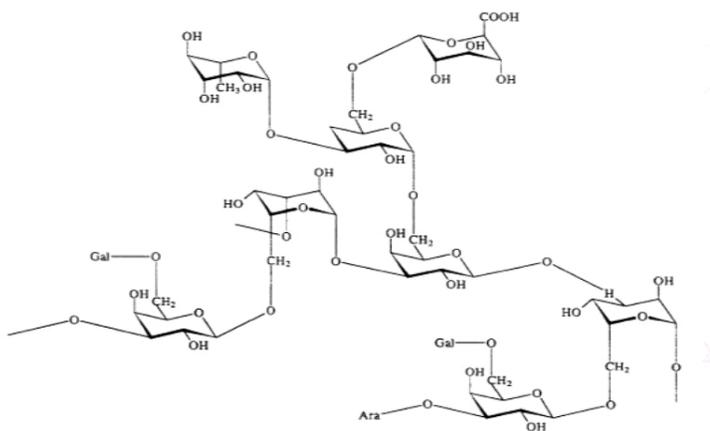


Figure 1-2. Generic polysaccharide structure of arabinogalactan portion of gum acacia (176)

Both the main and side chains contain arabinopyranose, arabinofuranose and rhamnopyranose with glucuronic acid and 4-O-methylglucuronic acids as terminating groups (173, 175). Based on photon correlation spectroscopy, it is thought that the majority of the gum has a highly branched, block-type structure which may explain why very high concentrations (>40%) of the gum are required before aqueous solutions become viscous (175). Refer to Figure 1-3 for the proposed arabinogalactan-protein fraction of gum acacia exhibiting the highly branched, complex structure.

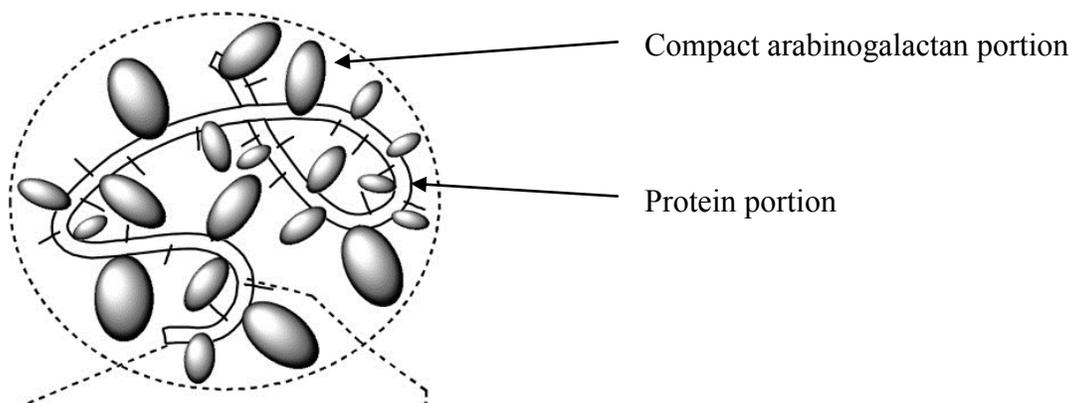


Figure 1-3. Proposed arabinogalactan-protein fraction of gum acacia. Adapted from (177)

Depending on the variety, age, and environment the trees are grown in, the chemical composition of gum acacia can vary (173).

Gum acacia is highly soluble in water (1 g dissolves in 2 ml water) and is well fermented. Using a human fecal slurry *in vitro* system, approximately 95% of the gum acacia was fermented as measured by short chain fatty acid analysis and substrate disappearance (178, 179). Fermentability of gum acacia has also been shown *in vivo* via stool output, SCFA analysis, fecal microflora, and breath hydrogen tests (178, 180). Although data is limited in human studies, gum acacia shows promise in increasing stool weight and fecal moisture. No differences in transit time have been observed. Gum acacia is well tolerated up to doses of 40 g/day and some studies have observed increases in fecal bifidobacteria and lactobacilli. Table 1-2 summarizes the effects of gum acacia on gut function and tolerance in human subjects.

Table 1-2. Effects of gum acacia on gut function and tolerance in human subjects.

Fiber	Stool weight	Transit time or stool frequency	Fecal short chain fatty acid	Fecal pH	Fecal moisture or consistency	Fecal bacteria	Tolerance
Gum acacia	<p><i>Increased stool weight compared to semi-controlled diet</i></p> <p>-15 g/day gum acacia (178)</p>	<p><i>No change in stool frequency compared to habitual diet</i></p> <p>-Semi-controlled diet, 15 g/day gum acacia (178)</p> <p>-Uncontrolled diet, up to 40 g/day gum acacia (181)</p>	<p><i>No human data</i></p> <p><i>In vitro</i></p> <p>-Slowly, but extensively fermented. Less than 2% constituent sugars remain after 48 h fermentation (179)</p>	<p><i>No difference compared to semi-controlled diet</i></p> <p>-15 g/day gum acacia (178)</p>	<p><i>Increased fecal moisture compared to semi-controlled diet</i></p> <p>-15 g/day gum acacia (178)</p>	<p><i>Differences in groups of bacteria</i></p> <p>-Semi-controlled diet, increased bifidobacteria and lactic acid producing bacteria, 15 g/day gum acacia (178)</p> <p>-Uncontrolled diet, increased bifidobacteria and lactobacilli, optimal 10 g/day gum acacia. PCR. (181)</p>	<p><i>No difference in GI symptoms compared to habitual diet</i></p> <p>-Uncontrolled diet, up to 40 g/day gum acacia (181)</p> <p>-Semi-controlled diet, 15 g/day gum acacia (178)</p>

Outer pea fiber

Pea hull fiber or outer pea fiber is obtained from grinding the hulls of the field pea (*Pisum sativum*) (182, 183). The resulting 'high fiber flour' contains approximately 90-95% fiber (as determined by standard AOAC methods), 3-6% protein, 2% ash, and <2% lipids, starch and sugar (183, 184, 185). The amounts of insoluble to soluble fiber vary depending on particle size distribution and heat treatment, but have been approximated at 87% insoluble and 4% soluble in raw pea hulls that have an average size of 500 μm (184). As particle size decreased and with extrusion conditions, the soluble fraction peaked at 15% soluble fiber (184). Pea hulls are primarily composed of cellulose, along with xylose and arabinose-containing polymers and pectic substances (184, 186). The insoluble fraction contains primarily cellulose (80%), xylose (10%), and arabinose (3%) and the soluble fraction primarily uronic acids (77%) i.e. pectin (186). The structure of the primary component cellulose is β (1, 4) linked glucose units. Refer to Figure 1-4 for generic cellulose and pectin structures. Cellulose and pectin are represented since they are found in the highest concentrations in the insoluble and soluble fractions of outer pea hull fiber.

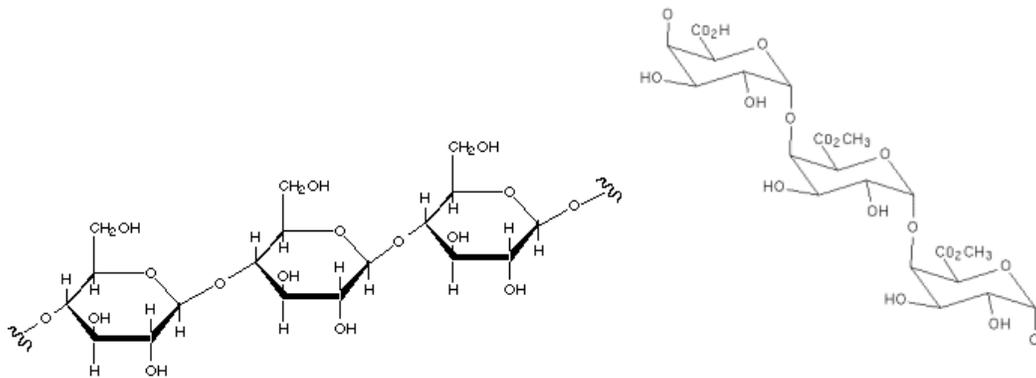


Figure 1-4. Generic structures of cellulose (left) and pectin (right) found in outer pea hull fiber. (9)

Pea hull fiber is a complex mixture of insoluble and soluble fibers. As a whole, approximately 77% of a pea fiber was remaining after a 48 hour fermentation and produced a total of $\sim 1240 \mu\text{mol SCFA/g}$ of substrate as opposed to a highly fermented substrate gum acacia $\sim 9400 \mu\text{mol SCFA/g}$ in an *in vitro* system utilizing human fecal slurries (179). These results were also corroborated in a pig model where both cellulose and xylose portions of the pea fiber had a high resistance to fermentation whereas the pectic portion was readily fermentable as measured by substrate recovery in the feces and SCFA analysis (7). Since a large portion of pea hull fiber is cellulose, other properties such as water binding capacity/hydration may be of importance. In a manuscript comparing relative hydration properties, pea hull fiber (particle size unknown) had a water binding capacity of 5.2 g water/g dry solids (185) with others reporting 6.6 g water/g dry weight (178) relative to cellulose 4.8 g water/g dry weight and wheat fiber 4.4 g water/g dry weight (185). Another study using similar water binding capacity measurements found that pea hulls of 500 μm average size had a binding capacity of 7.1

ml water/g dry weight vs. pea hulls of 80 μm average size had a binding capacity of 4.6 ml water/g dry weight (184). The authors suggest that the smaller particles led to a decreased water binding capacity due to the collapse of the fiber matrix. As far as particulate viscosity, using 8% wt/vol of fiber and water, the viscosity of pea hull fiber was 3.63 mPa/sec at a shear rate of 122/sec while cellulose was 3.65 and wheat fiber 1.54 (particle size not reported) (185).

Data from outer pea hull fiber human feeding studies is extremely limited. From the few studies performed, pea hull fiber shows promise in increasing stool weight, but no effects were seen on transit time. Refer to Table 1-3 for a summary of the effects of pea hull fiber on gut function and tolerance in human subjects.

Table 1-3. Effects of outer pea hull fiber on gut function and tolerance in human subjects.

Fiber	Stool weight	Transit time or stool frequency	Fecal short chain fatty acid	Fecal pH	Fecal moisture or consistency	Fecal bacteria	Tolerance
Pea hull fiber	<p><i>Increased stool weight compared to habitual diet</i></p> <p>-30g/day pea hull fiber (187)</p>	<p><i>No difference in transit time compared to habitual diet</i></p> <p>-30g/day pea hull fiber (187)</p> <p><i>No change in colonic motility by high amplitude propagated contractions</i></p> <p>-15 g/day pea fiber (188)</p> <p><i>Increased bowel frequency in elderly residents compared to habitual diet</i></p> <p>-4 g/day pea fiber in 16 g total (182)</p>	<p><i>No human data</i></p> <p><i>In vitro</i></p> <p>-Poorly fermented, 77% of fiber remaining after 48 h fermentation (179)</p> <p>-Slowly and poorly fermented, 2% utilized after 6 h, and 22% at 24 (187)</p>	<p><i>No human data</i></p> <p>Based on poor fermentability would not expect acid to change.</p>	<p><i>No human data</i></p> <p>Higher water binding capacity than wheat bran (6.6. g water/g dry weight vs. 3.8) (187)</p>	<p><i>No human data</i></p> <p>Based on poor fermentability would not expect bacteria to change.</p>	<p><i>No differences in diet acceptability compared to habitual diet</i></p> <p>-4 g/day pea fiber in 16 g total (182)</p>

Rationale for a Fiber Blend

Dietary fiber includes a wide variety of compounds and based on physicochemical properties, each provides a different physiological effect. By supplementing enteral nutrition with a blend of fibers, the resulting fiber profile more closely resembles the mixed fibers supplied from a normal diet (81). Additionally, by utilizing a fiber blend that contains both insoluble and well fermented soluble fibers, the beneficial effects associated with fecal bulking and fiber fermentation will theoretically be achieved (49). Soluble fibers with known prebiotic properties provide additional benefits by stimulating the growth of health promoting bacteria. Although some well fermented fibers cause gas and bloating in individuals, the use of a blend of fibers with a range of complexity (and thus speed of fermentation), may attenuate this. Finally, by incorporating fibers with varying fermentation profiles, the health benefits associated with fermentation (such as short chain fatty acid production) will theoretically be prolonged throughout the large intestine.

Inulin and fructo-oligosaccharides are well fermented, non-viscous, soluble fibers. Gas and bloating, however, are common side effects of fructan consumption in doses above 5-8 g (171). As a prebiotic, fructans are the most well researched fibers. Not as well studied, but showing promise as a prebiotic is gum acacia. Gum acacia is fermented more slowly than FOS and inulin and when blended with FOS has been shown to increase the tolerance compared to inulin alone (189). As a gum, acacia is unique since it is non-viscous in solutions up to 40% (9, 175). Outer pea hull fiber provides insoluble fiber that has *in vitro* water binding capabilities greater than that of wheat bran, the gold standard in fecal bulking (187). Additionally, it provides insoluble fiber without adding color, flavor, gluten, or soy to the product.

The novel fiber blend contains a 50:50 ratio of insoluble:soluble fiber containing pea hull fiber, inulin, fructo-oligosaccharides, and gum acacia.

CHAPTER TWO

Healthy subjects experience bowel changes on enteral diets: addition of a fiber blend attenuates stool weight and gut bacteria decreases without changes in gas¹

Executive Summary

Background: Tube-fed patients frequently suffer from abnormal bowel function which affects intestinal bacteria and quality of life. Dietary fiber affects laxation and can be fermented by gut bacteria to produce metabolites which influence gut health and fecal moisture.

Objective: The aim of this study was to compare the effects of a fiber blend fortified enteral formula (FB, 15 g/L) and a fiber-free formula (FF), and habitual diet on bowel function, fecal bacteria and quality of life.

Design: In a randomized, double-blind, crossover design, 20 healthy subjects consumed both FF and FB for 14 d with a 4-wk washout. A 5-day fecal collection was used to assess stool output, whole gut transit time (WGTT), total bacteria, bifidobacteria, lactobacilli, clostridia, and bacteroides. Bacteria were quantified via fluorescence in-situ hybridization. Subject gastrointestinal quality of life index (GIQLI) and side effects were also measured.

Results: On formula diets, 5-day fecal output decreased by more than 55% from habitual diet, but was 38% higher on FB than FF ($p=0.0321$). WGTT was approximately 1.5 times longer on formula diets than habitual diet ($p<0.0004$). Total bacteria declined from habitual diet on FF ($p<0.004$), but not on FB. Numbers of bifidobacteria and lactobacilli declined from habitual diet on both formula diets, but bifidobacteria was higher on FB compared to FF ($p<0.0001$). Bacteroides and

clostridia numbers did not change between diets. GIQLI and incidence of gas symptoms did not differ between formulas.

Conclusions: Addition of a fiber blend moderated decreases in stool weight and gut bacteria observed in healthy subjects consuming FF. These results support adding mixed fiber sources to enteral nutrition products if no contraindication exists.

Introduction

Enteral nutrition (EN), or enteral tube feeding, is an effective means of nutritional support for patients who do not or cannot eat adequately for a variety of medical reasons (39). Administered in both hospital and home settings, EN use is frequently associated with gastrointestinal (GI) symptoms including diarrhea and constipation (29, 39). EN associated GI intolerance affects patient quality of life and is a complex phenomenon affected by situation (drug therapies, concurrent infections, etc.), but the colonic bacteria are also involved (29, 190). Gut bacteria are involved in bowel health as a normal flora provides competitive exclusion for potentially pathogenic organisms (e.g. *C.difficile*) and ferments carbohydrates and proteins reaching the colon to produce short chain fatty acids (SCFA) (17, 47). SCFAs affect colonic water absorption and decrease fecal pH which affects bacterial growth in the colon (45, 56). Disruption of gut bacteria homeostasis (dysbiosis) is thought to contribute to many bowel ailments and previous studies have shown changes in gut bacteria while on enteral nutrition (48, 129, 190, 191).

A wide range of compounds are considered dietary fiber, and depending on physiochemical properties, have different physiological effects. Some fibers increase fecal bulk and/or hold water which facilitates colonic motor activity and promotes normal laxation (10). Others are fermented in the distal colon to produce SCFAs

which through their effects on colonic fluid absorption, may prevent or ameliorate diarrhea-like symptoms (45). By using a blend of fibers to fortify EN, the physiological effects of fiber such as bowel function moderation, SCFA production and healthy gut microbiota maintenance are maximized (192). Blends may also be used to minimize the contraindications of gas and bloating (192). Furthermore, adding fibers that selectively stimulate the growth and activity of bacteria which improve host health (“prebiotics”) may help to attenuate the growth of potentially harmful bacteria (14). Most prebiotic studies have focused on increases in bifidobacteria (144).

The aim of this study was to compare the effects of a fiber blend fortified enteral formula (FB), a fiber-free formula (FF) and habitual diet on bowel function, fecal bacteria and quality of life in healthy subjects. The primary objective of the trial was to compare the stool weight of the subjects consuming these diets. The secondary objectives were to evaluate the effects of the diets on modification of the major groups of gut bacteria, whole-gut transit time (WGTT), Bristol stool form, fecal moisture, fecal pH and subjective tolerance.

Subjects and Methods

Subjects. Healthy men and women were recruited on the University of Minnesota campus to a prospective, randomized, double-blind, crossover trial. Exclusion criteria included: age <18 or >75, BMI <23 or >29, any self-reported disease, use of medication (except contraception and certain over the counter medications), smoking, >2 h exercise per week, consumption of >20 g fiber/day, pregnancy or lactation, use of laxatives, use of antibiotics in the past 6 mo., use of pre or probiotic supplements in

the past 2 mo., use of probiotic foods in the past month, participation in another clinical trial during the last 4 weeks prior to the beginning of the study, and known allergy or sensitivity to formula ingredients. Subject eligibility was determined via questionnaire and an initial visit to obtain physical measurements. Refer to *Appendix B* for the eligibility screening questionnaire. Habitual diet daily fiber intake was screened via a fruit, vegetable, and fiber frequency questionnaire and measured using 2 d diet records entered into Nutrition Data System for Research (University of Minnesota, Version 2011) (193). A sample size calculation indicated that 20 subjects were required to detect a 21 g/day difference in fecal weight (80% power, 0.05 significance) based on a previous enteral study (48). Refer to *Appendix A* for the sample size/power calculation. Written informed consent was obtained from each subject and the study was approved by the University of Minnesota Institutional Review Board for Human Research Protection Program and registered at clinicaltrials.gov. The informed consent form is listed in *Appendix C*.

Subject Protocol. During the study, subjects were asked to maintain their normal activity level and refrain from any exclusion criteria behaviors (laxatives, prebiotic/probiotic foods, etc.). Approved over the counter medication was recorded in subject records. Subjects consecutively completed a run-in period of 14 days on habitual diet, 14 days on enteral formula diet 1, 28 days on habitual diet (washout phase), and enteral formula diet 2 for 14 days. Enteral formula diets were assigned in random order and the two treatment sequences (FF-FB or FB-FF) were given in equal numbers, stratified by gender. See *Appendix F* for the randomization schedule. Enteral formula was orally consumed as the sole source of nutrition with no other dietary intake except *ad libitum* consumption of water and coffee, tea and non-

caloric/no-fiber containing beverages in similar amounts consumed during habitual diet.

Subject weight was recorded at the start and end of each period. Gastrointestinal quality of life was measured at the end of each period. Subjects recorded 2 day diet records during habitual diet periods and 14 days during the enteral formula periods. A capsule containing 20 radioopaque pellets for the measurement of transit time was consumed on Day 8 of each period. Subjects performed a 5-day total fecal collection the last five days of each period. For the fecal sampling, individuals collected every stool produced during the collection period and recorded the date and time of each stool. The last stool sample from each fecal collection was collected anaerobically by immediately placing it into a Bitran Speciman Storage Bag (Fisher Scientific, Pittsburgh PA) which contained an Anaeropack Anaero (Mitsubishi Gas Chemical America, New York NY). The anaerobically stored stool was placed on ice in an insulated cooler, delivered to the laboratory within 1 h of defecation, and processed immediately to maintain microbial viability and ensure standardized sample preparation and handling.

Enteral formula. Enteral formulas were nutritionally complete and identical in composition except for fiber content. Base formula contained 1500 kcal/L, 67.6 g protein/L, 64.8 g fat/L and 168 g carbohydrate/L. The fiber containing formula was the base formula supplemented with 15 g/L of fiber blend. The fiber blend contained a 50:50 insoluble: soluble mixture of short-chain fructo-oligosaccharides and inulin (Beneo, Morris Plains NJ), pea hull fiber (Nutri-Pea Limited, Manitoba Canada), and

gum acacia (Nexira, Somerville NJ). Refer to *Appendix D* for the complete nutrition information and ingredient list for the formulas.

Subjects received amounts of enteral formula based on their total energy expenditure (TEE). TEE was calculated using the Harris-Benedict equation for basal metabolic rate and adjusting with physical activity factors (194). Formula was provided in identically labeled 250 ml cans with the exception of product code. Formulas were assigned four different codes to ensure that both subjects and researchers were unaware of the formula type. All formula was vanilla flavored. Subjects were provided with excess formula of their prescription and were withdrawn from the study if the intake was below 75% of the target volume for two consecutive days. Formula consumption compliance was assessed via diet records, and subjects were not informed of the procedure to ensure accuracy.

Fecal collection and stool sample preparation. Five day fecal collections delivered to the lab were weighed, assessed for Bristol type and frozen at -20°C (91). The anaerobically packaged stool was weighed, assessed for Bristol type, mixed, and diluted 1:10 in phosphate buffered saline (PBS, 0.1 M, pH 7.2) using a 10 g aliquot. The fecal:PBS mixture was homogenized in a stomacher for 2 min and the slurry was aliquoted for fecal bacteria measurements. The remaining sample was frozen at -80°C until subsequent testing.

Mean transit time, stool frequency, and Bristol stool form. Subjects consumed a single dose of 20 radioopaque (polythene) pellets placed into a size 00 food grade gelatin capsule and recorded the time consumed. Subsequent stool samples were then

collected for 5 days. The stool samples produced were x-rayed to determine pellet content. Mean transit time was calculated as the sum of the product of the number of pellets in a stool and the time of excretion (of that stool) which was divided by the total number of pellets recovered (as determined by x-ray) (82). Stool frequency was the number of stools produced per 5 day collection period. Stool form was visually determined by comparison to the Bristol scale pictorial and verbal descriptors. The Bristol scale includes seven types of formed-ness ranging from 1 (most formed-separate hard lumps) to 7 (least formed-watery, no solid pieces) (91). See *Appendix G* for the Bristol stool chart.

Fecal moisture. Anaerobically collected fecal samples were thawed and re-mixed. Aliquots of 2-5 g were measured in triplicate and spread into a thin layer in pre-dried (24 h at 105°C) and pre-weighed 64 mm diameter aluminum pans. Prepared stool samples were placed in a 105°C for 24-48 h, removed and cooled under desiccant. Stool weight loss was recorded and used to determine moisture content (98, 195).

Enumeration of fecal bacterial populations by fluorescence in situ hybridization. Fecal sample preparation and fixation was performed as described by Costabile (121). Triplicate 375 µL aliquots of the 1:10 fecal slurry were added to 1125 µL of 4% paraformaldehyde (w/v, pH 7.2) and fixed at 4°C for 4-8 h. Samples were washed with 0.2 µm filtered PBS (twice), re-suspended in 300 µL of 1:1 (v/v) PBS:ethanol mixture, and stored at -80°C until analysis.

Hybridization was performed using conditions outlined in probeBase and as described by Martin-Pelaez (196, 197). Probes were used to target total bacteria (EUB338 5'-

GCT GCC TCC CGT AGG AGT-3') (198), bifidobacteria (BIF164 5'-CAT CCG GCA TTA CCA CCC-3') (199), lactobacilli-enterococci (LAB158 5'-GGT ATT AGC AYC TGT TTC CA-3' (200), bacteroides (BAC303 5'-CCA ATG TGG GGG ACC TT-3' (201) and the *Clostridium coccooides-Eubacterium rectale* group (EREC482 5'-GCT TCT TAG TCA RGT ACC G-3' (202). All probes were cyanine-3 (Cy-3) labeled and synthesized by Sigma Aldrich. Fixed fecal samples were thawed on ice, and 20 μ L of appropriately diluted sample was pipetted onto Teflon and poly-L-lysine-coated, six-well (10 mm diameter each) slides (Tekdon Inc., Myakka City, FL USA). For lactobacilli analysis, slides were treated with 50 μ L of lysozyme (2 mg/ml in 1mM Tris/HCl) at 37°C for 30 min and rinsed with water after drying to permeabilize cells. All samples were dehydrated in a series of ethanol concentrations for 3 min each (50%, 80%, 96%). A probe/hybridization buffer mixture (5 ng/ μ L) was applied to each well and the hybridization was performed in a sealed, humidified chamber for 4 h. Slides were washed in 50 ml of wash buffer containing 50 ng/ μ L of 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) for 15 min, and then 2-3 s in ice-cold water. Slides were dried using compressed air. Five μ L of polyvinyl alcohol mounting medium with 1,4-diazabicyclo(2-2-2)octane antifade was added to each well. Slides were stored at 4°C in the dark for a maximum of 3 days until being counted.

Slides were evaluated using a Nikon E800 Hyperspectral microscope equipped with a XCite mercury lamp and a Photometrics HQ2 charge-coupled device camera. The Cy-3 labeled probe and DAPI stained slides were visualized using a filter cube; excitation filter 555-585 nm and emission filter 590-650 nm (Cy-3) and excitation filter 385-415 nm and emission filter 450-470 nm (DAPI). Bacterial populations were

quantified as in Martin-Pelaez (196). Total bacteria were counted using digitally captured images. All other groups of bacteria were counted without imaging.

Gastrointestinal symptoms and quality of life. Gastrointestinal quality of life index (GIQLI) was measured using a validated 36 question questionnaire that measures physical well-being, mental well-being, digestion and defecation (132). The GIQLI also addresses general gastrointestinal symptoms such as diarrhea, constipation, abdominal pain and nausea. For each question the most desirable option is 4 points with the least desirable option assigned a 0. The GIQLI is the overall sum of the points with a maximum score of 144. To calculate separate scores for gas/bloating, constipation, and bowel urgency, scores of questions relating to those areas were summed separately. Refer to *Appendix E* for the complete GIQLI.

Statistical analysis

The two sequence groups (FB-FF vs FF-FB), dietary fiber and demographic characteristics were compared by ANOVA. Responses to the treatments and habitual diet were compared by linear contrasts in mixed-effects linear models, where period (1-4), gender and treatment were fixed effects and subject (intercept) was a random effect to model the within-subject correlation for repeated measurements. The assumptions of equal carryover and no period-treatment interaction were tested for each outcome. Analysis of bacterial counts was done and reported on the \log_{10} scale. Data collected during the initial habitual diet and washout periods were combined and adjusted for period effects and used to report habitual diet values. Data analysis was performed in SAS (Version 9.2) and differences were considered significant at $P < 0.05$.

Results

Cohort. Twenty-four healthy subjects were enrolled in the study. Twenty subjects (10 men, 10 women) completed the study and 4 dropped out (2 men, 2 women) within the first 2 days of the first liquid diet and did not complete the GIQLI for the liquid diet. One man dropped because he was unable to consume enteral formula as a sole source of nutrition and another because he found his breath to be malodorous when on the formula. One woman dropped due to dislike of the formula and another was unable to fully comply with study procedures.

We report results only for the 20 who completed the study. Subject baseline demographic characteristics and diet information are listed in **Table 2-1**. Baseline characteristics of the two sequence groups were compared, and no statistical differences were found.

Enteral formula intake and compliance. Enteral formula prescription was based on individual energy needs and actual intake was calculated from subject diet records during the formula periods. Subjects were considered to be compliant if they consumed at least 75% of their energy needs, and did not consume below 75% for 2 consecutive days. All subjects who completed the study were compliant. There was no overall difference in reported formula intake between fiber-free or fiber blend containing formula. Table 2-1 lists the total and gender specific formula energy and fiber intake. Most subjects lost weight during each 14 day enteral formula period. There was no difference in weight loss between subjects consuming fiber free formula (1.2 kg +/- 0.3 SE) and fiber blend formula (1.7 kg +/- 0.3 SE, $p = 0.21$).

Fecal output and characteristics. Subjects on FB had a 38% higher mean 5-day fecal output compared to FF ($p=0.0321$, **Table 2-2**). Compared to habitual diet, mean 5-day fecal output was reduced by 55% on FB and by 67% on FF (both $p<0.0001$). Gender was a significant predictor for stool weight and values reported are gender adjusted. Whole gut mean transit time was approximately 1.5 times longer during formula diets compared to habitual diet (both $p<0.001$, Table 2-2), but was not significantly different between the formulas ($p=0.2570$). Stool frequency (stools/5 d) was higher on habitual diet than the formula diets (both $p<0.001$) and there was no difference between formulas. Fecal moisture values were 4% (wet basis) higher on FF than both habitual diet and FB (both $p<0.0001$, Table 2-2), which did not differ. Mean Bristol stool scores indicated that the stools produced on FF were least formed, intermediate on FB and most formed on habitual diet (all $p<0.0001$).

Overall gastrointestinal quality of life scores for subjects consuming formula diets were the same between formulas ($p=0.8573$), but lower during habitual diet (both $p<0.001$, **Table 2-3**). Incidence of constipation and gas/bloating symptoms were not different between formulas or between either formula and habitual diet. Incidence of symptoms related to increased bowel urgency/uncontrolled stool was lowest on habitual diet, intermediate on FB, and highest on FF (all $p<0.02$).

Total bacteria declined from habitual diet on FF but not on FB (**Table 2-4**). Numbers of bifidobacteria and lactobacilli declined on both FF and FB, and the decline was significantly greater in FF. Numbers of bacteroides did not change, but there was a trend toward increased numbers of clostridia on FF vs. habitual diet ($p=0.0528$).

Finally, mean fecal pH for both formulas was 7.5; higher than habitual diet pH 6.5 (p<0.0001, Table 2-4).

Discussion

Subjects on FB had increased 5-day stool weight (total fecal output) compared to FF even though FF had higher moisture content. Whelan et al measured similar increases in fecal weight in healthy subjects consuming standard and fiber fortified enteral formula (48). Both formulas, however, reduced stool weight to less than 50% of habitual diet levels despite receiving a mean fiber dose of 18.9 g/day on FB formula and 16.0 g/day on habitual diet. While fecal weight is affected by fiber intake, the water the fiber binds, fecal microbe weight, and short-chain fatty acid concentration, other factors such as personality have also been associated with fecal output (49, 58, 59). Fecal output reductions occurring when consuming standard and/or fiber containing enteral formula compared to habitual diet have also been documented in other studies (48, 79, 81). Slavin et al observed that 60 g/day of soy fiber added to enteral nutrition was necessary to increase stool weight to self-selected diet levels (79). Additional factors contributing to the marked decrease in stool weight compared to habitual diet may be decreased particle size of the isolated fibers (10), naturally occurring FOS and resistant starch not measured by typical fiber analytical methods (203), and fiber type/proportions in the blend (different fibers affect fecal weight differently) (58).

Whole gut transit time influences water and SCFA absorption from stool and previous human feeding studies have observed inverse correlations with stool weight and transit time (61). In general, the longer feces remain in the large intestine, the more

water is absorbed which may lead to a highly formed stool (49, 197). In this study, mean transit time was approximately 1.5 times longer for subjects consuming formula diets compared to habitual diets. Silk et al observed significantly longer WGTT on fiber free formula than habitual, but saw no difference between habitual and fiber containing formula. Fiber doses on that fiber containing formula, however, averaged 30 g/day (87).

Fecal moisture values were higher on FF compared to FB possibly due to decreased fermentation and SCFA production by the gut bacteria. SCFA increase sodium and water uptake in the colon and may lead to a decreased moisture content (as seen in the fiber blend formula) (45, 111). Moisture values were similar for FB and habitual diet. Bristol stool formed-ness followed similar trends as fecal moisture; habitual diet and FB were more normally formed while FF was less formed and trended toward diarrhea. FF had the highest moisture content and least formed stool which is consistent that individuals restricted to fiber free enteral nutrition have more frequent diarrhea (29, 44). Furthermore, Bowling et al demonstrated that high energy density enteral nutrition causes net secretion in the distal colon (204).

Overall GIQLI was decreased from habitual diet on both formula diets, which wasn't surprising due to the high level of dietary restriction and social impact of being on a liquid diet. Wierdsma et al also observed decreased GIQLI for patients on enteral nutrition versus healthy controls (129). GIQLI was not different between formulas in this study, although Wierdsma found that patients receiving fiber free formula had decreased GIQLI versus fiber containing formula (129).

Symptoms related to constipation and gas/bloating did not differ between any diets. Similar results were observed by Whelan et al that found no differences between nausea, bloating and flatulence for habitual, fiber free or fiber containing formula (48). Since no increases in gas/bloating incidence were found, it suggests that the constituents of the fiber blend are fermented in a manner that is well tolerated in individuals. Greater gas volume is perceived more in the proximal bowel compared to the distal colon, so rapidly fermented fibers would be expected to cause more gastrointestinal intolerance than those that are more slowly fermented (205). Using a blend of fibers with various chain lengths may attenuate gas production and this has been demonstrated in previous *in vitro* studies (128, 206).

Formula diets appear to increase the incidence symptoms of bowel urgency, although the FB had less than the FF. Symptoms related to bowel urgency were highest on FF, intermediate on FB and lowest on habitual. This is in agreement with other studies that suggest individuals on enteral nutrition frequently have abnormal bowel function such as diarrhea (29, 44, 204). Although diarrhea is not well defined in the literature, urgent bowel movements are typically correlated with diarrhea.

Numbers of total bacteria were significantly reduced in individuals consuming FF compared to habitual diet which may be due to decreased substrates for bacterial metabolism and has also been measured in a similar enteral nutrition study (48).

Between formulas, the numbers of bacteria were higher on FB (although not significant), and other studies have observed more bacteria in individuals consuming fiber formula (48, 123).

The most marked changes in bacterial populations were observed in the bifidobacteria. Compared to habitual diet, bifidobacteria were reduced by 2 log₁₀ when consuming FF but were partially restored (increased) when consuming FB. Again, prebiotics, such as FOS and inulin are known to selectively stimulate the growth of bifidobacteria (14), and other enteral studies have observed decreases in bifidobacteria on fiber free formula (48, 129). Although prebiotics are also suggested to increase lactobacilli levels, this study found no differences in populations between the formulas and a reduction in numbers from habitual diet. Clostridia increased from habitual diet when on FF, but the changes were not significant and no other differences were measured. Bacteroides populations were not different among any treatments and this was also observed by Whelan et al (48).

Finally, pH was higher on both formula diets vs. habitual diet. This was not surprising since total bacterial populations (and thus fermentation) were decreased on formula, but it was hypothesized that the fiber blend would have a somewhat decreased pH compared to fiber free as seen in other studies (48).

Conclusions

Significant bowel function changes occur when healthy subjects transition to an exclusive enteral diet. Subjects consuming a formula supplemented with a fiber blend, however, had increased fecal weight, a moderately formed stool and less negative symptoms related to bowel urgency/uncontrolled stool without a difference in gas/bloating symptoms compared to fiber-free formula. Overall GIQLI was not different between the test formulas. These results support the addition of mixed fiber sources to enteral products.

¹The authors wish to thank Nestle Health Science S.A. (Lutry, Switzerland) for supplying the experimental diets. We would also like to thank Prof. Glenn Gibson and Dr. Adele Costabile from the Department of Food and Nutritional Sciences, The University of Reading UK, for training us on the FISH method.

The manuscript “Healthy subjects experience bowel changes on enteral diets; addition of a fiber blend attenuates stool weight and gut bacteria decreases without changes in gas” by Katie Koecher (KK), William Thomas (WT), and Joanne Slavin (JS) was submitted to a peer reviewed journal and is currently under review. JS, WT, and KK designed research; KK conducted research; WT and KK analyzed data; KK, WT, and JS wrote the paper; JS had primary responsibility for final content. All authors read and approved the manuscript.

Table 2-1. Subject baseline demographics and diet characteristics of 20 subjects consuming fiber free formula, fiber blend formula, and habitual diet.

	Age (y)	Body Mass Index (kg/m²)	Habitual Diet Energy Intake¹ (kcal/d)	Formula Diets Energy Intake² (kcal/d)	Habitual Diet Fiber Intake¹ (g/d)	Formula Diet Fiber Intake² (g/d)
Women	25.7	25.3	1914	1615	16.8	16.2
(n=10)	(12.4)	(2.8)	(580) ^a	(204)	(4.2)	(1.9) ^b
Men	25.8	26.2	2223	2225	15.1	21.6
(n=10)	(7.3)	(2.3)	(599)	(347) ^c	(6.5)	(3.8) ^d
Total	25.8	25.8	2069	1928	16.0	18.9
(n=20)	(9.9)	(2.5)	(594)	(417)	(5.3)	(4.0)

Values are means (standard deviation)

¹Habitual diet energy and fiber intake are reported as an average of the 2 day diet records from the baseline and washout periods. Values were generated using the Nutrition Data System for Research (Version 2011).

²Formula diet energy intake is reported as an average of the 14 day diet records combined over FF and FB. Formula fiber intake is reported as an average of the 14 day diet records from FB.

^aIn women, energy intake on habitual diet is different from formula diet (p=0.009).

^bFiber intake on formula diet is different between men and women (p=0.0009).

^cEnergy intake on formula diets is different between men and women (p<0.0001).

^dIn men, fiber intake on habitual diet is different from formula diet (p=0.0153).

Table 2-2. Fecal output, stool characteristics and mean transit time of 20 subjects consuming fiber free formula, fiber blend formula, and habitual diet.

	Habitual diet	Fiber free formula	Fiber blend formula
Fecal output¹ (g/5 d)	825 (56) ^a	267 (33) ^b	368 (33) ^c
Mean transit time (h)	59 (7) ^a	101 (10) ^b n=19	87 (10) ^b n=19
Stool frequency (n stools/5 d)	5.3 (0.3) ^a	3.9 (0.3) ^b	3.7 (0.3) ^b
Fecal Moisture (% wet basis)	74.5 (0.9) ^a	78.3 (1.4) ^b	73.6 (1.4) ^a
Bristol stool score²	3.2 (0.2) ^a	5.1 (0.3) ^b	4.2 (0.3) ^c

Values are means (standard error). Groups were compared in each row. Means with no letters in common were significantly different ($p < 0.05$); means sharing a letter were not significantly different.

¹Fecal output values were adjusted for gender.

²Bristol scores range from 1 (most formed, separate hard lumps) to 7 (least formed, watery no solid pieces).

Table 2-3. Gastrointestinal quality of life index (GIQLI) and incidence of gastrointestinal symptoms of 20 subjects consuming fiber free formula, fiber blend formula and habitual diet.

	Habitual diet	Fiber free formula	Fiber blend formula
GIQLI¹	129.9 (1.9) ^a	123.5 (2.4) ^b	123.2 (2.4) ^b
Gas-bloat score¹	17.3 (0.4) ^a	17.3 (0.4) ^a	18.0 (0.4) ^a
Bowel urgency score¹	15.5 (0.3) ^a	13.1. (0.6) ^b	14.4 (0.6) ^c
Constipation score¹	3.8 (0.1) ^a	3.7 (0.1) ^a	3.6 (0.1) ^a

Values are means (standard error). Groups were compared in each row. Means with no letters in common were significantly different ($p < 0.05$); means sharing a letter were not significantly different.

¹Higher scores indicate more favorable outcomes; symptoms with lower scores occur at a higher incidence.

Table 2-4. Log₁₀ fecal bacteria populations and pH of 20 subjects consuming fiber free formula, fiber blend formula and habitual diet.

	Habitual diet	Fiber free formula	Fiber blend formula
Total bacteria¹	11.58 (0.08) ^a	11.28 (0.10) ^b n=19	11.38 (0.10) ^{ab} n=19
Bifidobacteria¹	7.29 (0.16) ^a	5.07 (0.19) ^b	6.87 (0.19) ^c
Lactobacilli¹	6.49 (0.12) ^a n=19	6.10 (0.15) ^b n=18	6.11 (0.15) ^b n=18
Clostridia¹	8.36 (0.06) ^a	8.52 (0.07) ^a	8.42 (0.07) ^a
Bacteroides¹	8.51(0.06) ^a	8.40 (0.07) ^a	8.50 (0.07) ^a
pH	6.52 (0.07) ^a	7.49 (0.09) ^b	7.54 (0.09) ^b

¹log₁₀ cells/g stool.

Values are means (standard error). Groups were compared in each row. Means with no letters in common were significantly different ($p < 0.05$); means sharing a letter were not significantly different.

CHAPTER THREE

Coupling fermentation results from a 24 h batch *in vitro* system with fecal measurements and subjective tolerance from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia and pea fiber¹

Executive Summary

Fiber fermentation in the gut increases short chain fatty acid (SCFA) and gas production. Patients receiving fiber-free enteral nutrition frequently suffer from abnormal bowel function and may benefit from the addition of fiber. *In vitro* systems estimate fermentation and tolerance of fiber while human studies are the gold standard in nutrition. The objectives of this trial were to compare the *in vitro* fermentation profiles of fructo-oligosaccharides (FOS), inulin, gum acacia, and pea fiber alone or blended using a 24 h batch model and relate these findings to *in vivo* results. The fiber blend was added to an enteral formula (15g/L) and fed to 20 healthy human subjects for 14 days in a randomized, crossover, double blind study with a 28 day washout. Stool samples were collected during the human study to determine fecal acids while gas symptoms were assessed via questionnaire. The *in vitro* fermentation of the fiber blend resulted in a delayed pH decrease and gas and SCFA production compared to the FOS and inulin. Human samples had higher total SCFA on the fiber formula compared to the fiber free formula ($p=0.029$), and both formulas yielded lower SCFA than habitual diet (both $p<0.0001$). Mean fecal pH for both formulas was 7.5; higher than habitual diet pH 6.5 ($p<0.0001$). No differences in the frequency of gas and bloating were found between any diet. By blending fibers, a slower fermentation was observed *in vitro* and was well tolerated in human subjects. Fiber

addition to enteral formula increases fecal short chain fatty acids which may reflect increased fermentation.

Introduction

The human large intestine contains up to 10^{12} bacterial cells/g contents with over 400 different species represented (55). Gut bacteria ferment non-digestible carbohydrates to produce short chain fatty acids (SCFAs) and hydrogen, carbon dioxide, and methane gases. Proteins may also be fermented if sufficient carbohydrates are not available, and this results in branched chain fatty acids and other products (56, 107).

The major SCFAs in the colon are acetate, propionate and butyrate although branched chain fatty acids and other products are found in lesser amounts (56). Gas is a product of fermentation and large amounts produced *in vivo* may cause bloating and discomfort (128).

SCFA are thought to be readily absorbed in a normal colon via both nonionic diffusion and ionic exchange and up to 95% of SCFA are thought to be absorbed from colonic contents (45, 109). In total, SCFAs reduce fecal pH, stimulate electrolyte and colonic fluid absorption, provide energy to the host and may affect gut transit time (72, 111). Individually, butyrate is the preferred form of fuel for colonocytes and plays a role in cell differentiation and proliferation (113). Acetate and propionate are absorbed and utilized by the liver (although to different degrees) with acetate the major acid entering systemic circulation (115, 117).

Diarrhea and selective bacterial overgrowth are common complications that occur with enteral nutrition (EN) (48, 123). Besides increasing fiber intake, addition of

fiber to enteral formulas has been shown to decrease diarrhea and colonization of potentially pathogenic bacteria through the increased production of SCFAs and decreases in colonic pH (46). By using a blend of fibers to fortify formula, the fiber profile more closely resembles a mixed diet (192). Furthermore, by blending fibers with various physicochemical properties, a variety of physiological benefits may be achieved with minimal gas and bloating.

Fecal SCFAs reflect the amount of SCFA left after microbial production and colonocyte absorption. Batch *in vitro* systems allow fermentation modeling without absorption and may help to estimate potential health benefits and gastrointestinal tolerances of fibers *in vivo* (207). Well controlled, blinded and randomized intervention human studies are the “gold standard” for human nutrition research.

The objective of the *in vitro* study was to compare the fermentation profiles of different dietary fibers alone or blended using a 24 h batch system. The fiber blend was then added to an enteral nutrition product and fed to human subjects. The objective of the human study was to compare the effects of fiber-free enteral formula, a fiber blend-fortified formula and habitual diet on fecal acids and gas symptoms in healthy subjects.

Methods and Materials

In vitro fermentation using human fecal inoculums

Potential human fecal sample donors were recruited on the University of Minnesota campus. Subjects were screened using a questionnaire. Inclusion criteria was self-reported absence of disease (“healthy”), consumption of a non-specific western diet,

and no antibiotic use for three months prior to the study. Fecal samples were collected using a Commode Specimen Collection System (Sage, IL, USA) lined with a 2 mm thickness plastic bag. Samples were immediately placed into an anaerobic bag (Remel, Lenexa, KS) containing an AnaeroPouch anaerobic sachet (Mitsubishi Gas Company, Tokyo Japan), sealed with a Pouch Clip (Remel, Lenexa, KS) and placed on ice in an insulated container to maintain microbial viability and ensure standardized sample preparation. Fecal samples were delivered to the laboratory and processed within 2 h.

Fiber samples were provided by Nestle Health Science S.A. (Lutry, Switzerland) in coded pouches, and investigators were blinded to the treatments. The fibers tested were fructo-oligosaccharides (FOS, Beneo, Morris Plains NJ), inulin (Beneo, Morris Plains NJ), gum acacia (Nexira, Somerville NJ), outer pea fiber (Nutri-Pea Limited, Manitoba Canada), and a 50:50 soluble: insoluble blend of FOS, inulin, gum acacia, and outer pea fiber. Half a gram (0.5g) of each fiber or blend was weighed and placed into sterilized 100 mL serum bottles. Forty mL of sterile trypticase peptone fermentation media was added to each bottle and fibers were allowed to hydrate for 12 h at 4°C (208). The fiber:media slurry was heated to 37°C and 10 mL fecal inoculum was added to each bottle along with 0.8 mL Oxyrase® (Oxyrase Inc., Mansfield, OH USA) to remove oxygen from the environment. Fecal inoculum was prepared by pooling three fecal samples with phosphate buffer solution (1:6 w/v) and then mixing two parts reducing solution (950 mL of distilled water, 6.25 g of cysteine hydrochloride, 40 mL 1N sodium hydroxide, 6.25 g of sodium sulfide nonahydrate) to 15 parts diluted fecal inoculum (v/v) (209). Bottles were flushed with carbon dioxide

and sealed. Sealed bottles were placed in a 37°C shaking water bath and allowed to ferment for 0, 4, 8, 12, and 24 hours (triplicate bottles for each timepoint).

In vitro pH, gas volume and SCFA

At each timepoint, aliquots were taken for measurement of pH, gas volume and SCFAs. Gas volume was measured by piercing the rubber cap of the sealed fermentation bottle with a 50 mL or 100 mL glass syringe fitted with a stainless steel needle and allowing the gas to fill the syringe (210). The pH of the fermented treatments was measured with a calibrated pH probe, and following pH measurement, 1 mL of copper sulfate (200 g/L) was added into the serum bottles to inhibit further fermentation. A two mL aliquot was removed from each triplicate for SCFA measurement and frozen at -20°C until subsequent analysis.

Enteral nutrition human feeding study

Subjects.

Healthy men and women were recruited on the University of Minnesota campus. Exclusion criteria included: age <18 or >75, BMI <23 or >29, any self-reported disease, use of medication (except contraception and certain over the counter medications), smoking, >2 h exercise per week, consumption of >20 g fiber/day, pregnancy or lactation, use of laxatives, use of antibiotics in the past 6 mo., use of pre or probiotic supplements in the past 2 mo., use of probiotic foods in the past month, participation in another clinical trial during the last 4 weeks prior to the beginning of the study, and known allergy or sensitivity to formula ingredients. Subject eligibility was assessed via questionnaire and physical measurements. Refer to *Appendix B* for the eligibility screening questionnaire. Fiber intake was screened using a fruit,

vegetable, and fiber frequency questionnaire (193). Written informed consent was obtained from each subject and the study was approved by the University of Minnesota Institutional Review Board for Human Research Protection Program. The informed consent form is listed in *Appendix C*.

Subject protocol

During the study, subjects consecutively consumed habitual diet for 14 days (run-in period), enteral diet 1 for 14 days, habitual diet for 28 days (washout phase), and enteral diet 2 for 14 days. Enteral formula diets were randomly assigned and the two treatment sequences were given in equal numbers, stratified by gender. See *Appendix F* for the randomization schedule. Subjects exclusively consumed (orally) formula during the enteral periods except *ad libitum* consumption of water and coffee, tea and non-caloric/no-fiber containing beverages in similar amounts consumed during normal diet. Subjects were provided with formula in excess of their total energy needs as calculated by the Harris-Benedict equation and adjusting with physical activity factors (194). Subjects were allowed to consume formula *ad libitum* above their calculated needs, but were withdrawn from the study if their intake was below 75% of the target volume for two consecutive days. Formula was provided in 250 mL cans that were identically labeled with the exception of four different product codes (to ensure blinding).

Subjects recorded diet records for 2 days during habitual diet and 14 days during the enteral formula periods. Subjects anaerobically collected a fecal sample on the last day of each period as described in the *in vitro* section. Samples were delivered to the laboratory within 1 h of defecation, and processed immediately. Gas symptoms were

assessed via the gastrointestinal quality of life index questionnaire which was administered at the end of each period (132). Refer to *Appendix E* for the complete GIQLI.

Enteral formula and diet measurements

Enteral formulas were nutritionally complete and identical in composition except for fiber content. Base formula contained 6276 kJ/L, 67.6 g protein/L, 64.8 g fat/L, 168 g carbohydrate/L and fiber containing formula was base supplemented with 15 g/L of fiber blend. The fiber blend was composed of a 50:50 insoluble: soluble mixture of FOS, pea hull fiber, gum acacia, and inulin. Refer to *Appendix D* for the complete nutrition information and ingredient list for the formulas.

Enteral formula energy and protein intake was calculated from 14-day diet records during the formula periods. Enteral formula fiber intake was calculated from 14-day diet records during the fiber formula period. Habitual diet reports were generated by entering the diet records into Nutrition Data System for Research (University of Minnesota, Version 2011). Habitual diet energy, fiber, and protein intake values were averaged from the 2-day diet records recorded during the run-in and washout phases.

Human subject fecal sample preparation

Anaerobically packaged fecal samples were kneaded for 2 min to homogenize. A 10 g aliquot of fecal sample was diluted 1:10 (w/w) in phosphate buffered saline (PBS, 0.1 M, pH 7.2). The fecal: PBS mixture was homogenized in a stomacher for 2 min and the slurry was aliquoted for SCFA. The remaining sample was used for pH measurement via calibrated pH probe.

In vitro and human subject short chain fatty acid measurement

SCFA were completed in triplicate using a modified Schneider method (123). *In vitro* fermentation liquid aliquots were thawed at 4 °C. To the fermentation liquid, 1.6 mL of distilled water and 0.4 mL 50% sulfuric acid was added and vortex mixed. Two mL of diethyl ether and 2 µL 2-ethylbutyric acid (99%, internal standard) was added to each tube. Tubes were sealed, vortex mixed and orbitally shaken at 300 rpm for 45 minutes. Samples were centrifuged at 3000 rpm for 5 minutes and the supernatant was transferred to a test tube containing calcium chloride to remove residual water. The final sample was filtered through a 0.2 µm nylon filter and placed into a 2 mL glass gas chromatography vial and sealed.

For the human subjects samples, approximately 2 mL of PBS:stool slurry was centrifuged at 10,000 rpm for 10 min. To 1 mL of fecal supernatant, 0.4 mL 50% sulfuric acid and 2 mL of ether containing 1 µL/mL 2-ethyl butyric acid (99%, internal standard) was added. The sample was sealed, vortex mixed for 10 s and shaken at 300 rpm for 45 min. The ether was retained, excess water removed with calcium chloride, and the final sample placed into a 2 mL glass gas chromatography vial and sealed.

All SCFA samples were analyzed using a HP 5890 gas chromatograph (in split mode) equipped with a 30 m x 0.53 mm x 1µm film thickness Stabilwax-DA (Restek Corporation, Bellefonte PA) column. The injection port was 220°C, the detector 240°C, oven program 110°C for 0.5 min followed by 10°C/min to 180°C and then 50°C/min to 240°C, hold 3 min. Acetate, propionate, and butyrate were quantified for

in vitro samples. Acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were quantified for *in vivo* samples. All quantification was performed using standard curves generated from known amounts of acids and internal standard.

Statistical Analysis

All data analysis was performed in SAS (Version 9.2). *In vitro* longitudinal repeated measurements were compared between fibers using a mixed-effects linear model, with a random intercept to model within-fiber correlation. Gas volume was log transformed before analysis. To adjust for multiple comparisons, differences were considered significant at $P < 0.005$.

Baseline characteristics of human subject data were compared using a t-test. All other human subject data was compared using longitudinal regression models with random subject effect to model the within-subject correlation. For each outcome, period, gender and unequal carryover effects were tested for. Data collected during the initial habitual diet and washout periods were combined and adjusted for period effects. Differences were considered significant at $P < 0.05$.

Results

In vitro fermentation study

Three volunteers (aged 24-32; one male, two female) provided fecal samples for the fermentation. Samples were pooled and used in the fermentation.

All fiber:fecal slurries produced more than 3 mL gas during the 24 h fermentation except the control and pea fiber (**Figure 3-1**). At 4 h, gas production was the highest

for inulin followed by 10 times less gas for FOS and gum acacia and essentially no gas from the fiber blend. By 8 h, inulin and FOS produced approximately 20 times more gas than the fiber blend. At 12 h, inulin and FOS continued to have the highest gas production (and statistically similar) which was three times higher than the gum acacia and five times more than the fiber blend. At the final 24 h timepoint, FOS and inulin produced the most (and statistically similar) amount of gas, followed by gum acacia (which was similar to FOS levels). The blend produced intermediate amounts at approximately a quarter of that produced by inulin and FOS. Gas volume values for each fiber and statistical significance can be found in **Table 3-1**.

Due to the fermentation media used, the initial pH of the system was approximately 9, and no statistical differences were found between any fibers at time 0 (**Table 3-2**). At 4 h, the FOS and inulin both had dropped approximately 3 pH units from baseline (time 0) and were significantly lower than all other fibers. Statistically different, but somewhat higher than FOS and inulin was the fiber blend (pH 6.5), followed by approximately pH neutral values for the outer pea, gum acacia and control. At 8 h, the inulin and FOS continued to have the lowest (and statistically similar) pH, followed by (in increasing pH) fiber blend and gum acacia. Outer pea and control remained approximately pH neutral. By 12 h, FOS and inulin had the lowest (and similar) pH at approximately 5.9 with increased and similar values for the fiber blend and gum acacia (~pH 6.5), followed by the pH neutral outer pea and control. At the end of the 24 h fermentation, the control continued to remain approximately neutral followed by (highest to lowest) outer pea, fiber blend, inulin and gum acacia and FOS.

Total SCFA production (sum of acetate, propionate, and butyrate) from the *in vitro* fermentation is shown in **Figure 3-2**. At baseline (time 0), all fiber samples had similar SCFA values except fiber blend which was 7-10 mmol/L lower than the rest. At 4 h, FOS and inulin had the highest (and similar) SCFA values. Gum acacia and outer pea had intermediate concentrations of SCFA while the control and outer pea fiber had the lowest values. By 8 h, inulin had the highest production which was statistically different from all other fibers. FOS, fiber blend, gum acacia and outer pea had intermediate values with the control the lowest. At 12 h inulin continued to have the highest SCFA followed by (in decreasing concentration) FOS, gum acacia, fiber blend, control and outer pea. At the conclusion of the fermentation, inulin produced the highest SCFA which was statistically similar to FOS. Gum acacia produced similar amounts of SCFA as FOS and these were not statistically distinguishable from fiber blend, pea fiber or control. Total SCFA values for each fiber and statistical significance can be found in **Table 3-3**.

Enteral nutrition human feeding study

Twenty-four subjects were enrolled, and twenty subjects (10 men, 10 women) completed the study. The mean age and BMI of the women and men did not significantly differ. Overall mean subject age was 25.8 (+/- 9.9 SD) with a BMI of 25.8 (+/- 2.5 SD). Baseline characteristics of the two sequence groups were also compared, and no statistical differences were found. Subject diet characteristics are listed in **Table 3-4**.

pH and SCFA

Human samples were analyzed for both SCFA and branched chain fatty acids (BCFA). Total SCFA refers to total amounts of acetate, propionate, and butyrate. Total BCFA refers to amounts of isobutyrate, isovalerate and valerate. SCFA and BCFA are reported per mL of fecal water.

Total SCFA were approximately 2 times higher during the habitual diet phase than during the formula phases ($p < 0.0001$, **Table 3-5**). Between the formula periods, SCFA on the fiber blend was 1.1% higher than the fiber free formula ($p = 0.029$). Individual SCFA also differed; butyrate proportion was approximately 1.6 times higher in the habitual diet compared to both formulas (both $p < 0.0001$), and 1% higher in the fiber free formula compared to the fiber formula ($p = 0.041$). Propionate was in a lower proportion in habitual diet than both formula diets (both $p < 0.03$), but did not differ between formulas. Acetate was also in a lower proportion during habitual diet than both formulas (both $p < 0.0001$) and did not differ between formulas. Total BCFA were 1% higher in habitual diet compared to the fiber blend formula ($p = 0.046$), but fiber free did not statistically differ from habitual diet or fiber blend formula. Formula diet pH did not differ, and both were a pH unit higher than habitual diet (both $p < 0.001$).

The incidence of gas and bloating symptoms was monitored during each diet. Symptom scores were as follows (higher scores indicate more favorable outcomes); habitual 17.3 (± 0.4 SE), fiber free formula 17.3 (± 0.4 SE), and fiber blend formula 18.0 (± 0.4 SE). Incidence of gas and bloating symptoms were not different between formulas or between either formula and habitual diet.

Discussion

Fiber source and structure, as well as the bacterial populations present, affect fiber fermentation patterns in both *in vitro* and *in vivo* models (10, 56). Fermentation leads to the production of acids, which are considered to be a beneficial product of fermentation, but also gas which can lead to reduced tolerance of fiber *in vivo*. Greater gas volume is perceived more in the proximal bowel compared to the distal colon, so rapidly fermented fibers would be expected to cause more gastrointestinal intolerance than those that are more slowly fermented (205). Human studies have found that dietary supplementation with short chain fibers such as FOS and inulin lead to increased side effects (170, 171, 211). In contrast, complex molecules, such as gum acacia are tolerated at doses up to 40 g/day, and a 1:1 combination of gum acacia and FOS fed to humans led to reduced gastrointestinal side effects than FOS alone (178, 189). Others have reported use of blends with fibers of varying chain length to attenuate gas production *in vitro* (128, 206).

Our results demonstrate similar trends in gas production. Fermentation of soluble shorter chain, single fibers (FOS and inulin) typically led to higher gas production at earlier time points (4 and 8 hours). In contrast, gas production was almost undetectable at these time points for the larger and more complex soluble gum acacia, insoluble pea fiber and fiber blend. Pea fiber has been shown to be poorly fermented and would be expected to contribute minimally to gas production (179). While the fiber blend contains inulin, FOS, and gum acacia which are known to be fermentable, it also contains pea fiber which changed the fermentation pattern as a whole and resulted in delayed gas production until 12 and 24 hours. The slower fermentation could lead to improved tolerance *in vivo*, as gas is perceived more in the proximal

bowel (205). The results of the human feeding study were parallel to the *in vitro* gas production from the fiber blend. No differences in gas and bloating were detected between any diet suggesting that the fiber blend did not induce symptoms beyond subject's normal levels and was well tolerated at the dose delivered.

In general, *in vitro* pH changes were consistent with changes in gas production. pH values were lower at earlier time points for substrates comprised entirely of rapidly fermented, short chain fructans (FOS, inulin). Hernot et al found the greatest decrease in pH for FOS and inulin after 8 hours fermentation, which is consistent with our results (206). Addition of higher molecular weight molecules resulted in a delayed decrease in pH consistent with more gradual fermentation. Gum acacia maintained a relatively high pH until 24 hours, where it decreased to values lower than the single fiber treatments. Similarly, the blend had a significantly lower pH than the control and pea fiber which can be attributed to delayed fermentation of the gum acacia fraction of the blend.

Fecal pH of the human samples, however, was not as definitive. Fecal pH during the fiber formula and the fiber free formula was the same despite an average fiber dose of 19 g on the fiber formula. Although a significant portion is the minimally fermentable pea fiber, it is not clear why the fecal pH was not decreased. A possible explanation is an increase in bicarbonate from the proposed SCFA-bicarbonate absorption mechanism (45). Furthermore, pH was lower during the habitual diet than during the formula diets despite receiving an average of 3 g less fiber per day. This may be explained by the shorter transit times during the habitual diet (data not presented) which would allow for less time for SCFA absorption to occur.

In vitro total SCFA production followed a similar pattern as gas and pH, although significant differences between treatments were more difficult to detect due to high standard errors. Among the single fiber treatments, total SCFA levels were higher at early time points for inulin and FOS compared to gum acacia and pea fiber. Later in the fermentation, total levels were difficult to statistically distinguish, but inulin produced the greatest SCFA levels overall. Since SCFAs are a product of fermentation, these results are consistent with rapid fermentation of short chain molecules, and delayed fermentation of fibers with higher molecule weight. More gradual fermentation may translate into increased SCFA production throughout the entire colon, which may have additional health benefits (166).

In vivo SCFA are more complex. In mixed diets, bacteria ferment carbohydrates to primarily produce linear SCFA while protein fermentation leads to the production of BCFA and less desirable metabolites such as ammonia, phenol, and indole (56). Absorption of SCFA is thought to occur via ionic exchange and nonionic diffusion and in human rectal studies, absorption rates can be manipulated by changing electrolyte composition (212). Furthermore, in an animal model, acetate absorption rates increased with increasing concentration (124). Fecal SCFA, then, reflect residual SCFA after both production and absorption. Fecal SCFA of the subjects on habitual diet was approximately 2 times higher than both of the formulas, although it should be noted that the whole gut transit times were approximately 1.5 times longer on the formula diet versus the habitual diet. Longer transit times may influence SCFA and water absorption (213). Between formulas, the fiber blend formula had increased SCFA concentration compared to the fiber free formula and transit time was

not different. This may be due to more substrate present for bacteria to ferment, although because concentration may influence absorption, it is difficult to make a conclusion. Similarly, it is difficult to draw conclusions regarding the proportions of SCFA measured in the fecal samples since differences may be due to production or absorption. BCFA are produced during protein fermentation, and although they are not detrimental, reflect a process that also produces phenols, indoles, etc. which are undesirable. Little has been published on BCFA in human samples. Measured levels were the highest on habitual diet despite similar protein intakes between the habitual and formula diets. Again, transit time may have contributed to differences. Between the two formulas, it was surprising that the fiber free samples did not have higher BCFA since the diet was devoid of fiber and the substrates reaching the colon would be undigested formula, and endogenous mucus, sloughed off cells, and digestive secretions (56). Although these substances would also be present during the fiber containing formula, carbohydrate fermentation is preferred over protein metabolism and it was hypothesized that BCFA would be found in lower amounts (119).

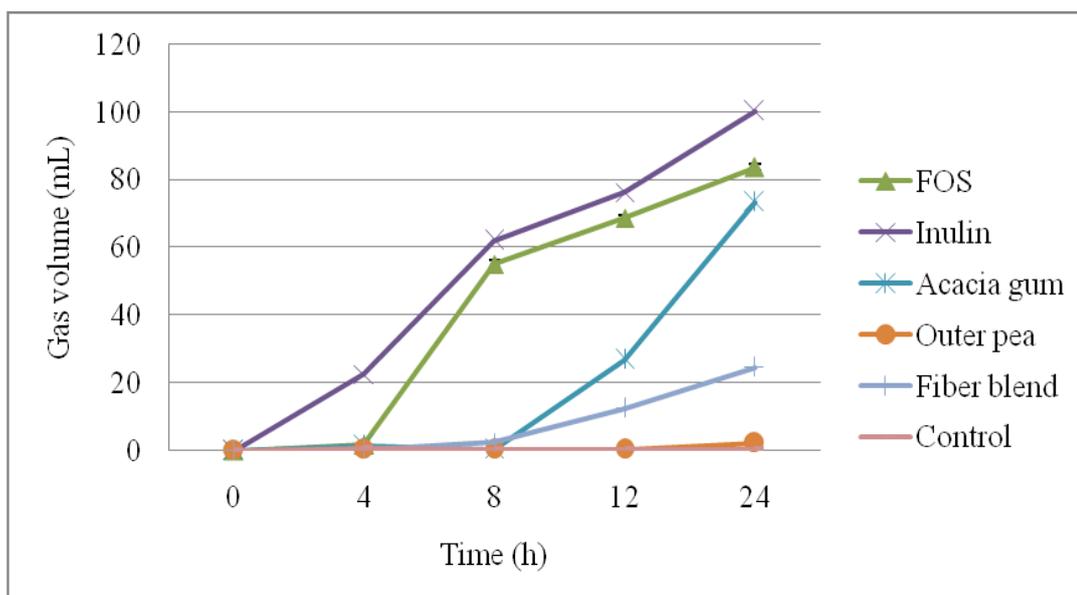
Conclusions

Overall, the fermentation of fibers with a range of physicochemical properties yielded differing profiles of pH, gas and SCFA. By blending insoluble and soluble fibers, an intermediate fermentation profile was observed and this blend was well tolerated when fed to healthy human subjects. During the human intervention study, fecal SCFA was increased on the fiber containing formula compared to the fiber free which may have indicated increased fermentation in the gut.

¹The authors wish to thank Nestle Health Science S.A. (Lutry, Switzerland) for providing the isolated fibers, the liquid diets in the human study and the financial support.

“Coupling fermentation results from a 24 h batch *in vitro* system with fecal measurements and subjective tolerance from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia and pea fiber” by Katie Koecher (KK), Jackie Noack (JN), Derek Timm (DT), Abby Klosterbuer (AK), William Thomas (WT) and Joanne Slavin (JS) will be submitted for publication. JN, DT, and AK performed and collected the data for the *in vitro* portion of the study.

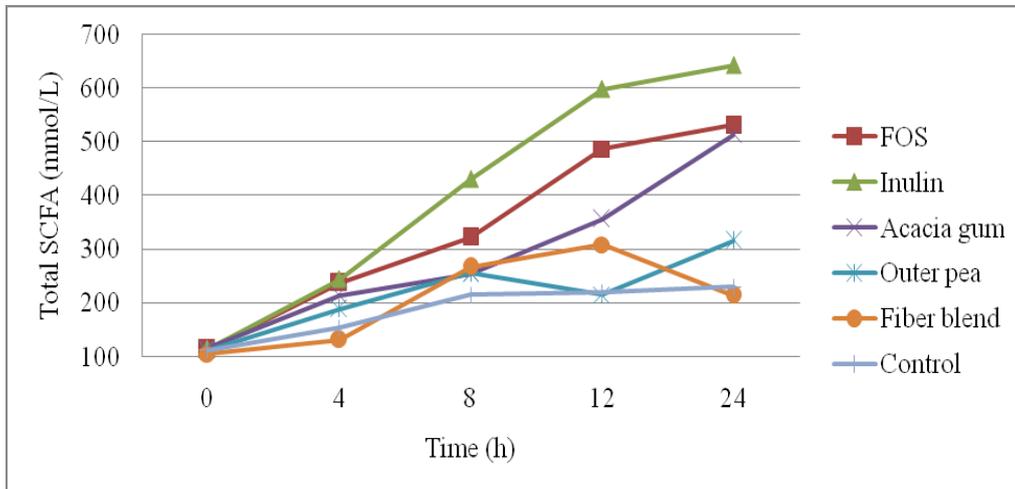
Figure 3-1. Gas volume produced during 24 h batch *in vitro* fermentation of fibers^{1,2}



¹Values are geometric means.

²FOS: Fructo-oligosaccharides

Figure 3-2. Total short chain fatty acids produced during 24 h batch *in vitro* fermentation of fibers^{1,2}



¹Values are means. Total short chain fatty acids refers to the sum of acetate, propionate, and butyrate.

²FOS: fructo-oligosaccharides

Table 3-1. Gas volume produced during 24 h *in vitro* fermentation of fibers¹

Gas volume				
(mL)				
Fiber	4 h	8 h	12 h	24 h
Inulin	22.6 (6.5, 78.4) ^{bc}	62.2 (40.1, 96.6) ^c	76.3 (66.8, 87.2) ^d	100.3 (90.0, 111.8) ^d
FOS	1.8 (0.5, 6.1) ^{ac}	55.2 (35.6, 85.7) ^c	68.7 (60.1, 78.4) ^d	83.7 (75.1, 93.2) ^{ad}
Gum acacia	1.6 (0.5, 5.6) ^{ab}	0.5 (0.3, 0.8) ^a	26.9 (23.5, 30.7) ^a	73.5 (66.0, 81.9) ^a
Fiber blend	0.5 (0.1, 1.7) ^a	2.5 (1.6, 3.9) ^b	12.6 (11.0, 14.4) ^b	24.6 (22.1, 27.4) ^b
Outer Pea	0.5 (0.1, 1.7) ^a	0.5 (0.3, 0.8) ^a	0.5 (0.5, 0.5) ^c	2.3 (2.1, 2.6) ^e
Control	0.9 (0.4, 2.2) ^a	0.5 (0.4, 0.7) ^a	0.5 (0.5, 0.6) ^c	0.5 (0.5, 0.5) ^c

¹Geometric mean (95% CI). Different letters indicate significantly different values (p < 0.005) within each time point (column).

Table 3-2. pH during 24 h batch *in vitro* fermentation of fibers¹

pH					
Fiber	0 h	4 h	8 h	12 h	24 h
FOS	9.09 (0.26) ^a	5.82 (0.10) ^c	5.62 (0.09) ^d	5.86 (0.08) ^c	6.04 (0.08) ^d
Inulin	9.01 (0.26) ^a	5.81 (0.10) ^c	5.81 (0.09) ^d	5.93 (0.08) ^c	5.88 (0.08) ^a
Gum acacia	9.02 (0.26) ^a	7.12 (0.10) ^a	6.79 (0.09) ^a	6.54 (0.08) ^a	5.87 (0.08) ^a
Outer pea Fiber	9.13 (0.26) ^a	6.98 (0.10) ^a	6.99 (0.09) ^c	6.85 (0.08) ^b	6.76 (0.08) ^e
blend	8.98 (0.26) ^a	6.5 (0.10) ^b	6.48 (0.09) ^b	6.43 (0.08) ^a	6.37 (0.08) ^b
Control	9.05 (0.26) ^a	7.07 (0.09) ^a	6.99 (0.08) ^c	6.91 (0.08) ^b	6.86 (0.07) ^c

¹Mean (SEM). Different letters indicate statistically different values within timepoints (column) at p<0.005.

Table 3-3. Total short chain fatty acids produced during 24 h *in vitro* fermentation of fibers¹

Total					
SCFA					
(mmol/L)					
Fiber	0 h	4 h	8 h	12 h	24 h
FOS	115 (4) ^a	238 (28) ^a	322 (23) ^a	487 (34) ^{ac}	531 (59) ^{acd}
Inulin	115 (4) ^a	244 (28) ^a	431 (23) ^c	598 (59) ^c	643 (59) ^c
Gum	116 (4) ^a	214 (29) ^{ac}	255 (23) ^{ab}	356 (34) ^{abd}	513 (59) ^{ad}
acacia					
Outer pea	112 (4) ^{ab}	189 (29) ^{cd}	256 (23) ^a	215 (34) ^d	316 (70) ^d
Fiber	105 (4) ^b	131 (28) ^b	267 (23) ^{ab}	309 (34) ^{bd}	214 (59) ^{bd}
blend					
Control	112 (3) ^a	154 (27) ^{bd}	215 (18) ^b	220 (24) ^{bd}	230 (54) ^{bd}

¹Mean (SEM). Total short chain fatty acids refers to the sum of acetate, propionate and butyrate. Different letters are significantly different ($p < 0.005$) within time points (column).

Table 3-4. Energy, fiber and protein intake of 20 human subjects consuming enteral formula and habitual diet¹

	Habitual			Formula		
	Energy²	Fiber²	Protein²	Energy³	Fiber⁴	Protein³
	(kJ/d)	(g/d)	(g/d)	(kJ/d)	(g/d)	(g/d)
Women	8008	16.8	71.5	6757	16.2	72.8
(n=10)	(2426)	(4.2)	(21.4)	(853)	(1.9)	(9.2)
Men	9301	15.1	102.4	9309	21.6	100.3
(n=10)	(2506)	(6.5)	(27.9)	(1451)	(3.8)	(15.6)
Total	8656	16.0	89.6	8067	18.9	86.5
(n=20)	(2485)	(5.3)	(29.2)	(1745)	(4.0)	(18.8)

¹Mean (SD).

²Habitual diet energy, fiber and protein intake was calculated by combining 2-day diet records from the run-in and washout periods. Values were generated by entering the diet records into Nutrition Data System for Research (University of Minnesota, Version 2011)

³Enteral formula energy and protein intake was calculated from 14-day diet records during the formula periods.

⁴Enteral formula fiber intake was calculated from 14 day diet records during the fiber formula period.

Table 3-5. Fecal short chain fatty acids and pH of human subjects consuming fiber free formula, fiber blend formula and habitual diet¹

	Habitual diet	Fiber free formula	Fiber blend formula
Total SCFA² (mmol/L)	80.9 (2.7) ^a	39.7 (2.3) ^b	43.5 (2.3) ^c
Acetate %	39.5 (0.6) ^a	49.2 (1.0) ^b	50.2 (0.9) ^b
Propionate %	29.7 (1.1) ^a	31.6 (1.3) ^b	31.5 (1.3) ^b
Butyrate %	30.7 (1.1) ^a	19.2 (0.8) ^b	18.3 (0.8) ^c
Total BCFA³ (mmol/L)	11.5 (1.2) ^a	10.5 (1.1) ^{ab}	10.1 (1.1) ^b
pH	6.52(0.07) ^a	7.50(0.09) ^b	7.54 (0.09) ^b

¹Mean (SEM). Different letters indicate statistically different values between diets (within row comparisons) at p<0.05.

²Total SCFA refers to the sum of acetate, propionate and butyrate.

³Total BCFA refers to the sum of isobutyrate, isovalerate, and valerate.

References

1. Hipsley EH. Dietary “fibre” and pregnancy toxemia. *Br Med J.* 1953;4:420.
2. Anonymous. The definition of dietary fiber. *Cereal Foods World.* 2001;46(3):112-26.
3. Food and Nutrition Board. Dietary reference intakes for energy, carbohydrates, fiber, fat, protein and amino acids. Institute of medicine of the national academies; Washington, DC: The National Academies Press; 2002.
4. Codex Alimentarius Commission. Guidelines on nutrition labelling. 1985, Revision 1993. Amendment 2003, 2006, 2009, 2010. Report No.: CAC/GL 2-1985.
5. McCleary B. Dietary fibre analysis. *Proc Nutr Soc.* 2003;62:3-9.
6. Brodribb AJ, Groves C. Effect of bran particle size on stool weight. *Gut.* 1978;19(1):60-3.
7. Canibe N, Knudsen KEB. Degradation and physicochemical changes of barley and pea fibre along the gastrointestinal tract of pigs. *J Sci Food Agric.* 2001;82:27-39.
8. Thomas LH, Forsyth VT, Sturcova A, Kennedy CJ, May RP, Altaner CM, et al. Structure of cellulose microfibrils in primary cell walls from collenchyma. *Plant Phys.* 2013;161(1):465-76.
9. BeMiller J, Whistler R. Carbohydrates. In: Fennema O, editor. *Food Chemistry.* 3rd ed. New York, NY: Marcel Dekker; 1996. p. 157-224.
10. Eastwood M, Morris E. Physical properties of dietary fiber that influence physiological function: A model for polymers along the gastrointestinal tract. *Am J Clin Nutr.* 1992;55:436-42.
11. Talaro K. Microbial metabolism: The chemical crossroads of life. In: Reidy P, editor. *Foundations in Microbiology.* 5th ed. New York, NY: McGraw Hill Higher Education; 2006. p. 217-52.
12. Bourquin L, Titgemeyer E, Fahey G. Vegetable fiber fermentation by human fecal bacteria: Cell wall polysaccharide disappearance and short-chain fatty acid production during *in vitro* fermentation and water-holding capacity of unfermented residues. *J Nutr.* 1993;123:860-9.
13. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J Nutr.* 1995;125:1401-12.
14. Gibson GR, Probert HM, Van Loo J, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutr Res Rev.* 2004;17:259-75.

15. de Vrese M, Schrezenmeir J. Probiotics, prebiotics and synbiotics. *Adv Biochem Eng Biotechnol.* 2008;111:1-66.
16. Schrezenmeir J, de Vrese M. Probiotics, prebiotics and synbiotics-approaching a definition. *Am J Clin Nutr.* 2001;73:361S-364S.
17. Charalampopoulos D, Rastall R, editors. *Prebiotics and probiotics science and technology.* New York, USA: Springer; 2009.
18. Gibson GR, Rastall RA, editors. *Prebiotics: Development and application.* West Sussex, England: John Wiley and Sons; 2006.
19. Gibson GR, Roberfroid M, editors. *Colonic microbiota, nutrition and health.* Dordrecht, The Netherlands: Kluwer Academic Publishers; 1999.
20. Reid G, Sanders M, Gaskins H, Gibson G, Mercenier A, Rastall R, et al. New scientific paradigms for probiotics and prebiotics. *J Clin Gastroenterol.* 2003;37(2):105-18.
21. Roberfroid M. Prebiotics: The concept revisited. *J Nutr.* 2007;137:830S-7S.
22. Makivuokko H, Nurminen P. *In vitro* methods to model the gastrointestinal tract. In: Ouwehand A, Vaughan E, editors. *Gastrointestinal Microbiology.* 1st ed. New York, NY: Taylor and Francis; 2006. p. 237-53.
23. Henriksson A. Animal models for the human gastrointestinal tract. In: Ouwehand A, Vaughan E, editors. *Gastrointestinal Microbiology.* 1st ed. New York, NY: Taylor and Francis; 2006. p. 253-72.
24. Roberfroid M, Gibson G, Hoyles L, McCartney A, Rastall R, Rowland I, et al. Prebiotic effects: Metabolic and health benefits. *Br J Nutr.* 2010;104(S2):S1-S63.
25. Hughes R, Rowland I. Nutritional and microbial modulation of carcinogenesis. In: Fuller R, Perdigon G, editors. *Gut Flora, Nutrition, Immunity and Health.* 1st ed. Oxford, UK: Blackwell Publishing; 2003. p. 208-36.
26. Bartel B, Gau A. Nosocomial diarrhea: a review of pathophysiology, etiology, and treatment strategies. *Am J Clin Nutr.* 2012;40(1):130-8.
27. Kandler O. Carbohydrate metabolism in lactic acid bacteria. *Antonie van Leeuwenhoek.* 1983;49:209-24.
28. Toure R, Kheadr E, Lacroix C, Moroni O, Fliss I. Production of antibacterial substances by bifidobacterial isolates from infant stool active against listeria monocytogenes. *J Appl Microbiol.* 2003;95:1058-69.
29. Stroud M, Duncan H, Nightingale J. Guidelines for enteral feeding in adult hospital patients. *Gut.* 2003;52(Suppl VII):1-12.

30. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letters*. 2002;217(2):133-9.
31. Wexler HM. Bacteroides: The good, the bad, and the nitty-gritty. *Clin Microbiol Rev*. 2007;20:593-621.
32. Wick E, Sears C. Bacteroides spp. and diarrhea. *Curr Opin Infect Dis*. 2010;23(5):470-4.
33. Chang JY, Antonopoulos DA, Kalra A, Tonellia A, Khalife WT, Schmidt TM, et al. Decreased diversity of the fecal microbiome in recurrent clostridium difficile associated diarrhea. *J Infect Dis*. 2008;197:435-8.
34. Roberfroid MB, Van Loo JA, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. *J Nutr*. 1998;128:11-9.
35. Rycroft CE, Jone MR, Gibson GR, Rastall RA. Fermentation properties of gentio-oligosaccharides. *Lett Appl Microbiol*. 2001;32:156-61.
36. Rao V. The prebiotic properties of oligofructose at low intake levels. *Nutr Res Rev*. 2001;21:843-8.
37. Lochs H, Allison SP, Meier R, Pirlich M, Kondrup J, Schenider S, et al. Introductory to the ESPEN guidelines on enteral nutrition: Terminology, definitions and general topics. *Clin Nutr*. 2006;25:180-6.
38. Mueller C, Bloch AS. Intervention: Enteral and parenteral nutrition support. In: Mahan LK, Escott-Stump S, editors. *Krause's Food and Nutrition Therapy*. 12th ed. St Louis, MO: Saunders Elsevier; 2008. p. 506-31.
39. Elia M, Engfer M, Green C, Silk D. Systematic review and meta-analysis: The clinical and physiological effects of fibre-containing enteral formulae. *Aliment Pharmacol Ther*. 2008;27(2):120-45.
40. American Society for Parenteral and Enteral Nutrition. Enteral nutrition practice recommendations. VI. Enteral nutrition administration. *J Parenter Enteral Nutr*. 2009;33(2):122-16753.
41. Ireton-Jones C, Delegge MH, Eppenson LA, Alexander I. Management of the home parenteral nutrition patient. *Nutr Clin Pract*. 2003;18:310-7.
42. Agency for healthcare research and quality: Healthcare costs and utilization project [Internet].: U.S. Department of Health and Human Services; 2013. Available from: <http://hcupnet.ahrq.gov/>
43. Benya R, Layden TJ, Morbarhan S. Diarrhea associated with tube feeding: The importance of using objective criteria. *J Clin Gastroenterol*. 1991;13(2):167-72.

44. Shankardass K, Chuchmach S, Chelswick K, Stefanovich C, Spurr S, Brooks J, et al. Bowel function of long-term tube-fed patients consuming formulae with and without dietary fiber. *J Parenter Enteral Nutr.* 1990;14:508-12.
45. Binder H. Role of colonic short-chain fatty acid transport in diarrhea. *Annu Rev Physiol.* 2010;72:297-313.
46. Walker A, Duncan S, Leitch C, Child M, Flint H. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol.* 2005;71:3692-700.
47. Chow WL, Lee Y. Mucosal interactions and gastrointestinal microbiota. In: Ouwehand A, Vaughan E, editors. *Gastrointestinal Microbiology*. 1st ed. New York, NY: Taylor and Francis; 2006. p. 123-36.
48. Whelan K, Judd P, Preedy VR, Simmering R, Jann A, Taylor M. Fructo-oligosaccharides and fiber partially prevent the alterations in fecal microbiota and short-chain fatty acid concentrations caused by standard enteral formula in healthy humans. *J Nutr.* 2005;135(8):1896-902.
49. American Dietetic Association. Position of the American Dietetic Association: Health implications of dietary fiber. *J Am Diet Assoc.* 2008;108(10):1716-31.
50. Meier R, Gassull M. Consensus recommendations on the effects and benefits of fibre in clinical practice. *Clin Nutr Suppl.* 2004;1(2):73-80.
51. Marlett JA, McBurney MI, Slavin JL. Position of the American Dietetic Association: Health implications of dietary fiber. *J Am Diet Assoc.* 2002;102:993-1000.
52. O'May GA, Reynolds N, Smith AR. Effect of pH and antibiotics on microbial overgrowth in the stomachs and duodena of patients undergoing percutaneous endoscopic gastroenterology feeding. *Appl Environ Microbiol.* 2005;71:3059-65.
53. O'May GA, Reynolds N, Macfarlane GT. Effect of pH on an *in vitro* model of gastric microbiota in enteral nutrition patients. *Appl Environ Microbiol.* 2005;71:4777-83.
54. Kerckhoffs APM, Samson M, van Berge Henegouwen GP, Akkermans LMA, Nieuwenhuijs VB, Visser MR. Sampling microbiota in the human gastrointestinal tract. In: Ouwehand AC, Vaughan EE, editors. *Gastrointestinal Microbiology*. 1st ed. New York, NY: Taylor and Francis; 2006. p. 25-50.
55. Guarner F, Malagelada J. Gut flora in health and disease. *Lancet.* 2003;361:512-9.
56. Cummings J, Macfarlane G. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol.* 1991;70:443-59.
57. Tso P. Gastrointestinal secretion, digestion and absorption. In: Rhoades R, Tanner G, editors. *Medical Physiology*. 2nd ed.; 2003. p. 481-513.

58. Cummings J. The effect of dietary fibre on fecal weight and composition. In: Spiller GA, editor. *CRC Handbook of Dietary Fibre in Human Nutrition*. CRC Press; 1993. p. 263-333.
59. Tucker DM, Sandstead HH, Logan GM, Klevay LM, Mahalko J, Johnson L, et al. Dietary fiber and personality factors as determinants of stool output. *Gastroenterol*. 1981;81:879-83.
60. Lampe J, Fredstrom S, Slavin J, Potter J. Sex differences in colonic function: A randomized trial. *Gut*. 1993;34:531-6.
61. Stephen AM, Wiggins H, Englyst H. The effect of age, sex and level of intake of dietary fiber from wheat on large-bowel function in thirty subjects. *Br J Nutr*. 1986;56:349-67.
62. Eastwood M, Robertson J, Byrdon W, MacDonald D. Measurement of water-holding properties of fibre and their faecal bulking ability. *Br J Nutr*. 1983;50:539-47.
63. Eastwood M, Brydon W, Baird J, Elton R, Helliwell S, Smith J, et al. Fecal weight and composition, serum lipids, and diet among subject aged 18 to 80 years not seeking health care. *Am J Clin Nutr*. 1984;40:628-34.
64. Stasse-Wolthuis M, Albers H, van Jeveren J, Jong W, Hautvast J, Hermus R, et al. Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colonic function. *Am J Clin Nutr*. 1980;33:1745-56.
65. Lampe JW, Slavin J, Melcher E, Potter JD. Effects of cereal and vegetable fiber feeding on potential risk factors for colon cancer. *Cancer Epidemiol Biomarkers*. 1992;1:207-11.
66. Tomlin J, Read N. The relation between bacterial degradation of viscous polysaccharides and stool output in human beings. *Br J Nutr*. 1988;60:467-75.
67. Wrick K, Robertson J, Van Soest P, Lewis B, Rivers J, Roe D, et al. The influence of dietary fiber source on human intestinal transit and stool output. *J Nutr*. 1983;113:1464-79.
68. Hinton JM, Lennard-Jones J, Young A. A new method for studying gut transit times using radiopaque pellets. *Gut*. 1969;10:842-7.
69. Cummings JH, Jenkins D, Wiggins HS. Measurement of the mean transit time of dietary residue through the human gut. *Gut*. 1976;17:210-8.
70. Rao S, Camilleri M, Hasler W, Maurer A, Parkman H, Saad R, et al. Evaluation of gastrointestinal transit in clinical practice: Position paper of the American and European Neurogastroenterology and Motility Societies. *Neurogastroenterol Motil*. 2011;23:8-23.
71. Stephen A, Wiggins H, Cummings J. Effect of changing transit time on colonic microbial metabolism in man. *Gut*. 1987;28:601-9.

72. Fukumoto S, Tatewaki M, Tadanori Y, Fujimiya M, Mantyh C, Voss M, et al. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am J Physiol Regul Integr Comp Physiol*. 2003;284:R1269-76.
73. Yajima T. Contractile effect of short-chain fatty acids on the isolated colon of the rat. *J Physiol*. 1985;368:667-78.
74. Lederle FA, Busch DL, Mattox KM, West MJ, Aske DM. Cost effective treatment of constipation in the elderly: A randomized double-blind comparison of sorbitol and lactulose. *Am J Med*. 1990;89:597-601.
75. Schneeman BP. Dietary fiber and gastrointestinal function. *Nutr Res*. 1998;18(4):625-32.
76. Various. The digestion and absorption of food. In: Widmaier EP, Raff H, Strang KT, editors. *Vander's Human Physiology*. 11th ed. ; 2008. p. 528-65.
77. McRorie J, Zorich N, Riccardi KB, L., Fillon T, Wason S, Giannella R. Effects of olestra and sorbitol consumption on objective measure of diarrhea: Impact of stool viscosity on common gastrointestinal symptoms. *Regul Toxicol Pharmacol*. 2000;31:59-67.
78. Martelli H, Devroede G, Ahran P. Some parameters of large bowel in normal man. *Gastroenterol*. 1978;75:612-8.
79. Slavin J, Nelson N, McNamara E, Cashmere K. Bowel function of healthy men consuming liquid diets with and without dietary fiber. *J Parenter Enteral Nutr*. 1985;9:317-21.
80. Timm DA, Thomas W, Boileau T, Williamson-Hughes PS, Slavin JL. Polydextrose and soluble corn fiber increase five-day fecal wet weight in healthy men and women. *J Nutr*. 2013;143(4):473-8.
81. Klosterbuer A, Hullar MA, Li F, Traylor E, Lampe JW, Thomas W, et al. Gastrointestinal effects of resistant starch, soluble maize fiber and pullulan in healthy adults. *Br J Nutr*. 2013;7:1-7.
82. Marlett J, Slavin J, Brauer P. Comparison of dye and pellet gastrointestinal transit time during controlled diets differing in protein and fiber levels. *Dig Dis Sci*. 1981;26(3):208-13.
83. Timm D, Willis H, Thomas W, Sanders L, Boileau T, Slavin J. The use of a wireless motility device (SmartPill(R)) for the measurement of gastrointestinal transit time after a dietary fibre intervention. *Br J Nutr*. 2011;105(9):1337-42.
84. Stevens J, VanSoest P, Robertson J, Levitsky D. Mean transit time measurement by analysis of a single stool after ingestion of multicolored plastic pellets. *Am J Clin Nutr*. 1987;46:1048-54.

85. Gear JS, Brodribb AJ, Ware A, Mann J. Fibre and bowel transit times. *Br J Nutr.* 1981;45:77-82.
86. Evans R, Kamm M, Hinton J, Lennard-Jones J. The normal range and a simple diagram for recording whole gut transit time. *Int J Colorectal Dis.* 1992;7:15-7.
87. Silk D, Walters ER, Duncan H, Green C. The effect of a polymeric enteral formula supplemented with a mixture of six fibres on normal human bowel function and colonic motility. *Clin Nutr.* 2001;20:49-58.
88. Whelan K, Judd P, Preedy V, Taylor M. Covert assessment of concurrent and construct validity of a chart to characterize fecal output and diarrhea in patients receiving enteral nutrition. *J Parenter Enteral Nutr.* 2008;32(2):160-8.
89. Whelan K, Judd P, Taylor M. Assessment of fecal output in patients receiving enteral tube feeding: Validation of a novel chart. *Eur J Clin Nutr.* 2004;58(7):1030-7.
90. Wenzl H, Fine K, Schiller L, Fordtran J. Determinants of decreased fecal consistency in patients with diarrhea. *Gastroenterol.* 1995;108:1729-38.
91. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol.* 1997;32:920-4.
92. Horvath A, Dziechciarz P, Szajewska H. Glucomannan for abdominal pain-related functional gastrointestinal disorders in children: A randomized trial. *World J Gastroenterol.* 2013;19(20):3062-8.
93. Bishop S, Young H, Golsmith D, Buldock D, Chin M, Bellomo R. Bowel motions in critically ill patients: A pilot observational study. *Crit Care Resusc.* 2010;12(3):182-5.
94. Lane M, Czyzewski D, Chumpitazi B, Shulman R. Reliability and validity of a modified bristol stool form scale for children. *J Pediatr.* 2011;159(3):437-41.
95. Markland A, Palsson O, Goode P, Burgio K, Busby-Whitehead J, Whitehead W. Association of low dietary intake of fiber and liquids with constipation: evidence from the National Health and Nutrition Examination Survey. *Am J Gastroenterol.* 2013;108(5):796-803.
96. Bradley R. Moisture and total solids analysis. In: Nielsen S, editor. *Food Analysis*, 3rd edition. ; 2003. p. 81-101.
97. Kerr KR, Vester Boler BM, Morris CL, Liu KJ, Swanson KS. Apparent total tract energy and macronutrient digestibility and fecal fermentative end-product concentrations of domestic cats fed extruded, raw beef-based, and cooked beef-based diets. *J Anim Sci.* 2012;90:515-22.
98. Chung Y, Hsu C, Ko C, Chan Y. Dietary intake of xylo-oligosaccharides improves the intestinal microbiota, fecal moisture, and pH value in the elderly. *Nutr Res.* 2007;27:756-61.

99. Scholtens P, Alles M, Willemsen L, van den Braak C, Bindels J, Boehm G, et al. Dietary fructo-oligosaccharides in healthy adults do not negatively affect faecal cytotoxicity: a randomised, double-blind, placebo-controlled crossover trial. *Br J Nutr.* 2006;95:1143-9.
100. Smith T, Pesti GM, Bakalli R, Kilburn J, Edwards HM. The use of near-infrared reflectance spectroscopy to predict the moisture, nitrogen, calcium, total phosphorus, gross energy, and phytate phosphorus contents of broiler excreta. *Poult Sci.* 2001;80:314-9.
101. Alam N, Meier R, Rausch T, Meyer-Wyss B, Hildebrand P, Schneider H, et al. Effects of a partially hydrolyzed guar gum on intestinal absorption of carbohydrate, protein and fat: A double-blind controlled study in volunteers. *Clin Nutr.* 1998;17:125-9.
102. Zimmaro D, Rolandelli R, Koruda M, Settle R, Stein T, Rombeau J. Isotonic tube feeding formula induces liquid stool in normal subjects: Reversal by pectin a. *J Parenter Enteral Nutr.* 1989;13:117-23.
103. Meier R, Beglinger C, Schneider H, Rowedder A, Gyr K. Effect of a liquid diet with and without soluble fiber supplementation on intestinal transit and cholecystokinin release in volunteers. *J Parenter Enteral Nutr.* 1993;17:231-5.
104. Lampe J, Effertz M, Larson J, Slavin J. Gastrointestinal effects of modified guar gum and soy polysaccharide as part of an enteral formula diet. *J Parenter Enteral Nutr.* 1992;16(538):544.
105. Millet S, van Oeckel M, Aluwe M, Delezie E, de Brabander D. Prediction of *in vivo* short-chain fatty acid production in hindgut fermenting mammals: Problems and pitfalls. *Crit Rev Food Sci Nutr.* 2010;50:605-19.
106. Macfarlane G, Macfarlane S. Factors affecting fermentation reactions in the large bowel. *Proceedings of the Nutrition Society.* 1993;52:367-73.
107. Awati A, Williams B, Bosch M, Gerrits W, Verstegen M. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. *J Anim Sci.* 2006;84(8):2133-40.
108. Hughes R, Magee E, Bingham S. Protein degradation in the large intestine: Relevance to colorectal cancer. *Curr Issues Intest Microbiol.* 2000;1(2):51-8.
109. Demigne C, Remesy C, Morand C. Short chain fatty acids. In: Gibson G, Roberfroid M, editors. *Colonic microbiota, nutrition and health.* 1st ed. Boston, MA: Kluwer Academic Publishers; 1999. p. 55-70.
110. Cummings JH. Short chain fatty acids in the human colon. *Gut.* 1981;22:763-79.
111. Binder H, Mahta P. Short-chain fatty acids stimulate active Na and Cl absorption *in vitro* in the rat distal colon. *Gastroenterol.* 1989;96:989-96.

112. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev.* 1990;70(2):567-90.
113. Roediger WE. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterol.* 1982;83:424-9.
114. Clausen MR, Mortensen PB. Kinetic studies on the metabolism of short-chain fatty acids and glucose by isolated rat colonocytes. *Gastroenterol.* 1994;106(2):423-32.
115. Chaia AP, Oliver G. Intestinal microflora and metabolic activity. In: Fuller R, Perdigon G, editors. *Gut Flora, Nutrition, Immunity and Health*. 1st ed. Oxford, UK: Blackwell Publishing; 2003. p. 77-98.
116. Wong J, de Souza R, Kendall C, Emam A, Jenkins D. Colonic health: Fermentation and short chain fatty acids. *J Clin Gastroenterol.* 2006;40(3):235-43.
117. Peters SG, Pomare EW, Fisher CA. Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. *Gut.* 1993;33:1249-52.
118. Duncan S, Louis P, Thomson J, Flint H. The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol.* 2009;11(8):2112-22.
119. Macfarlane GT, Gibson GR, Cummings JH. Comparison of fermentation reactions in different regions of the human colon. *J Appl Bacteriol.* 1992;72:57-64.
120. Baghurst PA, Baghurst KI, Record SJ. Dietary fibre, non-starch polysaccharides and resistant starch: A review. *Food Australia.* 1996;48:3-35.
121. Costabile A, Kolida S, Klinder A, Gietl E, Bauerlein M, Froberg C, et al. A double-blind, placebo-controlled, cross over study to establish the bifidogenic effect of a very-long-chain inulin extracted from globe artichoke (*Cynara scolymus*) in healthy human subjects. *Br J Nutr.* 2010;104:1007-17.
122. Patten G, Bird A, Topping D, Abeywardena M. Effects of convenience rice congee supplemented diets on guinea pig whole animal and gut growth, caecal digesta SCFA and *in vitro* ileal contractility. *Asia Pac J Clin Nutr.* 2004;13(1):92-100.
123. Schneider S, Girard-Pipau F, Anty R, van der Linde E, Philipsen-Geerling B, Knol J, et al. Effects of total enteral nutrition supplemented with a multi-fibre mix on faecal short-chain fatty acids and microbiota. *Clin Nutr.* 2006;25:82-90.
124. Rechkemmer G, von Engelhardt W. Concentration and pH dependence of short-chain fatty acid absorption in the proximal and distal colon of guinea pig (*Cavia porcellus*). *Comp Biochem Physiol A Comp Physiol.* 1988;91(4):659-63.

125. Robinson R, Feirtag J, Slavin J. Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects. *J Am Coll Nutr.* 2001;20(4):279-85.
126. Levitt MD. Intestinal gas. *Proc Nutr Soc.* 1985;44:145-6.
127. Levitt MD, Bond JH. Flatulence. *Ann Rev Med.* 1980;31:127-37.
128. Vester Boler B, Hernot D, Boileau T, Bauer L, Middelbos I, Murphy M, et al. Carbohydrates blended with polydextrose lower gas production and short-chain fatty acid production in an *in vitro* system. *Nutr Res.* 2009;29:631-9.
129. Wierdsma N, Van Bodegraven A, Uitdehaag B, Arjaans W, Savelkoul P, Kruijenga H, et al. Fructo-oligosaccharides and fibre in enteral nutrition has a beneficial influence on microbiota and gastrointestinal quality of life. *Scand J Gastroenterol.* 2009;44:804-12.
130. Revicki DA, Wood M, Wiklund I, Crawley J. Reliability and validity of the gastrointestinal symptom rating scale in patients with gastroesophageal reflux disease. *Quality of Life Research.* 1998;7(1):75-83.
131. Anderson RT, Aaronson NK, Wilkin D. Critical review of the international assessments of health-related quality of life. *Qual Life Res.* 1993;2(6):369-95.
132. Eypasch E, Williams J, Wood-Dauphinee S, Ure B, Schmulling C, Neugebauer E. Gastrointestinal quality of life index: Development, validation and application of a new instrument. *Br J Surg.* 1995;82:216-22.
133. DiBaise J, Zhang H, Crowell M, Krajmalnik-Brown R, Decker G, Rittmann B. Gut microbiota and its possible relationship with obesity. *Mayo Clin Proc.* 2008;83(4):460-9.
134. Corr S, Hill C, Gahan C. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. *Adv Food Nutr Res.* 2009;56:1-15.
135. Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN, et al. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Anim Health Res Rev.* 2008;9(2):217-25.
136. Wu G, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;Oct 7(334):105-8.
137. Harmsen H, Raangs G, Franks A, Wildeboer-Veloo AC, Welling G. The effect of the prebiotic inulin and the probiotic *Bifidobacterium longum* on the fecal microflora of healthy volunteers measured by FISH and DGGE. *Microb Ecol Health Dis.* 2002;14:219.
138. Jansen G, Wildeboer-Veloo A, Tonk R, Franks A, Welling G. Development and validation of an automated, microscopy-based method for enumeration of groups of intestinal bacteria. *J Microbiol Methods.* 1999;37:215-21.

139. Ben Amor K, Vaughan E. Molecular ecology of the human intestinal microbiota. In: Ouwehand A, Vaughan E, editors. *Gastrointestinal Microbiology*. 1st ed. New York, NY: Taylor and Francis; 2006. p. 1-23.
140. Backhed F, Ley RS, J., Peterson D, Gordon J. Host-bacterial mutualism in the human intestine. *Science*. 2005;307:1915-20.
141. Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol*. 1998;64(10):3854-9.
142. Kruse H, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr*. 1999;82:375-82.
143. Bouhnik Y, Vahedi K, Achour L, Attar S, Salfati J. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *J Nutr*. 1999;129:113-6.
144. Kolida S, Gibson G. Prebiotic capacity of inulin-type fructans. *J Nutr*. 2007;137:2503S-6S.
145. Bouhnik Y, et. al. Fecal recovery in human of viable bifidobacterium sp. ingested in fermented milk. *Gastroenterol*. 1992;102:875-8.
146. Ley RE, Turnbaugh PJ, Klein S, Gordon J. Microbial ecology: Human gut microbes associate with obesity. *Nature*. 2006;444(7122):1022-3.
147. Mueller ea. Differences in fecal microbiota in different European study populations in relation to age, gender and country: A cross-sectional study. *Appl Environ Microbiol*. 2006;22(2):1027-33.
148. Minelli ea. Bacterial fecal flora in healthy women of different ages. *Micro Ecol Health Dis*. 1993;6:43-51.
149. Zoetendal E, Akkermans A, Akkermans-can Vliet W, de Visser J, de Vos W. The host genotype affects the bacterial community in the human gastrointestinal tract. *Micro Ecol Health Dis*. 2001;13(3):129-34.
150. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiol*. 2010;156(11):3216-23.
151. Williams BL, Hornig M, Buie T, Bauman ML, Paik MC, Wick I, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS ONE*. 2011;6(9):1-21.
152. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: Composition and development. *Acta Paediatr*. 2003;441:48-55.

153. Blaut M, Collins M, Welling G, Dore J., van Loo J, de Vos W. Molecular biological methods for studying the gut microbiota: The EU human gut flora project. *Br J Nutr.* 2002;87(Suppl 2):S203-11.
154. O'Sullivan DJ. Methods for analysis of the intestinal microflora. *Curr Issues Intest Microbiol.* 2000;1(2):39-50.
155. Zoetendal E, Collier C, Koike S, Mackie R, Gaskins H. Molecular ecological analysis of the gastrointestinal microbiota: A review. *J Nutr.* 2004:465-72.
156. Amann R, Ludwig W., Schleifer K. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev.* 1995;59:143-69.
157. Daims H, Stoecker K, Wagner M. Fluorescence in situ hybridization for the detection of prokaryotes. In: Osborn AM, Smith C, editors. *Molecular Microbial Ecology.* New York, NY: Taylor and Francis; 2005. p. 213-39.
158. Lay C. Molecular tools for investigating the gut microbiota. In: Charalampopoulos D, Rastall R, editors. 1st ed. New York, NY: Springer Publishing; 2009. p. 33-78.
159. Sharp R, Ziemer C. Application of taxonomy and systematics to molecular techniques of intestinal microbiology. In: Gibson G, Roberfroid M, editors. *Colonic microbiota, nutrition and health.* 1st ed. Boston, MA: Kluwer Academic Publishers; 1999. p. 167-90.
160. Tasse L, Bercovici J, Pizzut-Serin S, Robe P, Tap J, Klopp C, et al. Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. *Genome Research.* 2010;20(11):1605-12.
161. Wu G, Lewis J, Hoffmann C, Chen Y, Knight R, Bittinger K, et al. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. *BMC Microbiol.* 2010;10(206):1-14.
162. Lamendella R, VerBerkmoes N, Jansson J. 'Omics' of the mammalian gut - new insights into function. *Curr Opin Biotechnol.* 2012;23:491-500.
163. Tunglund B. Fructooligosaccharides and other fructans: Structures and occurrence, production, regulatory aspects, food applications, and nutritional health significance. In: Eggleston G, Cote G, editors. *Oligosaccharides in Food and Agriculture.* Washington, DC: American Chemical Society; 2003. p. 135-52.
164. Rastall RA. Functional oligosaccharides: Application and manufacture. *Ann Rev Food Sci Tech.* 2010;1:305-39.
165. Franck A. Technological functionality of inulin and oligofructose. *Br J Nutr.* 2002;87:S287-91.

166. Stewart M, Timm D, Slavin J. Fructo-oligosaccharides exhibit more rapid fermentation than long-chain inulin in an *in vitro* fermentation system. *Nutr Res.* 2008;28:329-34.
167. Berggren A, Bjorck I, Margareta E, Nyman G. Short-chain fatty acid content and pH in caecum of rats given various sources of carbohydrates. *J Sci Food Agric.* 1993;63:397-406.
168. Gibson G, Beatty E, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterol.* 1995;108:975-82.
169. van Dokkum W, Wezendonk B, Srikumar T, van den Heuvel E. Effect of nondigestible oligosaccharides on large-bowel functions, blood lipi concentrations and glucose absorption in young healthy male subjects. *Eur J Clin Nutr.* 1999 53:1-7.
170. Slavin J, Feirtag J. Chicory inulin does not increase stool weight or speed up intestinal transit time in healthy male subjects. *Food Funct.* 2011;2(72):72-7.
171. Ripoll C, Flourie B, Megnien S, Hermand O, Janssens M. Gastrointestinal tolerance to an inulin-rich soluble roasted chicory extract after consumption in healthy subjects. *Nutr.* 2010;26:799-803.
172. FAO. Gum arabic, food and nutrition paper. Rome: 1999. Report No.: 52, Addendum 7.
173. Ali B, Ziada A, Blunden G. Biological effects of gum arabic: A review of some recent research. *Food Chem Toxicol.* 2009;47:1-8.
174. Islam AM, Phillips GO, Slijibo A, Snowden M, Williams P. A review of recent developments on regulatory, structural and functional aspects of gum arabic. *Food Hydrocoll.* 1997;11:357-65.
175. Randall RC, Phillips GO, Williams PA. Fractionation and characterization of gum from acacia senegal. *Food Hydrocoll.* 1989;3(1):65-75.
176. Gum arabic / gum acacia [Internet].: CE Roeper GmbH; 2013; cited July 23, 2013].
177. Mahendran T, Williams PA, Phillips GO, Al-Assaf S, Baldwin TC. New insights into the structural characteristics of the arabinogalactan-protein (AGP) fraction of gum arabic. *J Agric Food Chem.* 2008;56:9269-76.
178. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. Acacia gum is a bifidogenic dietary fibre with high digestive tolerance in healthy humans. *Microb Ecol Health Dis.* 2003;15:43-50.
179. Titgemeyer E, Bourquin L, Fahey G, Garleb K. Fermentability of various fiber sources by human fecal bacteria *in vitro*. *Am J Clin Nutr.* 1991;53:1418-24.

180. Bliss DZ, Stein TP, Schleifer CR, Settle RG. Supplementation with gum acacia fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. *Am J Clin Nutr.* 1996;63:392-8.
181. Calame W, Weseler A, Viebke C, Flynn CS, A. Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose dependent manner. *Br J Nutr.* 2008;100:1269-75.
182. Dahl W, Whiting S, Healey A, Zello G, Hildebrandt S. Increased stool frequency occurs when finely processed pea hull fiber is added to usual foods consumed by elderly residents in long-term care. *J Am Diet Assoc.* 2003;103(9):1199-202.
183. Aluko R, Mofolasayo O, Watts B. Emulsifying and foaming properties of commercial yellow pea (*Pisum sativum* L.) seed flours. *J Agric Food Chem.* 2009;57:9793-800.
184. Ralet M, Valle G, Thibault J. Raw and extruded fibre from pea hulls. part I: Composition and physico-chemical properties. *Carbohydr Polym.* 1993;20:17-23.
185. Turnbull C, Baxter A, Johnson S. Water-binding capacity and viscosity of Australian sweet lupin kernel fibre under *in vitro* conditions simulating the human upper gastrointestinal tract. *Int J Food Sci Nutr.* 2005;56(2):87-94.
186. Leterme P, Thewis A, van Leeuwen P, Monmart T, Huisman J. Chemical composition of pea fibre isolates and their effect on the endogenous amino acid flow at the ileum of the pig. *J Sci Food Agric.* 1996;72(1):127-34.
187. Cherbut C, Salvador V, Barry JL, Doulay F, Delort-Laval J. Dietary fibre effects on intestinal transit in man: Involvement of their physicochemical and fermentative properties. *Food Hydrocoll.* 1991;5:15-22.
188. Guedon C, Ducrotte P, Michel Antoine J, Denis P, Colin R, Lerebours E. Does chronic supplementation of the diet with dietary fibre extracted from pea or carrot affect colonic motility in man? *Br J Nutr.* 1996;76:51-61.
189. Goetze O, Fruehauf H, Pohl D, Giarre M, Rochat F, Ornstein K, et al. Effect of a prebiotic mixture on intestinal comfort and general wellbeing in health. *Br J Nutr.* 2008;100:1077-85.
190. Whelan K, Judd P, Tuohy K, Gibson G, Preedy V, Taylor M. Fecal microbiota in patients receiving enteral feeding are highly variable and may be altered in those who develop diarrhea. *Am J Clin Nutr.* 2009;89:240-7.
191. Robles Alonso V, Guarner F. Linking the gut microbiota to human health. *Br J Nutr.* 2013;109:S21-6.
192. Klosterbuer A, Roughead ZF, Slavin J. Benefits of dietary fiber in clinical nutrition. *Nutr Clin Pract.* 2011;26:625-35.

193. Block G, Gillespie C, Rosebaum EH, Jenson C. A rapid food screener to assess fat and fruit and vegetable intake. *Am J Prev Med.* 2000;18(4):284-8.
194. Frary CD, Johnson R. Energy. In: Mahan LK, Escott-Stump S, editors. *Krause's Food and Nutrition Therapy*. 12th ed. Saunders Elsevier; 2008. p. 22-38.
195. Anonymous. Loss on drying, moisture in meat, "B" air drying. AOAC official method 950.46. Association of Official Analytical Chemists. 1991.
196. Martin-Pelaez S, Gibson G, Martin-Orue S, Klinder A, Rastall R, LaRagione R, et al. *In vitro* fermentation of carbohydrates by porcine faecal inocula and their influence on *Salmonella typhimurium* growth in batch culture systems. *FEMS.* 2008;66:608-19.
197. University of Vienna, Department of Microbial Ecology [<http://www.microbial-ecology.net/probebase/>].
198. Daims H, Bruhl A, Amann R, Schleifer K, Wagner M. The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. *Syst Appl Microbiol.* 1999;22:434-44.
199. Langendijk P, Schut F, Jansen G, Raangs G, Kamphuis G, Wilkinson M, et al. Quantitative fluorescent in situ hybridisation of bifidobacterium spp. with genus specific 16S rRNA targeted probes and its application in fecal samples. *Appl Environ Microbiol.* 1995;61:3069-75.
200. Harmsen H, Elfferich P, Schut F, Welling G. A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent in situ hybridization. *Micro Ecol Health Dise.* 1999;11:3-12.
201. Manz W, Amann R, Ludwig W, Vancanneyt M, Schleifer K. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiol.* 1996;142:1097-106.
202. Franks A, Harmsen H, Raangs G, Jansen G, Schut F, Welling G. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol.* 1998;64:3336-45.
203. Slavin J. Impact of the proposed definition of dietary fiber on nutrient databases. *J Food Compost Anal.* 2003;16:287-91.
204. Bowling TE, Raimundo A, Grimble G, Silk D. Colonic secretory effect in response to enteral feeding in humans. *Gut.* 1994;35:1734-41.
205. Harder H, Serra J, Azpiroz F, Passos M, Aquade S, Malagelada JR. Intestinal gas distribution determines abdominal symptoms. *Gut.* 2003;52:1708-13.

206. Hernot D, Boileau TW, Bauer L, Middelbos I, Murphy M, Swanson K, et al. *In vitro* fermentation profiles, gas production rates and microbiota modulation as affected by ceratin fructans, galacto-oligosaccharides and polydextrose. *J Agric Food Chem.* 2009;57:1354-61.
207. Wisker E, Daniel M, Rave G, Feldheim W. Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: Comparative studies in human subjects and *in vitro*. *Br J Nutr.* 1998;80:253-61.
208. McBurney MI, Thompson LU. Effect of human faecal inoculum on *in vitro* fermentation variables. *Br J Nutr.* 1987;58:233-43.
209. Goering HK, Van Soest PJ. Forage fiber analysis: Apparatus, reagents, procedures and some applications. In: Department of Agriculture, editor. *Agriculture Handbook No. 379.* Washington, D.C.: US Government Printing Office; 1970. p. 1-20.
210. Timm DA, Stewart ML, Hospattankar A, Slavin JL. Wheat dextrin, psyllium, and inulin produce distinct fermentation patterns, gas volumes, and short-chain fatty acid profiles *in vitro*. *J Med Food.* 2010;13:961-6.
211. Gibson GR. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *J Nutr.* 1999;129:1438S-41S.
212. Holtug K, Hove H, Mortensen PB. Stimulation of butyrate absorption in the human rectum *in vivo*. *Scand J Gastroenterol.* 1995;30(10):982-8.
213. Billich C, Levitan R. Effects of sodium concentration and osmolality on water and electrolyte absorption from the intact human colon. *J Clin Invest.* 1969;48:1336-47.

Appendix A: Sample Size Calculation

The sample size of the human study was powered to detect differences in stool weight. The power/sample size calculation was based on the stool weight data reported in Table 4 of the Whelan et al paper (48) who compared enteral formula containing FOS and pea fiber with standard fiber-free formula. Whelan reported mean stool weight +/- standard deviations of:

Habitual diet	132.4 + 68.5 g/day
Fiber-free enteral formula	48.3 + 30.1 g/day
Fiber-containing enteral formula	73.2 + 37.5 g/day

Assuming standard deviations of 30 and 38 for the two treatments, and a within-subject correlation of 0.6, a sample of 20 subjects is required for this crossover to have at least 80% power to detect a difference of 21g/day at the 0.05 level between the two study treatments. This minimum detectable difference is smaller than the difference of 25 g/day found by (48).

The POWER Procedure

Paired t Test for Mean Difference

Fixed Scenario Elements	
Distribution	Normal
Method	Exact
Mean Difference	21
Standard Deviation 1	30
Standard Deviation 2	38
Correlation	0.6
Number of Pairs	20
Number of Sides	2
Null Difference	0
Alpha	0.05

Computed Power	
	Power
	0.814

Appendix B: Subject Eligibility Screening Questionnaire

Thank you for your interest in participating in our study. Before I can determine if you meet the criteria to participate in the study, I need your responses to the following questions. After receiving your responses, I will inform you whether or not you meet the criteria for the study. If you do not meet the criteria for the study, I will destroy the information collected.

NAME _____ DATE _____

DATE OF BIRTH : _____ AGE _____

HT _____ WT _____ (office use : BMI _____)

1. Do you smoke or chew tobacco now?
2. *For women*, are you currently pregnant or lactating?
3. Have you taken antibiotics within the last 6 months?
4. Do you currently take laxatives?
5. Are you currently taking any prescription medications other than oral contraceptives?
6. Do you take any over the counter medications such as aspirin, ibuprofen, etc.?

If yes, list;

7. Have you ever been diagnosed with the following diseases or conditions?

Diabetes (type I or type II), Cancer (any type), Kidney disease, Liver disease, Binge eating disorder, Ulcerative Colitis, Crohns Disease, Any other gastrointestinal conditions

8. Have you consumed probiotic foods, such as yogurt, kefir, kimchi, etc. in the last month?

If yes, how much and how frequently?

9. Think about your eating habits over the past year or so. About how often do you eat each of the following foods? Remember breakfast, lunch, dinner, snacks and eating out. Check one radio button for each food. Enter answers at:
<http://www.nutritionquest.com/wellness/free-assessment-tools-for-individuals/fruit-vegetable-fiber-screener/>

Fruits, Vegetables, and Grains	Less than 1/WEEK	Once a WEEK	2-3 times a WEEK	4-6 times a WEEK	Once a DAY	2+ a DAY
Fruit juice, like orange, apple, grape, fresh, frozen or canned. (Not sodas or other drinks)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
How often do you eat any fruit, fresh or canned (not counting juice?)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vegetable juice, like tomato juice, V-8, carrot	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Green salad	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Potatoes, any kind, including baked, mashed or french fried	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vegetable soup, or stew with vegetables	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Any other vegetables, including string beans, peas, corn, broccoli or any other kind	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fiber cereals like Raisin Bran, Shredded Wheat or Fruit-n-Fiber	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beans such as baked beans, pinto, kidney, or lentils (not green beans)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dark bread such as whole wheat or rye	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

10. Do you currently exercise more than 2 hr./week?

Please describe your exercise routine. If you do not have an exercise routine, please describe your activity.

11. Are you a vegetarian?

12. Do you have any food or other allergies?

If YES, what are they?

13. Do you take ANY supplements? This includes vitamin/mineral supplements, fiber supplements like Metamucil or Citrucel, pre or probiotic supplements, herbal supplements, etc.

If yes, please list the supplements and the dose/frequency:

14. Have you participated or are currently participating in a research study?

If yes, when?

15. Have you lost or gained weight in the past 3 months or are trying to gain or lose weight currently?

If yes, how much?

16. *For women*, have you gone through menopause?

17. This study requires you to consume a liquid nutrition product (similar to Ensure) for two 14 day periods exclusively (with the exception of non-caloric beverages such as coffee, tea, and water). Are you willing and able to do this?

18. This study will require you to collect fecal samples for the last five days of each study treatment (4 treatment periods). Are you willing and able to do this?

19. The fecal collection on the last day of each treatment period needs to be delivered to the study coordinator within 1 hr. of defecation. This means, during the entire study, four samples will need to be delivered within 1 hr. Are you willing and able to do this?

20. This study will require you to record diet records, consume a capsule of plastic pellets for a measurement, and fill out a 36 question questionnaire 4 different times. Are you willing and able to do this?

21. This study will include 6 visits to McNeal Hall on the St. Paul Campus of the University of Minnesota. Each visit will last approximately 30 min. Are you willing to do this? (We cover parking expenses)

22. Do you have reliable transportation?

23. Do you travel out of the Twin Cities area frequently?

24. Are there specific dates you will not be available (holidays, etc)?

25. After hearing about the study, how do you feel about the time commitment and effort involved to complete the study?

ADDRESS _____

CITY _____ STATE _____ ZIP _____

TELEPHONE NUMBERS: work (daytime): _____

Home (evening): _____

Email: _____

Best time of day to be reached _____

Comments:

Appendix C: Approved Subject Consent Form

GUT HEALTH RESPONSE TO A NEW FIBER BLEND STUDY CONSENT FORM

You are invited to participate in a research study of dietary fibers and their effects on gut health. You were selected as a possible participant because you are a healthy individual that meets the study population criteria. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

This study is being conducted by Joanne Slavin, Ph.D., RD and Katie Koecher, B.S. in the Department of Food Science and Nutrition. The Department of Food Science and Nutrition at the University of Minnesota is in the College of Food, Agricultural and Natural Resource Sciences. The study is funded by Nestle Research Center.

Part 1. Study Purpose

The purpose of the study is to examine the effects of dietary fiber on gut health. The results of the study will be used to assess the gut health benefits of adding fibers to a liquid food product. The fibers included in this study will be added to the liquid nutrition drink (complete nutrition source) and both the fibers and drink are food products currently on the market and are safe to consume. Fiber levels in the liquid nutrition drink, as part of a complete nutrition source, are in similar amounts to the recommended dietary guidelines.

Part 2. Study Procedures

In total, approximately 20 subjects will participate in this study. If you agree to participate, we would ask you to do as follows.

The study consists of six visits to the Food Science and Nutrition Department at the University of Minnesota while completing four pre-planned study phases in the subject's home (or site of choice). At each visit, subjects will receive information and materials for completing the corresponding phase of the study. The study is organized into four phases; regular diet (initial) for 14 days, liquid diet 1 (14 days), regular diet (washout-28 days), and liquid diet 2 (14 days). During each phase subjects will complete food intake records, consume a gelatin capsule of pellets for measurement of gastrointestinal transit time, fill out a gastrointestinal health survey (36 questions) and collect fecal samples during the last five days of each phase. At certain visits, subjects will deliver their completed records and fecal samples from the previous study phase. A schedule of the visits and corresponding events will be given to you in addition to thoroughly discussed.

Description of specific visit information:

The initial visit will include height and weight measurements. At the first visit, the stool sample collection kits, diet records, gastrointestinal survey, and the capsule of the pellets will be provided along with a schedule of events to assist you during the

regular diet (initial) phase. Diet records will be filled out during days 1-14, transit time pellet capsule will be consumed on day 8, the gastrointestinal (GI) survey will be filled out on day 14, and total fecal samples will be collected day 9-13. The fecal collection on day 13 will require a special collection procedure and will need to be delivered to the study coordinator within an hour of defecation. At the conclusion of the initial diet, subjects will have visit 2.

During visit 2, fecal samples, the GI survey and diet records will be delivered to the laboratory. In addition, during visit 2 the stool sample collection kits, diet records, gastrointestinal survey, transit time capsule, and liquid diet 1 will be provided for the next study phase (liquid diet 1). You will be given information on how much liquid diet to consume based on your height, weight and activity level such that you maintain body weight. The liquid diet is to be consumed exclusively except for non-caloric, non fiber containing, liquids such as sugar free flavoring, coffee, tea, and water. Again, the liquid diet is a complete nutrition source. During liquid diet 1 phase, diet records will be filled out for days 14-28, transit time capsule will be consumed on day 22, the GI survey will be filled out on day 28, and total fecal samples will be collected days 23-27. The fecal collection on day 27 will require a special collection procedure and will need to be delivered to the study coordinator within an hour of defecation. At the conclusion of the liquid diet 1 phase, subjects will have visit 3.

During visit 3, fecal samples and the survey and diet records will be delivered to the laboratory. In addition, during visit 3, the stool sample collection kits, diet records, gastrointestinal survey, and transit time capsule will be provided along with a schedule of events to assist you during the regular diet (washout phase). The washout phase will last 28 days, and diet records will be filled out during days 42-56, transit time capsule will be consumed on day 50, the gastrointestinal survey will be filled out on day 56 and total fecal samples will be collected days 51-55. The fecal collection on day 55 will require a special collection procedure and will need to be delivered to the study coordinator within an hour of defecation. At the conclusion of the washout phase, subjects will have visit 4.

During visit 4, fecal samples and the survey and diet records will be delivered to the laboratory. In addition, during visit 4 the stool sample collection kits, diet records, gastrointestinal survey, transit time capsule, and liquid diet 2 will be provided for the next study phase (liquid diet 2). You will be given information on how much liquid diet to consume based on your height, weight and activity level such that you maintain body weight. The liquid diet is to be consumed exclusively except for non-caloric, non fiber containing, liquids such as sugar free flavoring, coffee, tea, and water. Again, the liquid diet is a complete nutrition source. During liquid diet 2 phase, diet records will be filled out for days 56-70 transit time capsule will be consumed on day 64, the gastrointestinal survey will be filled out on day 70, and total fecal samples will be collected days 65-69. The fecal collection on day 69 will require a special collection procedure and will need to be delivered to the study coordinator within an hour of defecation. At the conclusion of the liquid diet 2 phase, subjects will have visit 5.

During visit 5, fecal samples and the survey and diet records will be delivered to the laboratory.

It will take approximately three months to complete the entire study. All procedures in this study have been completed by subjects before (none are experimental).

Part 3. Risks of Study Participation

The fibers used in this study are provided in amounts commonly taken in foods and are already used in commercial foods available in the United States. Addition of fiber to liquid nutrition products has no known risks, although some fibers may cause intestinal gas and loose stools in some individuals.

Part 4. Benefits of Study Participation

There is no direct benefit by participating in this study.

Part 5. Alternatives to Study Participation

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

Part 6. Study Costs/Compensation

Study related visits, procedures, and supplies (including the liquid nutrition drink) will be provided at no cost to you. Subjects will receive \$200 upon completion of first liquid diet phase. An additional \$800 payment will be disbursed to the subject upon completing the second liquid diet phase (completion of the entire study). *Please note, if you withdraw from the study, you are required to return any remaining study product to the researchers.*

Part 7. Research Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, seek immediate medical care and inform the study investigator/staff as soon as possible.

Part 8. Confidentiality

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by Nestle Research Center and by departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

Part 9. Protected Healthy Information

Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPAA authorization for details concerning the use of this information.

Part 10. Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University of Minnesota. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Part 11. Contacts and Questions

The researchers conducting this study are Joanne Slavin and Katie Koecher. You may ask any questions you have now, or if you have questions later, **you are encouraged to contact them at 612-624-7234 or 612-624-4793.**

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this office in writing or in person at University of Minnesota Medical Center, Fairview-Riverside Campus, 2200 Riverside Avenue, Minneapolis, MN 55454.

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

Statement of Consent

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

Signature of Subject _____

Date _____

Signature of Investigator _____

Date _____

Appendix D - Enteral Product Nutrition Information and Ingredient List

Control (fiber free) formula

Nutrition Information	1 x 8.45 fl oz can	
	Contents	Units
<i>Serving Size</i>	250	ml
Calories	375	
Protein	16.9	G
Carbohydrate	42.0	G
Fat	16.2	G
Sodium	322	MG
Potassium	536	MG
Vitamin A ¹	2680	IU
Vitamin C	80.4	MG
Thiamin	0.8	MG
Riboflavin	0.91	MG
Niacin	10.7	MG
Calcium	268	MG
Iron	4.82	MG
Vitamin D	107	IU
Vitamin E	16.1	IU
Vitamin B6	1.07	MG
Folic Acid	161	MCG
Vitamin B12	3.22	MCG
Phosphorus	268	MG
Iodine	40.2	MCG
Magnesium	107	MG
Zinc	8.04	MG
Copper	0.54	MG
Biotin	121	MCG
Pantothenic Acid	5.36	MG
Vitamin K	21.4	MCG
Choline	134	MG
Chloride	402	MG
Manganese	0.54	MG
Selenium	18.8	MCG
Chromium	32.2	MCG
Molybdenum	20.1	MCG
L-Carnitine	26.8	MG
Taurine	26.8	MG

Ingredients: Water, maltodextrin, sucrose, sodium caseinate (milk), canola oil, medium chain triglycerides, calcium caseinate, soybean oil, potassium citrate, calcium phosphate tribasic, magnesium chloride, sodium citrate, artificial flavor, soy lecithin, sodium ascorbate, choline chloride, taurine, l-carnitine, zinc sulfate, alpha tocopheryl acetate, niacinamide, ferrous sulfate, calcium pantothenate, copper gluconate, BHA/BHT (to preserve freshness), pyridoxine hydrochloride, thiamine hydrochloride, manganese sulfate, beta carotene, riboflavin, vitamin A palmitate, folic acid, biotin,

chrominum chloride, potassium iodide, sodium molybdate, sodium selenite, phytonadione, cyanocobalamin, cholecalciferol

Fiber blend formula

Nutrition Information	1 x 8.45 fl oz can	
	Contents	Units
<i>Serving Size</i>	250	ml
Calories	375	
Protein	16.9	G
Carbohydrate ¹	42.0	G
Fat	16.2	G
Sodium	322	MG
Potassium	536	MG
Vitamin A ²	2680	IU
Vitamin C	80.4	MG
Thiamin	0.8	MG
Riboflavin	0.91	MG
Niacin	10.7	MG
Calcium	268	MG
Iron	4.82	MG
Vitamin D	107	IU
Vitamin E	16.1	IU
Vitamin B6	1.07	MG
Folic Acid	161	MCG
Vitamin B12	3.22	MCG
Phosphorus	268	MG
Iodine	40.2	MCG
Magnesium	107	MG
Zinc	8.04	MG
Copper	0.54	MG
Biotin	121	MCG
Pantothenic Acid	5.36	MG
Vitamin K	21.4	MCG
Choline	134	MG
Chloride	402	MG
Manganese	0.54	MG
Selenium	18.8	MCG
Chromium	32.2	MCG
Molybdenum	20.1	MCG
L-Carnitine	26.8	MG
Taurine	26.8	MG

Ingredients: Water, maltodextrin, sucrose, sodium caseinate (milk), canola oil, medium chain triglycerides, calcium caseinate, soybean oil, pea fiber, potassium citrate, calcium phosphate tribasic, magnesium chloride, gum acacia, oligofructose, sodium citrate, inulin, artificial flavor, soy lecithin, sodium ascorbate, choline chloride, taurine, l-carnitine, zinc sulfate, alpha tocopheryl acetate, niacinamide,

ferrous sulfate, calcium pantothenate, copper gluconate, BHA/BHT (to preserve freshness), pyridoxine hydrochloride, thiamine hydrochloride, manganese sulfate, beta carotene, riboflavin, vitamin A palmitate, folic acid, biotin, chromium chloride, potassium iodide, sodium molybdate, sodium selenite, phytonadione, cyanocobalamin, cholecalciferol

Appendix E - Gastrointestinal Quality of Life Index

The below gastrointestinal quality of life index is modified for “healthy subjects” as defined by (132). Scoring is listed below; subjects were given a version with no numerical values associated with the answers.

1. How often during the past 2 weeks have you had pain in the abdomen?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

2. How often during the past 2 weeks have you had a feeling of fullness in the upper abdomen?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

3. How often during the past 2 weeks have you had bloating (sensation of too much gas in the abdomen)?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

4. How often during the past 2 weeks have you been troubled by the excessive passage of gas through the anus?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

5. How often during the past 2 weeks have you been troubled by strong burping or belching?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

6. How often during the past 2 weeks have you been troubled by gurgling noises from the abdomen?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

7. How often during the past 2 weeks have you been troubled by frequent bowel movements?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

8. How often during the past 2 weeks have you found eating to be a pleasure?

All of the time 4 most of the time 3 some of the time 2 a little of the time
1 never 0

9. Because of your health, to what extent have you restricted the kinds of food you eat?

Very much 0 much 1 somewhat 2 a little 3 not at all 4

10. During the past 2 weeks, how well have you been able to cope with everyday stresses?

Extremely poorly 0 poorly 1 moderately 2 well 3 extremely
well 4

11. How often during the past 2 weeks have you been sad about your health?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

12. How often during the past 2 weeks have you been nervous or anxious about your health?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

13. How often during the past 2 weeks have you been happy with life in general?

Never 0 a little of the time 1 some of the time 2 most of the time 3 all of
the time 4

14. How often during the past 2 weeks have you been frustrated about your health?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

15. How often during the past 2 weeks have you been tired or fatigued?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

16. How often during the past 2 weeks have you felt unwell?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

17. Over the past week, have you woken up in the night?

Every night 0 5-6 nights 1 3-4 nights 2 1-2 nights 3 never 4

18. Due to your health, have you been troubled by changes in your appearance?

A great deal 0 a moderate amount 1 somewhat 2 a little bit 3 not at all
4

19. Due to your health, how much physical strength have you lost?

A great deal 0 a moderate amount 1 somewhat 2 a little bit 3
not at all 4

20. Due to your health, to what extent have you lost your endurance?

A great deal 0 a moderate amount 1 somewhat 2 a little bit 3
not at all 4

21. Due to your health, to what extent do you feel unfit?

Extremely unfit 0 moderately unfit 1 somewhat unfit 2 a little unfit 3
fit 4

22. During the past 2 weeks, how often have you been able to complete your normal daily activities (school, work, household)?

All of the time 4 most of the time 3 some of the time 2 a little of the time
1 never 0

23. During the past 2 weeks, how often have you been able to take part in your usual patterns of leisure or recreational activities?

All of the time 4 most of the time 3 some of the time 2 a little of the time
1 never 0

24. During the past 2 weeks, how much have you been troubled by the medical treatment of your health?

Very much 0 much 1 somewhat 2 a little 3 not
at all 4

25. To what extent have your personal relations with people close to you (family or friends) worsened because of your health?

Very much 0 much 1 somewhat 2 a little 3 not
at all 4

26. To what extent has your sexual life been impaired (harmed) because of your health?

Very much 0 much 1 somewhat 2 a little 3 not
at all 4

27. How often during the past 2 weeks have you been troubled by fluid or food coming up into your mouth (regurgitation)?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

28. How often during the past 2 weeks have you felt uncomfortable because of your slow speed of eating?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

29. How often during the past 2 weeks have you had trouble swallowing your food?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

30. How often during the past 2 weeks have you been troubled by urgent bowel movements?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

31. How often during the past 2 weeks have you been troubled by diarrhea?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

32. How often during the past 2 weeks have you been troubled by constipation?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

33. How often during the past 2 weeks have you been troubled by nausea?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

34. How often during the past 2 weeks have you been troubled by blood in the stool?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

35. How often during the past 2 weeks have you been troubled by heartburn?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

36. How often during the past 2 weeks have you been troubled by uncontrolled stools?

All of the time 0 most of the time 1 some of the time 2 a little of the time
3 never 4

Appendix F - Randomization schedule: With product coding and after unblinding

ID	Women	Women	Unblinded
1	4H2 - 3W8	BA	Fiber-Control
2	9J1 - 5E1	BA	Fiber-Control
3	3W8 - 4H2	AB	Control-Fiber
4	5E1 - 9J1	AB	Control-Fiber
5	4H2 - 3W8	BA	Fiber-Control
6	3W8 - 4H2	AB	Control-Fiber
7	9J1 - 5E1	BA	Fiber-Control
8	3W8 - 4H2	AB	Control-Fiber
9	5E1 - 9J1	AB	Control-Fiber
10	4H2 - 3W8	BA	Fiber-Control

ID	Men	Men	Unblinded
21	9J1 - 5E1	BA	Fiber-Control
22	3W8 - 4H2	AB	Control-Fiber
23	5E1 - 9J1	AB	Control-Fiber
24	4H2 - 3W8	BA	Fiber-Control
25	3W8 - 4H2	AB	Control-Fiber
26	9J1 - 5E1	BA	Fiber-Control
27	5E1 - 9J1	AB	Control-Fiber
28	4H2 - 3W8	BA	Fiber-Control
29	9J1 - 5E1	BA	Fiber-Control
30	3W8 - 4H2	AB	Control-Fiber

Appendix G - Bristol Stool Chart

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 the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. Entirely Liquid

Appendix H - Flow Chart

VISITS	V0	V1	HOME	V2	HOME	V3	HOME	V4	HOME	V5
DAYS	0	1	1 to 14	14 (+/-2)	14 - 28	28 (+/-2)	28 - 56	56 (+/-2)	56 - 70	70 (+/-2)
PHASE			Habitual Diet		Blinded treatment 1		WASHOUT		Blinded treatment 2	
MEASURES:										
Informed consent	X									
Delivery of stool sample collection kits, diet records, radio opaque pellets and GI tolerance diaries/ instruments		X		X		X		X		
Diet records			-----		-----		Day 42-56		-----	X
Gastrointestinal tolerance			-----		-----		Day 42-56		-----	X
Stool sample collection			Day 9-13	Fresh sample	Day 23-27	Fresh sample	Day 51-55	Fresh sample	Day 65-69	Fresh sample
Radio opaque pellets consumption			Day 8		Day 22		Day 50		Day 64	
Gastrointestinal quality of life (TBD)				X		X		X		X
Collection of diet records				X		X		X		X
Collection of tolerance instrument				X		X		X		
Microbiota				X		X		X		X
Fecal Moisture				X		X		X		X
Stool weight				X		X		X		X
Transit time				X		X		X		X
Stool pH				X		X		X		X
Short chain fatty acids				X		X		X		X
Height	X									
Body weight	X	X		X		X		X		X
Bristol stool scale				X		X		X		X
Distribution of product for next phase				X				X		
Compliance				X		X		X		X
Health History / Status	X	X		X		X		X		X
Concomitant Medication	X	X		X		X		X		X
AE / SAE Assessment		X		X		X		X		X
Randomization / IP Assignment				X				X		