THE EFFECT OF A PREBIOTIC WITH A PROBIOTIC ON SYMPTOMS AND QUALITY OF LIFE IN ULCERATIVE COLITIS

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ABSTRACT

The medical management of ulcerative colitis in children often requires aggressive pharmacological therapy or colonic resection. We hypothesized that synbiotic therapy, consisting of *B. longum* R0175 and inulin would improve symptoms and quality of life (QOL) in children diagnosed with ulcerative colitis.

Consenting pediatric subjects (8-18 years; n = 9) with ulcerative colitis in remission were provided synbiotic therapy, (Probiotic: *Bifidobacterium longum* R0175 2.0 x 10^{10} cfu/day; Prebiotic: 15 g/day of inulin) (n = 4) or placebo (maltodextrin + ascorbic acid capsule; 15 g/day of non-resistant maltodextrin) (n = 5) for ten months in a pilot study (Phase I). After ten months, the study was unblinded and synbiotic therapy was administered to eight pediatric subjects (Phase II). In attempt to increase sample size, three adult subjects with active UC were recruited and provided the synbiotic therapy (Phase III). In all phases of the research, QOL was measured using the Short Inflammatory Bowel Disease Questionnaire (SIBDQ). The SIBDQ was administered at baseline and every two months. Subjects kept a daily records of symptoms (stool consistency and frequency, presence of blood and mucous, presence of abdominal pain and overall feeling).

Phase I QOL scores were significantly better for those receiving the synbiotic therapy versus the placebo ($p = 0.014$). Severe symptoms occurred in 60% of the control subjects, where as subjects receiving synbiotic therapy did not experience severe symptoms ($p = 0.032$). Phase II QOL scores were significantly better post-treatment with synbiotic therapy ($p=0.034$). One subject (steroid dependant) was able to wean off Prednisone® while receiving the synbiotic therapy; she remained in remission and was symptom free for over 26 months. In Phase III, synbiotic therapy did not induce remission in the adult subjects with active UC. No adverse effects were reported.

Synbiotic therapy consisting of *Bifidobacterium longum* R0175 and inulin, when provided in addition to conventional treatment, appears to be a safe and effective strategy for managing pediatric ulcerative colitis in remission. Further clinical trials are warranted to confirm these preliminary results.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS

AAD	Antibiotic Associated Diarrhea
CDD	*C. Difficile* Associated Diarrhea
CFU	Colony Forming Units
CRP	C - Reactive Protein
DE	Dextrose Equivalents
DP	Degree of Polymerization
ESR	Erythrocyte Sedimentation Rate
FOS	Fructo-oligosaccharides
GBF	Germinated Barley Foodstuff
HRQOL	Health Related Quality of Life
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire
IBS	Irritable Bowel Syndrome
LGG	*Lactobacillus* GG
NHPD	Natural Health Products Directorate
NEC	Necrotizing Enterocolitis
NOAEL	No Observed Adverse Effect Level
RFIPC	Rating Form of Inflammatory Bowel Disease Patient Concerns
SAS	Sulfasalazine
SCCAI	Simple Clinical Colitis Activity Index
SCFA	Short Chain Fatty Acids
SIBDQ	Short Inflammatory Bowel Disease Questionnaire
SRB	Sulfate Reducing Bacteria
SF-36	Short Form 36
UC	Ulcerative Colitis
UCDAI	Ulcerative Colitis Disease Activity Index
5-ASA	5-Aminosalicylates

CHAPTER 1
INTRODUCTION

1.1 Background

Ulcerative Colitis (UC) is an inflammatory bowel disease (IBD) characterized by intestinal inflammation and ulcerations within the mucosa of the colon (Friedman & Blumberg, 2005). The current hypothesis is that IBD is an inappropriate response to either the endogenous microbial flora within the intestine, with or without some component of autoimmunity (Friedman & Blumberg, 2005). Pathogenic bacteria have been suggested to evoke an inflammatory response in the gut mucosa (Cummings, McFarlane & McFarlane, 2003), that the mucosal immune system fails to control (Friedman & Blumberg, 2005).

Children tend to have a high incidence of severe type of UC (Tomomosa et al., 2004). The symptoms of UC are diarrhea, rectal bleeding, passage of mucus, tenesmus, abdominal pain, weight loss, lethargy and poor appetite (Wylie & Sarigol, 1998).

Synbiotic therapy is a novel treatment used to induce a favorable intestinal environment in the host (Gibson & Roberfroid, 1995) and aims to generate synergistic effects by combining prebiotic and probiotic treatments. Modulation of the bacteria in the colon, through the use of prebiotics and probiotics, may be useful in modifying the disease state (Furrie et al., 2005). Recent case reports have shown that synbiotic therapy significantly improved the intestinal bacteria (inducing domination of anaerobic bacteria and suppression of pathogenic bacteria), improved short chain fatty acids in the feces and improved nutritional status in seven patients with refractory enterocolitis (Kanamori et al., 2004). In a single short-term study, synbiotic therapy has been shown to improve inflammatory markers, reduce inflammation in the rectal mucosa and regenerate colonic epithelial tissue in patients with active UC (Furrie et al., 2005).

Hypotheses for the cause of the disease also center on initiation and perpetuation of mucosal damage by exogenous agents, such as bacterial sulfides (Roediger, Moore & Babidge, 1997). There is increasing evidence to suggest that hydrogen sulfide (H_2S),

produced by intestinal sulfate-reducing bacteria may be involved in the pathogenesis of chronic diseases such as UC (Roediger, Duncan, Kapaniris & Millard, 1993; Pitcher & Cummings, 1996). *In vitro* studies have demonstrated that when butyrate is selectively inhibited in the colonocytes by hydrogen sulfide, the colonocytes develop lesions that closely mirror biochemical lesions reported in UC (Roediger et al., 1993). High concentrations of hydrogen sulfide (Pitcher, Beatty & Cummings, 2000; Levine, Ellis, Furne, Springfield & Levitt, 1998) and sulfate reducing bacteria have been found in patients with active UC (Roediger et al., 1997). Patients with active UC have been found to have higher amounts of sulfate-reducing bacteria with clinically active disease compared with patients in remission (Ohge et al., 2005; Pitcher et al., 2000). Sulfate levels in western Canada are particularly high; out of 428 sampling locations across Alberta and Saskatchewan, 13% had sulfate concentrations of greater than 500 mg/L (Health Canada, 1994).

There is considerable evidence that inflammatory bowel disease (IBD) impairs health related quality of life (HRQOL) (Casellas, Lopez-Vivancos, Badia, Vilaseca, & Malagelada, 2001). Chronic gastrointestinal symptoms and required treatments of IBD impose psychological and social stresses on young patients. Response to treatment may not always mirror an improvement in well-being, thus there has been increasing emphasis on the assessment of HRQOL in patients with chronic diseases. The Short Inflammatory Bowel Disease Questionnaire (SIBDQ) has been developed and validated to assess HRQOL for patients with IBD (Irvine, Zhou, & Thompson, 1996; Jowett, Seal, Barton & Welfare, 2001).

1.2 Purpose

Results of well-designed clinical trials indicate that probiotic or prebiotic therapy (used independently) may effectively maintain remission in UC (Haskey & Dahl, 2006). However, there is little evidence that examines the effect of synbiotic therapy in UC. Only one clinical has examined the effect of synbiotic therapy in adult patients with active UC (Furrie et al., 2005). To date, there have been no clinical trials that have examined the effect of synbiotic therapy in pediatric patients with UC.

It is believed that supplementation with synbiotic therapy may be a safe and effective strategy to maintain remission in UC. Thus, it is necessary to evaluate the clinical efficacy and the effect on quality of life of synbiotic therapy (Probiotic: *Bifidobacteria longum* R0175; Prebiotic: inulin) in maintaining remission in UC patients. In addition, with the high levels of sulfate found in the water supply in Saskatchewan, there is value in examining whether low sulfate water will also benefit UC patients.

1.3 Objectives

The objectives of the study are as follows:

1. *To determine if the consumption of inulin and Bifidobacterium longum R0175 will have an effect on quality of life and symptoms in subjects with UC.*
2. *To determine if the consumption of the placebo will have an effect on quality of life and symptoms in subjects with UC.*
3. *To compare the number of subjects experiencing a relapse of UC during the ten month period between the two treatment groups.*
4. *To assess tolerance to the study medications (Bifidobacteria longum R0175 and inulin) and record adverse events.*
5. *To assess compliance to the prescribed treatment protocol in the UC subjects. If compliance is an issue, possible solutions will be suggested.*

1.4 Hypotheses

The hypotheses of this study are as follows:

1. *Pediatric subjects with UC that are treated with the synbiotic therapy (Probiotic: Bifidobacteria longum R0175; Prebiotic: inulin) will experience improved of quality of life and reduced symptoms (abdominal pain, blood, mucous, stool frequency and stool consistency) compared to the pediatric subjects consuming the placebo.*

2. *Pediatric subjects with UC that are treated with the synbiotic therapy will relapse less frequently than pediatric subjects with UC receiving the placebo.*
3. *Synbiotic therapy will induce remission in both pediatric and adult subjects with active UC.*

1.5 Limitations

There are limitations associated with this clinical trial. The researchers did not feel it was ethical to pursue invasive medical procedures such as colonoscopy and biopsy in subjects of such a young age. Data collection was limited to non-invasive procedures such as self-reporting in the study diaries, thus endoscopic and histological findings were not collected. The data obtained from the study diaries was an expression of the subjects recording practices which may be inherently limited. The researchers did not examine the dietary habits of the subjects.

CHAPTER 2
LITERATURE REVIEW

2.1 ULCERATIVE COLITIS

2.1.1 Clinical Presentation

Ulcerative Colitis (UC) is one of two chronic inflammatory diseases of the gastrointestinal tract. In UC, the inflammatory response is limited to the mucosa and submucosa of the colon. It normally manifests in the rectum and spreads throughout the large intestine (Friedman & Blumberg, 2005). Symptoms of UC include diarrhea, rectal bleeding, passage of mucus, tenesmus, abdominal pain, weight loss, lethargy and poor appetite (Wylie & Sarigol, 1998). If UC is left untreated, it can lead to dehydration, malnutrition and anemia. Patients with UC may also present with extraintestinal manifestations, such as decreased linear growth velocity in pediatric patients, arthritis and skin lesions (Gremse & Crissinger, 2002). The severity of symptoms usually correlates with the severity of the disease.

2.1.2 Epidemiology

There are a number of studies that describe the incidence of IBD in the world. The highest incidence rates for IBD have been reported in Northern Europe, the United Kingdom and North America (Loftus, 2004). However, incidence rates are increasing in other areas of the world.

Bernstein et al. (2006) recently studied the epidemiology of IBD in Canada. Bernstein et al. (2006) estimated that in the year 2000, approximately 81,000 Canadians were living with UC. The estimated incidence rate was 11.8/100,000 person-years and the prevalence was 194/100,000 for the adult population. The peak age of onset in the adult population is usually during the second and third decades of life. There are no differences in incidence for gender. There appears to be a difference in incidence rates of UC between urban and rural dwellers across Canada with a total urban: rural incidence rate ratio of 1.13 (Bernstein et al., 2006).

Bernstein et al. (2006) reported that the incidence of pediatric UC is highest in Nova Scotia (5.7) and lowest in British Columbia (3.2). The incidence rate is higher for girls than boys (3.6). The prevalence of pediatric UC is highest in Alberta (30.7) and lowest in British Columbia (17.5). The incidence of pediatric UC in Saskatchewan was 4.2/100,000 person-years and prevalence was 18.1/100,000. The age of onset of UC in pediatric patients is usually in late adolescence (Gremse & Crissinger, 2002).

2.1.3 Etiology and Pathogenesis

The etiology and pathogenesis of UC is unknown (Chudleigh & Hunter, 2003; Gremse & Crissinger, 2002). The current hypothesis is that IBD is an inappropriate response to either the endogenous microbial flora within the intestine, with or without some component of autoimmunity (Friedman & Blumberg, 2005). It is believed that patients with IBD suffer from dysbiosis (Penner & Fedorak, 2005). Dysbiosis describes the abnormalities in intestinal bacterial content and host-bacterial interaction.

Current research is suggesting that IBD may also have an infectious etiology. Pathogenic bacteria may evoke an inflammatory response in the gut mucosa (Cummings, Macfarlane & Macfarlane, 2003), that the mucosal immune system fails to control (Friedman & Blumberg, 2005). The pathogenic bacteria involved in this process include species belonging to the genera *Yersinia, Shigella, Salmonella, Campylobacter, Clostridium, Aeromonas* and pathogenic *Escherichia coli* (Cummings et al., 2003; Steidler, 2001). These organisms are able to either penetrate the gut epithelium or proliferate the epithelial surface evoking the inflammatory response (Cummings et al., 2003).

2.1.4 Diagnostic Evaluation

Combinations of laboratory, radiographic and endoscopic tests are used in the diagnosis of UC. Diagnostic tests for UC provide the evidence to confirm the diagnosis of UC and to rule out other gastrointestinal conditions that mirror UC.

Initial investigations in a patient with symptoms of UC involve excluding enteric infection through examination of the stool for bacterial and viral pathogens (e.g., *Salmonella*, *Shigella, Campylobacter, Aeromonas, Plesiomonas, Yersinia, Escherichia*

coli 0157:H7, *Clostridium Difficile* or *Entamoeba histolytica*) (Bremner, Griffiths & Beattie, 2004; Chudleigh & Hunter, 2003). The symptoms of bacterial and parasitic gastroenteritis are very similar to those of UC (Chudleigh & Hunter, 2003).

Routine laboratory investigations include complete blood count, serum chemistry profile (e.g., electrolytes, blood urea nitrogen, creatinine, albumin and liver enzymes), iron status and inflammatory markers (e.g., C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), IgG and IgA antibodies) (Bremner, Griffiths & Beattie, 2004). Hypoalbuminemia, anemia, elevated CRP, elevated ESR, leukocytosis and thrombocytosis are often present in UC (Chudleigh & Hunter, 2003). Electrolyte disturbances are common when severe diarrhea is present. Iron-deficiency is associated with the loss of blood through stools.

Endoscopy is indicated in all cases of UC for diagnosis and to assess disease extent (Bremner, Griffiths & Beattie, 2004; Chudleigh & Hunter, 2003). Often distal gastrointestinal tract findings may be indistinguishable in UC and Crohn's disease; therefore diagnostic testing of pediatric IBD includes evaluation of the proximal gastrointestinal tract with radiographs, small bowel follow through and esophagogastroduodenoscopy (Chudleigh & Hunter, 2003). The addition of proximal gastrointestinal endoscopy with biopsy improves the diagnostic accuracy. Crohn's disease tends to involve the entire gastrointestinal tract and extends into the intestinal wall from the mucosa to the serosa. Histology is required to confirm the diagnosis and distinguish UC from Crohn's disease and other gastrointestinal conditions (Bremner, Griffiths & Beatty, 2004).

2.1.5 Measurements of Disease Activity in UC

Disease severity is assessed using symptomology, laboratory investigations and endoscopy. Multiple disease activity indices for UC have been devised, but none have been rigorously validated (Higgins, Schwartz, Mapili, & Zimmermann, 2005). Most clinical trials use multiple disease activity indices since there is no clear gold standard (Higgins, Schwartz, Mapili, & Krokos, et al., 2005). The medical management of UC is based on disease severity.

2.1.5.1 Truelove and Witts Classification of UC

Truelove and Witts (1955) were the first to classify UC patients into three categories, those with mild, moderately and severe disease (Table 2.1).

Table 2.1 Truelove and Witts Classification of UC

Disease Severity	Symptoms
Mild	< 4 bowel movements/day Passage of blood in stool less than daily No systemic symptoms
Moderate	> 5 bowel movements/day with blood present Tenesmus, colicky pain, malaise Low grade or intermittent fever Abdominal tenderness in left lower quadrant Anemia, hypoalbuminemia, fecal leukocytes
Severe	> 6 bowel movements/day with blood present Abdominal tenderness, tachycardia Fever, weight loss, hypoalbuminemia

Adapted from Truelove SS, Witts LI. (1955). *BMJ* 2, 1041.

2.1.5.2 Powell-Tuck Activity Score

Powell-Tuck et al. (1978) developed the Powell-Tuck Activity Score for clinical trials of corticosteroid treatment to include a wider range of symptoms than Truelove and Witts classification of UC. The Powell-Tuck Activity Score includes endoscopy, a general health question and examination for abdominal tenderness (Table 2.2). A score of < 3.5 indicates that the patient is in remission (Higgins et al., 2005). The Powell-Tuck Activity Score is widely used but has never been validated (Higgins, Schwartz, Mapili & Krokos, et al., 2005).

Table 2.2 Scoring System for the Powell-Tuck Activity Score

Symptom	Score
Bowel frequency (day)	
3-6	1
>6	2
Stool consistency	
formed	0
semi-formed	1
liquid	2
Abdominal pain	
before/after BM	1
prolonged	2
Anorexia	1
Nausea/Vomiting	1
General Health	
normal	0
slightly impaired	1
activities restricted	2
unable to work	3
Extracolonic manifestations	
one/mild	1
more than one/severe	2
Abdominal tenderness	
mild	1
marked	2
rebound	3
Body temperature	
<37.1	0
37.1-38	1
>38	2
Blood in stool	
trace	1
more than trace	2
Sigmoidoscopy	
non-hemorrhagic	0
friable	1
spontaneous bleed	2

2.1.5.3 Ulcerative Colitis Disease Activity Index (UCDAI)

Sutherland et al. (1987) developed the UCDAI (Table 2.3). The UCDAI score is the sum score of four parameters, each scoring between 0 and 3, making 12 the worst score. According to the sum score, UC activity is classified as follows: sum score 0–2 (no activity); 3–5 (mild activity); 6–8 (moderate activity); 9–12 (severe activity). A score of <2.5 indicates the patient is in remission (Higgins, Schwartz, Mapili & Krokos, et al., 2005).

Table 2.3 Ulcerative Colitis Disease Activity Index

Variable	Score
Stool frequency (day)	
normal	0
1-2 stools/day > normal	1
3-4 stools/day > normal	2
>4 stools/day > normal	3
Rectal bleeding	
none	0
streaks of blood	1
obvious blood	2
mostly blood	3
Mucosal appearance	
normal	0
mild friability	1
moderate friability	2
exudation, spontaneous bleeding	3
Physician's rating of the disease	
normal	0
mild	1
moderate	2
severe	3

2.1.5.4 Seo index

In 1992, Seo et al. developed a numerical disease activity index for UC to correlate with Truelove and Witts' classification (Table 2.4). The activity index (AI) is expressed as follows: AI = 60 x blood stool + 13 x bowel movements + 0.5 x ESR - 4 x Hb - 15 x albumin + 200. Index values below 150, values between 150 and 220, and values above 220 nearly corresponded to mild, moderate, and severe disease, respectively, in Truelove and Witts' classification. A score of <120 indicates the patient is in remission (Higgins, Schwartz, Mapili & Krokos, et al., 2005).

Table 2.4 Seo Activity Index

Variable	Score	Weighting
Bloody stool		x 60
Little or none	0	
Present	1	
Bowel movements/day		x 13
≤ 4	1	
5-7	2	
≥ 8	3	
Erythrocyte sedimentation rate (mm/h)		x 0.5
Hemoglobin (g/dl)		
Albumin (g/dl)		x - 4
Constant		x -15
		200

2.1.5.5 Simple Clinical Colitis Activity Index (SCCAI)

Walmsley et al. (1998) developed the SCCAI, a survey of six questions that assesses the symptoms of UC (Table 2.5). Laboratory values and endoscopy are not always available for immediate evaluation; therefore the intent was to develop an index that could be scored immediately without the use of laboratory indices. The SCCAI has been validated against an established activity index (e.g., Powell-Tuck). The SCCAI is designed to be used as an initial assessment of outpatients, by non-specialist physicians and by patients themselves as guide to modify treatment or the need to seek further advice (Walmsley et al., 1998). A score of <2.5 indicates the patient is in remission (Higgins, Schwartz, Mapili & Krokos, et al., 2005).

Table 2.5 Clinical Scoring System for the SCCAI

Symptom	Score
Bowel frequency (day)	
1-3	0
4-6	1
7-9	2
>9	3
Bowel frequency (night)	
1-3	1
4-6	2
Urgency of defecation	
Hurry	1
Immediately	2
Incontinence	3
Blood in stool	
Trace	1
Occasionally frank	2
Usually frank	3
General Well Being	
Very well	0
Slightly below par	1
Poor	2
Very poor	3
Terrible	4
Extracolonic features	1 point per manifestation

2.1.6 Current Medical Management of Ulcerative Colitis in Children

Children with UC have a high incidence of severe type illness and even when it occurs mildly, it often progresses to one of the more severe forms of colitis (Tomomasa et al., 2004). In a review of 171 children, 43 percent had mild disease, 57 percent had disease that was moderate or severe (Hyams, 1994). Children require individualized treatment that takes numerous factors into account: the specific disease manifestations (e.g., location of inflammation in the intestines, duration, and prior response to therapy), the psychosocial adaptation of the child and family, and the child's age and size (Crohn's and Colitis Foundation of America, 2006). Children are in a period of rapid growth, therefore long term treatment with steroids may inhibit growth and should be avoided as much as possible (Tomomasa et al., 2004). Children are also moving through a period of emotional development, therefore quality of life must also be carefully evaluated (Tomomasa et al., 2004; Bremner, Griffiths, & Beattie, 2004). In addition to pharmacological therapy, the consequences of UC on peer and family relationships, school attendance and emotional well-being should be included as part of the

comprehensive management of this disorder (Chudleigh & Hunter, 2003). Since the etiology of UC is unknown, the goals of medical therapy in pediatric UC patients are to control symptoms, prevent relapses, maintain growth and promote pubertal development (Tomomasa et al., 2004; Chudleigh & Hunter, 2003).

The treatment approaches for children with UC are based on adult experience (Crohn's and Colitis Foundation of America, 2006). Pharmacological therapy for UC involves suppression of the inflammatory processes in order to control symptoms and prevent relapses (Chudleigh & Hunter, 2003). The type of pharmacological agent used is determined by the severity of the disease and response to treatment (Chudleigh & Hunter, 2003). 5-Aminosalicylates (5-ASA) preparations, such as Sulfasalazine® and mesalamine are used to induce and maintain remission in pediatric UC patients, but corticosteroids such as Prednisone® are often prescribed in conjunction with 5-ASA compounds for patients with moderate to severe colitis (Chudleigh & Hunter, 2003). Corticosteroids are most effective in the treatment of active UC versus UC in remission (Steidler, 2001; Wyllie & Sarigol, 1998). Pediatric patients that do not respond to 5-ASA and corticosteroids may be effectively treated with immunomodulatory therapy such as aziathioprine, mercaptopurine, methotrexate, cyclosporine or tacrolimus (Tomomasa et al., 2004; Chudleigh & Hunter, 2003). The pharmacological agents described have a number of side effects such as growth suppression, hypertension, decreased bone mineral density, cataracts, glaucoma, hyperglycemia, bone marrow suppression, increased risk of infection and increased risk for certain cancers (Wyllie & Sarigol, 1998). Unfortunately, pediatric patients with the more severe forms of UC often will have chronic intermittent symptoms and some will have continuous symptoms despite being in remission (Hyams et al., 1996).

Colonoscopy during follow-up can determine disease progression and assess response to treatment (Bremner, Griffiths & Beatty, 2004). It is used in children that have UC that is difficult to treat, as repeat endoscopy can help determine the subsequent treatment choice (Bremner, Griffiths & Beatty, 2004). If there is disease progression with the initial therapy, treatment can then be intensified.

A colectomy is used for patients where pharmacologic therapies have been unsuccessful or the unacceptable side effects of medications must be avoided. The

primary benefit of a colectomy is the control of symptoms without the use of medications (Chudleigh & Hunter, 2003). A colectomy is curative for UC, although the postoperative complications can be problematic (Bremner et al., 2004). The most common complication of a colectomy is pouchitis, a non specific inflammation of the ileal reservoir (Mahadevan & Sandborn, 2003). Despite the complications associated with a colectomy, almost all children with severe UC report improved quality of life after colectomy (Tomomasa et al., 2004).

2.1.7 Current Nutrition Management of Ulcerative Colitis in Children

Nutrition therapy plays an adjunctive role in the medical management of pediatric UC, as the success of nutrition as a primary therapy is limited (Kelly, 1999). The goals of nutrition therapy for pediatric UC are to correct specific nutrient deficiencies and prevent growth failure (Chudleigh & Hunter, 2003). The provision of adequate nutrition is essential to promotion of optimal healing.

In UC, undernutrition is often caused by a reduced intake of calories and protein, altered taste sensation, either from side-effects from medication, nutrient deficiencies or sitophobia in patients who associate food intake with diarrhea or abdominal pain (Oviedo & Farraye, 2001). Weight loss has been reported to be as high as 60% to 75% in patients with UC (Kelly, 1999). Iron deficiency anemia occurring from blood loss is reported in about 80% of UC patients (Kelly, 1999). Medications used in the therapy for UC are associated with the development of nutritional deficiencies (Oviedo & Farraye, 2001). Sulfasalazine® contributes to folate malabsorption and corticosteroids suppress intestinal absorption of calcium.

Current practice recommends a daily multivitamin supplement, as well as folic acid supplementation (1 mg/day) in all patients with UC (Oviedo & Farraye, 2001). Adequate dietary calcium is encouraged. In patients with limited dietary intake of calcium, a calcium supplement is recommended. A vitamin D supplement at a minimum dose of 400 IU/day is suggested (Oviedo & Farraye, 2001). Oral nutrition supplements are often prescribed to assist patients in meeting protein and caloric needs. Nutrition support may be necessary for patients who cannot voluntarily increase their intake and who are unable to meet their nutrition requirements via oral intake.

There is no specific diet for the prevention or management of UC at this time (Oviedo & Farraye, 2001). Individuals with UC are instructed to find out their tolerance to foods on a trial-and-error basis (Oviedo & Farraye, 2001). Enteral or parenteral therapy, in addition to corticosteroid therapy, does not appear to increase the remission rate or reduce the need for a colectomy in UC patients (Kelly, 1999; Chudleigh & Hunter, 2003). However, in growing children, treatment with nocturnal enteral feeding has been successful in circumventing weight loss and correcting body composition (Kelly, 1999). Exclusion diets do not prevent or induce remission in patients with UC (Oviedo & Farraye, 2001). At the present time, a low fibre diet is prescribed during periods of active inflammation. A high fibre diet, including sources of insoluble and soluble fibre may be prescribed during periods of remission (Gremse & Crissinger, 2002).

2.2 HEALTH RELATED QUALITY OF LIFE (HRQOL)

2.2.1 Definition of Health Related Quality of Life

Health related quality of life (HRQOL) has been defined as the functional effect of an illness and its consequent therapy upon a patient, as perceived by the patient (Schipper, Clinch & Olweny, 1996). Health status of patients with chronic disease should not only include assessment of disease activity, but should also investigate the psychological state, cultural influences, degree of social support, effects of disease complications, previous surgery, and medication (Garrett, & Drossman, 1990). Changes induced by chronic diseases in different aspects of patients' lives are reflected in impairment in HRQOL (Alcala, Casellas, Fontanet, Prieto & Malagelada, 2004).

HRQOL is a term applied to a wide spectrum of both intangible and tangible parameters of perception and function in the physical, social and emotional domains (Irvine, 1993). The domains may range from negatively valued aspects of life, including death, to the more positively valued aspects of life such as happiness (Guyatt, Feeny & Patrick, 1993). Thus, patient perception of HRQOL is increasingly acknowledged to be a critical component in patient care (Alcala et al., 2004). UC, like many other chronic

diseases, significantly impairs HRQOL. During relapses, the perception of health is markedly impaired for all dimensions of HRQOL (Casellas et al., 2001).

2.2.2 Importance of Measuring HRQOL

Traditional medical management and follow-up of patients with UC is based on clinical symptoms, laboratory tests, endoscopic and histological findings (Martin, Leone, Fries & Naccarato, 1995). These findings fail to recognize the subjective experiences of the patients. In UC, HRQOL has been found to be significantly altered not only when the disease is active but even when it is in clinical remission and laboratory and endoscopic findings are essentially normal (Martin et al., 1995).

Clinicians and policymakers are recognizing the importance of measuring HRQOL in clinical medicine and research (Guyatt et al., 1993). Measurement of HRQOL can facilitate identification of patient needs, such as the necessity for psycho-social support (Irvine, 1993; Martin et al., 1995). HRQOL instruments can be used to detect subtle differences in disease states (Irvine, 1993). For example, Jowett et al. (2001) established that the Short Inflammatory Bowel Disease Questionnaire (a HRQOL tool) was able to detect clinically important changes in disease activity in patients living with IBD. HRQOL tools can be used in clinical research to assess outcomes, for example assessing new drugs (versus placebo) or different choices of drugs (Levine, 1993). HRQOL is recommended to be a required secondary outcome measure in clinical trials involving IBD (Sandborn et al., 2002). Finally, assessment of HRQOL provides a more in depth perspective of a patient's total health status and may offer important information to the clinician that may have otherwise been overlooked by traditional measures (Hjortswang et al., 2003). Measurement of HRQOL ultimately leads to improved patient care.

2.2.2.1 HRQOL in Childhood and Adolescence

Measurement of HRQOL is more difficult in children and adolescents than in adults as physical, emotional and intellectual functions are constantly changing with normal development. Chronic and unpredictable gastrointestinal symptoms and required treatments impose psychological and social stresses on young patients (Griffiths et al., 1999). Teenagers affected by growth stunting and delayed physical maturation are at

increased risk for psychosocial trauma and social isolation (Griffiths et al., 1999). IBD triggers emotional responses such as unfairness and frustration, and children with UC report anger and embarrassment related to the disease (Griffiths et al., 1999). IBD has a major impact on HRQOL in both children and adolescents and is a crucial component of monitoring the impact chronic disease has on a child's overall well-being.

2.2.3 Validated Tools for Measuring HRQOL in UC

Several tools have been designed to measure HRQOL in UC, with most tools being subjective and quantitative (Levine, 1993). The most widely used and internationally accepted HRQOL questionnaires for use in IBD are the Inflammatory Bowel Disease Questionnaire (IBDQ), Short Inflammatory Bowel Disease Questionnaire (SIBDQ), the Rating Form of Inflammatory Bowel Disease Patient Concerns (RFIPC) and Short Form General Health Survey (SF-36). IMPACT is a newly developed HRQOL questionnaire designed for use in children with IBD.

2.2.3.1 Inflammatory Bowel Disease Questionnaire

The IBDQ is a 32 item questionnaire that evaluates bowel symptoms, social functions, worries and concerns related to IBD (Guyatt et al., 1989). Reponses are graded on a seven point Likert scale, ranging from one (represents the "worst" aspect) to seven (representing the "best" aspect). The sum score is the total of the individual scores and ranges from 32 to 224, with the lowest score representing the poorest quality of life. The 32 questions are divided into four domains: bowel symptoms (10 questions), systemic responses (5 questions), emotional functions (12 questions) and social functions (5 questions). The IBDQ has been translated and validated into other languages such as Dutch, Swedish, English, Greek, Chinese, Korean and Spanish (Russel, 1997; Hjortswang et al., 2001; Cheung, Garratt, Russell & Williams, 2000; Pallis, Vlachonikolis & Mouzas, 2001; Leong et al., 2003; Kim et al., 1999; Lopez-Vivancos, Casellas, Badia, Vilaseca & Malagelada, 1999). The main drawback of the IBDQ is that it is very time consuming for practitioners to administer and interpret (Cassellas et al., 2004). The IBDQ is the most widely used tool to measure HRQOL in IBD and has repeatedly been validated in multiple settings, and it has become the gold

standard for measurement of quality of life in UC (Higgins, Schwartz, Mapili & Zimmerman, 2005).

2.2.3.2 Short Inflammatory Bowel Disease Questionnaire

Irvine et al. (1996) developed a shortened version of the IBDQ which is called the SIBDQ (Appendix 5). The SIBDQ uses ten questions derived from the IBDQ. The ten questions are divided into four domains: bowel symptoms (3 questions), systemic responses (2 questions), emotional functions (3 questions) and social functions (2 questions). Reponses are graded on a seven point Likert scale as described for the IBDQ. The total score ranges from 10 (worst health) to 70 (best health). The SIBDQ has been validated in UC (Irvine et al., 1996; Jowett et al., 2001). It is able to discriminate between both active and inactive disease (Irvine et al., 1996), as well as differentiate between different classes of disease activity (i.e., remission versus mild relapse) (Jowett et al., 2001). The SIBDQ is reproducible and consistent in a test-retest situation when the clinical situation remains changed, and is sensitive enough to respond to changes in disease activity for an individual (Jowett et al., 2001). Jowett et al. (2001) were able to demonstrate that a mean decrease of 11.8 points occurred for an individual when disease activity changed from remission to mild relapse or mild to moderate relapse. The SIBDQ is administered and scored quickly; therefore, it is ideal for use in clinical practice and in the research setting (Irvine et al., 1996).

2.2.3.3 Rating Form of Inflammatory Bowel Disease Patient Concerns

Drossman et al. (1991) developed the Rating Form of Inflammatory Bowel Disease Patient Concerns (RFIPC) to evaluate the worries and concerns associated with IBD. The RFIPC is a self administered questionnaire containing 25 items of concern to IBD patients. It takes the form of 'Because of your condition, how concerned are you with …?'. Each item is ranked from 0 to 100 (0= not at all, 100 = a great deal), on a visual analogue scale. The total score is calculated as an average of all concerns. The RFIPC has been validated for use in clinical care, as well as in research to evaluate the effects of interventions on IBD outcome.

2.2.3.4 Short Form General Health Survey

The SF-36 is a self-administered, widely used multipurpose survey of general health status developed by Ware and Sherbourne (1992). SF-36 is a 36 item health

survey that consists of multi-item scales touching on eight aspects of health: physical functioning, role limitations due to physical health, bodily pain, general health, vitality, social functioning, limitations due to emotional problems and mental health (Ware & Sherbourne, 1992). For each question, raw scores are coded, summed and transformed into a scale ranging from 0 to 100 with higher scores representing better health states. It is has been shown to be reliable and valid in surveying HRQOL in patients with a variety of chronic diseases (McHorney, Ware & Raczek, 1993). SF-36 has been widely used in studies examining HRQOL in IBD patients.

2.2.3.5 IMPACT

IMPACT is described as a "disease specific HRQOL questionnaire developed for use in pediatric IBD" (Otley et al., 2002). The questionnaire is targeted to children and adolescents with IBD between the ages of 10 to 17 years. IMPACT is a self-administered questionnaire that includes 36 questions encompassing six domains: bowel (6 concerns), body image (3 concerns), functional/social impairment (11 concerns), emotional impairment (11 concerns), tests/treatments (3 concerns) and systemic impairment (2 concerns) (Otley et al., 2002). A visual analogue scale is used for responses. A horizontal line is anchored by a range from worst (0 cm) to best function (10 cm) and patients are asked to mark where their feelings lie in response to each question. Each mark is measured and multiplied by .7 to give a maximal score of 7 per question, so the scores range from 0 to 231 with lower scores representing poor quality of life. IMPACT is designed as a descriptive tool to facilitate recognition of emotional and functional disability (Otley et al., 2002). This tool is also described as an evaluative tool that can be incorporated into clinical trials (Otley et al., 2002). Otley et al. (2002) reported that IMPACT is a valid and reliable reflection of HRQOL in older children (ages 10-17 years) with IBD.

IMPACT is a relatively new questionnaire available to researchers and clinicians. The first reported use of IMPACT in a clinical trial was in 2005 when Shepanski et al. studied the HRQOL in children and adolescents with IBD after attending a summer camp. Otley et al. (2006) used IMPACT to assess pediatric HRQOL during the first year after diagnosis.

2.3 ORIGIN OF THE INTESTINAL MICROFLORA

2.3.1 Development of Healthy Microflora

The development of the human intestinal microflora is a complicated process involving the microflora of the parent and the infant's immediate environment. The human fetus is sterile in utero, but during and after the birth process, the fetus is exposed to a variety of different species of microbes (Mackie, Sghir & Gaskins, 1999). It is well established that the type of birth delivery has a significant impact on the development of the intestinal microflora (Mackie, Sghir & Gaskins, 1999). Other factors that may contribute to the composition of the intestinal microflora are mother's dietary intake or probiotic use, gestational age, primary source of nutrition (bottle or breast-fed), neonatal health, immunological status, gastrointestinal transit time, pH and stress (Kopp-Hoolihan, 2001).

Dramatic differences have been noted in the microflora of infants born vaginally versus by Caesarean section. The newborn born vaginally first comes into with microflora in the birth canal. Studies have demonstrated that the gastric contents of newborns are very similar to that of the mother's cervix (Brook, Barett, Brinkman, Martin & Finegold, 1979), implying that the mother's microflora is passed to the infant. The microflora of infants born via Caesarean section is believed to be the result of exposure to environmental exposures from obstetrical equipment, air, hospital staff and other infants in the nursery (Lennox -King, O'Farrell, Bettelheim & Shooter, 1976). After birth, environmental, cutaneous and oral microbes will be passed from the mother to the infant through kissing, caressing and suckling (Mackie, Sghir & Gaskins, 1999).

Feeding practices have an impact on the composition of the intestinal microflora. The dominate organisms of formula fed infants are the *Enterobacter* species, where as the microflora of breast-fed infants is predominately *Bifidobacteria* (Mackie, Sghir & Gaskins, 1999). These differences are believed to be due to components in human milk (e.g., human milk oligosaccharides) that is currently not replicated in infant formulas (Mountzouris & Gibson, 2003). Following weaning and introduction of solids, the number and diversity of obligate anaerobes increases (Mackie, Sghir & Gaskins, 1999). By 24 months, the fecal microflora resembles that of an adult (Stark & Lee, 1982). Once the microflora is established, it is stable throughout life and it difficult to change

permanently. It continues to be manipulated throughout life for short periods of time through a variety of factors such as the environment, diet (prebiotics and probiotics), antibiotic therapy and gastrointestinal illness.

2.3.2 Microflora and Gastrointestinal Health

The intestinal microflora plays a crucial role in the gastrointestinal health of the host, which may be both beneficial and harmful to health. For example, specific strains of the healthy gut microflora, such as *Bifidobacterium* and *Lactobacillus* species have anti-inflammatory capabilities that prevent the colonization of the intestine by pathogenic strains of bacteria which are beneficial to health. Pathogenic bacteria (e.g., *Clostridium difficile*) may invade the intestinal microflora causing gastrointestinal illness which is harmful to health. The concept of manipulating the gastrointestinal microflora to prevent or treat illness is currently being promoted. Temporary alterations of the gastrointestinal flora can be achieved through the addition of prebiotics and probiotics in the diet. The addition of probiotics to dairy products and prebiotics to various food sources are just some examples.

2.4 PREBIOTICS

2.4.1 Definition

Prebiotics are defined as non-digestible food ingredients (dietary fibre) that beneficially affect the host by selectively stimulating the growth and/or activity of one of more limited number of bacteria in the colon and thus improve host health (Gibson & Roberfroid, 1995). For a food ingredient to be considered a prebiotic, it must meet several criteria. These criteria include: it must neither be hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract; be selectively fermented by one or a limited number of potentially beneficial bacteria in the intestine; and is able to alter the colonic microflora towards a healthier composition (Gibson & Roberfroid, 1995).

2.4.2 Types of Prebiotics

All prebiotics are described as short-chain carbohydrates with a degree of polymerization (DP) of between 2 and 60 units and are not digested by human or animal

digestive enzymes (Roberfroid, 2002). They are classified as dietary fibre and are widely used in a variety of food products. Lactulose, oligofructose (FOS), galacto-oligosaccharides, inulin, psyllium and germinated barley foodstuff are examples of prebiotics that have been demonstrated to selectively foster the growth of the *Bifidobacterium* and *Lactobacillus* species in the colons of healthy subjects (Kanauchi, Mitsuyama, Araki & Andoh, 2003). Prebiotics have also been shown to stimulate the production of lactate and short chain fatty acids (SCFA's), acetate, propionate and butyrate as end products of prebiotic fermentation (Delzenne, 2003). Recent data obtained from FOS intake indicate that the dose and duration of intake, the place where fermentation occurs (proximal or distal colon), are important factors influencing the prebiotic effect – namely the increase in *Bifidobacteria* (Delzenne, 2003).

2.4.3 Inulin and Fructooligosaccharides (FOS)

The most well studied prebiotics are inulin and FOS. Inulin is a term applied to a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates (Niness, 1999). A product with a DP from 2 to ≥60 is labelled as inulin (Roberfroid, 2001). Oligofructose or FOS is a subgroup of inulin, consisting of polymers with a DP of ≤10 units.

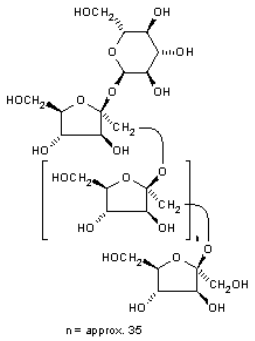
Inulin and FOS are present as plant storage carbohydrates in fruit and vegetables such as wheat, onion, banana, garlic, chicory, endive, Jerusalem artichoke and leek (Van Loo, Coussement, De Leenheer, Hoebregs & Smits, 1995). Human milk contains approximately 5-10 g/L of oligosaccharides (Bode, 2006). The inulin commercially available is synthesized from chicory root. The average daily consumption of inulin and FOS in Europe ranges between 3-11 g/day and 3-10 g/day in the American diet (Van Loo et al., 1995).

2.4.3.1 Chemical Structure of Inulin and FOS

Inulin is not simply one molecule; it is a polydisperse of ß (2-1) linkages (Phelps, 1965). The fructose units in the mixture of linear fructose polymers and oligomers are each linked by ß (2-1) bonds. The ß (2-1) bonds give inulin and FOS unique properties. These linkages prevent inulin from being digested like a typical carbohydrate and are

responsible for it reduced caloric value and dietary fibre effects (Niness, 1999). Figure 2.1 shows an example of the chemical structure of inulin (Stevens, 2006).

Figure 2.1 Chemical Structure of Inulin (Stevens, 2006)



2.4.3.2 Functional Properties of Inulin and FOS

The differences in chain length between inulin and oligosaccharides account for their distinct differences in functional properties. Inulin has a longer chain length than FOS; therefore inulin can form microcrystals when sheared in water or milk. These crystals create a smooth creamy texture and provide a fat-like mouth feel. Because of these characteristics, inulin has been used a fat-replacement in baked goods, fillings, dairy products, frozen desserts and dressings (Niness, 1999).

FOS is composed of short chain oligomers and possesses functional properties similar to sugar or glucose syrup. FOS acts in much the same way sugar does in baked goods, but it has fewer calories and enriches the product with fibre. FOS contributes body to dairy products, depresses the freezing point in frozen desserts, provides crispness to low-fat cookies and acts as a binder in granola bars (Niness, 1999). FOS is often added to yogurt to capitalize on the prebiotic effect, which may serve to enhance the action of the probiotic cultures typically added to yogurt (Niness, 1999).

Both inulin and FOS are used worldwide to add fibre to food products (Niness, 1999). Unlike other fibre, they add no “off flavours” and do not increase viscosity of the food product. These properties allow the formulation of high fibre foods that look and taste like standard food formulations.

2.4.3.3 Nutritional Properties of Inulin and FOS

Inulin has been used in other countries to replace fat, sugar and calories in food products such as ice cream, dairy products, candy and baked goods. Inulin has a lower caloric value as compared to other forms of carbohydrates due to the ß (2-1) linkages (Niness, 1999). The ß (2-1) bonds render inulin non-digestible by human enzymes, thus inulin can pass through the mouth, stomach and small intestine without being metabolized. All ingested inulin or FOS enters the colon where it fermented by the colonic microflora. The energy derived from the fermentation is largely a result of the production of SCFA and lactate, which are metabolized and contribute 1.5 kcal/gram of useful energy (Niness 1999).

Another important attribute of inulin is its action as a dietary fibre. Inulin and FOS influence intestinal function by increasing stool frequency (Gibson, Beatty, Wang & Cummings, 1995), increasing stool weight (Gibson et al., 1995) and decreasing fecal pH (Gibson & Roberfroid, 1995), which has been linked to suppression in the production of putrefactive substances in the colon.

2.4.4 Prebiotic Effects of Inulin and FOS

2.4.4.1 Bifidus Stimulation

The ultimate aim of supplementation of the human diet with prebiotics is the beneficial management of the gut microbiota (Kolida, Tuohy & Gibson, 2002). As mentioned previously, inulin escapes digestion in the upper part of the gastrointestinal tract and reaches the colon virtually intact. Once the inulin enters the colon, the resident bacteria namely *Bifidobacteria* and *Lactobacilli*, ferment the prebiotic.

The most well known actions of inulin are to stimulate the growth of *Bifidobacteria*. Health benefits ascribed to *Bifidobacteria* include the following: inhibiting the growth of harmful bacteria, stimulating components of the immune system, aiding in the synthesis of certain ions, synthesis of B vitamins, prevention of osteoporosis and cancer prevention (Niness 1999; Kolida et al., 2002). The bifidogenic effect of inulin and FOS has been well proven in both *in vitro* and *in vivo* (Gibson & Roberfroid, 1995; Delzenne 2003; Van Loo et al., 1995; Niness 1999; Gibson et al., 1995, Kolida et al., 2002; Kruse, Kleesen & Blaut, 1999). Dramatic positive shifts in the

microflora have shown through *in vivo* human studies at doses of inulin between 5 and 20 g/day over a 15 day period (Gibson et al., 1995; Wang & Gibson, 1993). Table 2.6 summarizes the *in vivo* human studies published to date that determine the bifidogenic effect of inulin and FOS.

Table 2.6 Summary of Human Studies that Provide Statistically Significant Evidence for the Bifidogenic Effect of Inulin and FOS

Reference	Prebiotic	Mode of study	Evidence of prebiotic effect
Kruse et al. 1999	Inulin	8 healthy humans	Increase in *Bifidobacteria*
Bouhnik et al. 1999	FOS	40 healthy humans	Increase in *Bifidobacteria* at 10 g/day
Gibson et al. 1995	FOS and Inulin	8 healthy humans	15 g of inulin/day led to *Bifidobacteria* becoming predominant in feces
Kleessen et al. 1997	Inulin and lactose	20 five elderly constipated patients	Increase in *Bifidobacteria*, decrease in putrefactive bacteria
Tuohy et al. 2001	FOS	31 healthy humans	Increase in *Bifidobacteria* at FOS supplemented at 6.6 g/day
Buddington et al. 1996	FOS	12 healthy humans	Increase in *Bifidobacteria*, no change in total bacteria levels

Kruse et al. (1999) studied the effect of inulin on fecal flora composition in eight healthy living subjects. Subjects consumed a typical Western diet (45% energy as fat, 40% energy as carbohydrate) followed by a fat reduced diet (30% energy as fat, 55% energy as carbohydrate) for a period of 64 days. Inulin was used as the fat replacement (maximum inulin consumed was 34 g/day). The control subjects consumed an identical diet but inulin was not used as fat replacement. A significant increase in *Bifidobacterial* populations was observed. Inulin significantly increased *Bifidobacteria* from 9.8 to 11.0 $\log_{10}$/gram of dry feces.

Bouhnik et al. (1999) assessed the tolerance and threshold dose of FOS in 40 healthy human subjects. The researchers found that fecal *Bifidobacteria* counts increased in seven days. The optimal dose for promoting bifidogenesis without significant side effects was 10 g/day of FOS.

Gibson & Wang (1995) studied the selective stimulation of *Bifidobacteria* by inulin and FOS in a 45-day study of eight healthy subjects. The subjects were fed control

diet of 15 g of sucrose for the first 15 days followed by 15 g of FOS for the next 15 days. Four subjects went on to receive 15 g of inulin for the final 15 days of the study. Both FOS and inulin significantly increased *Bifidobacteria*. Bacteriodes, clostridia and fusobacteria decreased when fed FOS, where as gram-positive cocci decreased when the subjects received the inulin.

Kleessen et al. (1997) studied the effects of lactose and inulin on the bowel habits of elderly constipated patients. Groups of 15 patients received lactose and groups of 10 patients received inulin for 19 days. The dosages of the lactose and inulin were initiated at 20 g/day and gradually increased to 40 g/day. Inulin increased *Bifidobacteria* significantly from 7.9 to 9.2 $\log_{10}$/g dry feces and decreased enterococci and enterobacteria frequency.

Tuohy et al. (2001) completed a double-blind, placebo controlled crossover trial using 31 healthy volunteers. Thirty-one volunteers consumed daily either three experimental biscuits (providing 6.6 g of FOS/day) or three placebo biscuits for a 21 day crossover period. *Bifidobacteria* significantly increased with the biscuits supplemented with the FOS. The researchers also found that *Bifidobacterial* numbers returned to pre-treatment levels within seven days, following discontinuation of the biscuits containing the FOS.

Buddington et al. (1996) studied the influence of FOS on the fecal flora in 12 healthy humans. The subjects were fed a control diet for 42 days of 4 g of FOS/day between days 7 and 32. Increases in fecal *Bifidobacteria* were noted when the subjects were on the control diet, with the highest density of *Bifidobacteria* occurring when the diet was supplemented with FOS.

On the basis of the results of well-designed human studies, it can be concluded that inulin administered at 5-15 g/day for a few weeks can change the composition of the fecal bacteria, especially the *Bifidobacteria*.

2.4.4.2 Fermentation

Carbohydrates such as inulin or FOS that reach the proximal colon are highly fermented. Inulin and FOS are so highly fermented that studies have been unable to recover any of these products in the feces (Cummings & Macfarlane, 2002). The lower DP oligomers (i.e., FOS) are believed to be fermented in the proximal colon and the

longer chain prebiotics (i.e., inulin) are fermented more distally in the colon (Kolida et al., 2002).

Fermentation of carbohydrates by colonic microflora is an anaerobic process producing gas (CO_2, H_2 and CH_4), short chain fatty acids (SCFA) and lactic acid (Cummings & Macfarlane, 2002). Lactic acid is further metabolized into SCFA. These gases are utilized by fecal bacteria, absorbed then excreted in breath or excreted in stools. The majority of SCFA are absorbed and only very small fraction is found in the stool. SCFA are an important source of energy for bacteria such as *Bifidobacteria* and *Lactobacilli*.

The SCFA have important effects on the intestinal tract. It is largely accepted that butyrate plays an essential role in maintaining colonic mucosa integrity by acting on epithelial cell metabolism, proliferation and differentiation (Cherbut, 2002). While prebiotics have been shown to be a source of SCFA both *in vivo* and *in vitro* in animals, *in vivo* studies in humans have been inconclusive (Gibson, Beatty et al., 1995; Kleessen et al., 1997; Cummings & Mcfarlane, 2002). Inulin and FOS are known to produce butyrate and therefore, may modulate intestinal barrier function (Cherbut, 2002). This hypothesis still needs to be tested in humans. However, short-chain FOS, known to produce substantial amounts of butyrate in the colon, has been shown to increase mucosal crypt height and epithelial cell density in neonatal pigs (Delzenne, 2003). Further studies in humans are needed to identify the exact mechanism of fermentation on mucosal cells.

2.4.4.3 Fecal bulking effect

Carbohydrates that reach the bowel have a mild laxative effect. The clearest demonstration of a mild laxative effect is in the controlled diet study completed by Gibson et al. (1995), which showed 15 g/day of inulin and FOS increased stool output. Dahl et al. (2005) confirmed that inulin fortification (13 g/day) produces significant fecal bulking in institutionalized individuals. In addition, inulin has been shown to stimulate bowel movements and increase stool frequency, in elderly constipated patients (Kleessen et al. 1997). Other human experiments using inulin and FOS have shown not been able to demonstrate an increase in stool output, but the studies did not control for diet, which would mask the small effects. Inulin and FOS are likely mild laxatives, but

because the effect is small it is difficult to detect except in carefully controlled studies (Cummings & Macfarlane, 2002).

2.4.5 Safety of Prebiotics

Inulin and FOS are present in the daily diet of many of the world's population (Van Loo et al., 1995). Up to 20 g/day/person may be ingested through the normal diet without noticeable side effects (Coussement, 1999). Historical literature provides no specific reports doubting the safety of inulin containing vegetables (Coussement, 1999). In fact, many inulin-containing vegetables (e.g., chicory, garlic and leek) have been noted to have beneficial effects on health.

The long history of mankind's safe use of inulin-containing foods is reflected by the fact that very little formal toxicity testing in laboratory animals has been reported (Coussement, 1999). Numerous publications in peer-reviewed clinical journals document careful studies with inulin and FOS in healthy subjects and patients with chronic diseases (Roberfroid, Gibson & Delzenne, 1993). The studies of subjects with various disease conditions and of differing ages provide additional assurance of the safety of inulin and FOS.

2.4.5.1 Tolerance

Tolerance of a nondigestible fibre is determined by the osmotic effect and the fermentation effect. The osmotic effect leads to an increased presence of water in the colon. Smaller molecules like sorbitol exert a higher osmotic pressure and bring more water into the colon (Roberfroid et al., 1993). The fermentation effect is caused by the production of fermentation products, mainly SCFAs and gases. Slow fermenting products such as inulin are easier to tolerate than FOS (Roberfroid et al., 1993).

It is difficult to determine the tolerance of fibre for an individual, as individuals have different tolerance and intestinal acceptability levels. For example, diarrhea is a symptom of poor tolerance, but soft stools may be acceptable or even a desired outcome. Flatulence is even more difficult to measure objectively, as what is acceptable for one person may be intolerable for another.

An approach, based on personal judgement of discomfort, was designed by Orafti et al. to determine the tolerance level of fermented carbohydrates such as inulin

and FOS (Roberfroid et al., 1993). A food is considered unacceptable, if it causes one or more of the following symptoms: too much flatulence, too much intestinal pressure, too much intestinal noise, too much intestinal cramping or diarrhea as observed by the individual (Roberfroid et al., 1993). The no observed adverse effect level (NOAEL) for inulin is proposed to be 30 g/day (Roberfroid et al., 1993). Orafti's tests and experience show that regarding the sensitivity to (totally) fermentable carbohydrates, three categories of people can be distinguished: 1) nonsensitive persons can consume 30 g/day without the undesirable actions described above; 2) sensitive persons can consume 10 g/day without undesirable actions but might experience undesirable reactions with 20 g/day; 3) very sensitive persons can experience undesirable reactions at doses of ≤10 g/day (Roberfroid et al., 1993). The values reported are based on adult volunteers. Little or no information is available concerning the acceptability of indigestible carbohydrates for children. A test with FOS completed by Cadranel et al. (1995) showed that daily doses of 3, 6, 9 g of FOS in drinks or confectionary products cause no significant undesirable effects in children aged 10 to 13 years of age (Roberfroid et al., 1993).

Inulin appears to be better tolerated than FOS. Inulin has rarely been shown to cause diarrhea (Roberfroid et al., 1993). The values that can be recommended as formulation doses for inulin, based on both tests with volunteers and the experience of the food industry are 10 g/day (Roberfroid et al., 1993), a value significantly less than the proposed NOAEL of 30 g/day.

2.4.6 Prebiotics and Infant Nutrition and Health

In the newborn breast-fed infant, a stable microflora is developed in the colon within five days of birth. Breast-fed infants are known to have a gastrointestinal flora that is dominated by *Bifidobacteria* and *Lactobacilli*. Although breast-fed infants and formula fed infants have a similar gastrointestinal flora at days three and four of life, there is a substantial difference in colonic flora after several weeks of life, which is dependant on mode of feeding (Harmsen et al., 2000). *Bifidobacteria* become predominant in breast-fed infants, but the formula fed infant's colonic flora is dominated by anaerobic bacteria such as bacteroides (Harmsen et al., 2000). More importantly, the

numbers of pathogenic bacteria such as clostridium and coliform are higher in infants receiving formula.

The mechanism that appears to play a role in the promotion of growth of *Bifidobacteria* in the colon of breast-fed infants are the oligosaccharides found in human milk. Human milk oligosaccharides are the third most abundant solid constituents of human milk (Picciano, 2001). Human milk oligosaccharides are postulated to play an immuno-protective role in the infant. Research is only beginning to understand the defense mechanisms of human milk oligosaccharides and its role in infant health. There is substantial evidence suggesting that oligosaccharide secretion in mother's milk is a very complex, variable and dynamic process (Harmsen et al., 2000). Cow's milk and infant formula have very low levels of oligosaccharides.

The addition of prebiotics (e.g., inulin, FOS and galacto-oligosaccharides) to infant formula stimulates the growth of *Bifidobacteria* and plays an immuno-protective role in the infant (Boehm et al., 2002; Vandenplas, 2002). Supplementation of a preterm formula with a mixture of FOS and galacto-oligosaccharides has been shown to stimulate the growth of *Bifidobacteria* and results in stool frequency and consistency similar to preterm infants fed human milk (Boehm et al., 2002). Similar results have been observed in term infants fed a combination of galacto-oligosaccharides and FOS versus standard infant formula (Hansjorg et al., 2003). The infants fed the formula containing the nondigestible oligosaccharides had higher numbers of *Bifidobacteria* compared with infants fed standard infant formula.

FOS supplementation may have potential immunomodulatory activity in the infant, although research is very preliminary. Infant cereal supplemented with FOS has been shown to decrease the occurrence of febrile illness, associated with diarrhea or respiratory illness (Saavedra & Tshernia, 2002). An additional study observed improved humoral immune response to measles vaccine in infants receiving infant cereal containing FOS (Firmansyah et al., 2001). The mechanisms of the observed clinical benefit have yet to be defined, but it is becoming apparent that modifications in intestinal flora by ingestion of prebiotics may yield immunological benefits (Saavedra & Tshernia, 2002).

2.4.7 Prebiotics and Gastrointestinal Disease

2.4.7.1 Inflammatory Bowel Disease

The effect of inulin has been tested in the rat model of distal colitis induced by dextran sulphate sodium, which histologically resembles human UC (Videla et al., 2001). Oral inulin prevented mucosal inflammation, as demonstrated by lower colonic lesion scores and the release of fewer inflammatory mediators in test rats as compared with controls (Videla et al., 2001).

Several small scale studies of prebiotics have been reported in UC patients (Table 2.7). Inulin has been tested in patients with ileal pouch-anal anastomosis. Compared with placebo, three weeks of dietary supplementation with 24 g of inulin increased butyrate concentrations, lowered pH, decreased numbers of *Bacteriodes fragilis*, and diminished concentrations of secondary bile acids in feces (Welters et al., 2002). This was endoscopically and histologically accompanied by a reduction of inflammation of the mucosa of the ileal reservoirs.

A multi-center open trial, completed over 24 weeks, has suggested that administration of 20-30 g/day of germinated barley foodstuff (GBF), provided in addition to base line therapy may be useful in the induction and maintenance of clinical remission in patients with mild to moderately active UC (Kanauchi et al., 2003). Patients consuming GBF showed significant decrease in clinical activity index scores and increased fecal concentrations of *Bifidobacteria*.

A 24-week trial found that psyllium was superior to the placebo in alleviating the gastrointestinal symptoms of patients with UC in remission (Hallert et al., 1991). In a randomized control trial, psyllium was equivalent to mesalamine, a commonly prescribed anti-inflammatory agent in maintaining remission in UC (Fernandez- Banares et al., 1994).

Hallert et al (2003) investigated the effects of oat bran on remission rates in patients with UC. This pilot study involved adding 60 g of oat bran, corresponding to 20 g of oat fibre, to the daily diet of 22 patients. During the intervention, the fecal butyrate concentrations increased significantly and reduced symptomology in patients reporting abdominal pain and reflux.

Davies et al. (1978) studies the effect of a high fibre diet on remission rates in UC patients in remission taking sulfasalazine® (SAS). The increased fibre was in the form of whole wheat bread, vegetables and a supplement of 25 g/day of bran. Fifteen patients continued on SAS and a regular diet and 20 patients were provided a high fibre diet and the SAS was stopped. The relapse rate of the high fibre group was 75% and 20% relapse rate with the patients that remained on the SAS. This study demonstrates that not all types of dietary fibre will have a prebiotic effect on the intestinal mucosa. It appears that only specific prebiotics play a role in maintaining remission in UC.

The human studies mentioned above, support the idea that prebiotics may prevent intestinal inflammation. More clinical trials with larger populations are necessary to demonstrate the effectiveness of prebiotics in the prevention of inflammation in IBD.

Table 2.7 Evidence for the Prebiotic Effect in Ulcerative Colitis

Authors	Prebiotic	Dosage (g/day)	Mode of study	Length of treatment	Evidence for prebiotic effect
Welters et al., (2002)	Inulin	24 g/day	20 patients with ileal pouch-anal anastomosis	3 weeks	Increased butyrate concentrations, lowered pH, decreased numbers of *Bacteriodes fragilis*, and diminished concentrations of secondary bile acids in feces.
Kanauchi et al., (2003)	Germinated barley food stuff (GBF)	20-30 g/day in addition to standard medical therapy	18 patients with moderately active UC	4 weeks	Patients consuming GBF showed significant decrease in clinical activity scores, increased concentration of *Bifidobacteria* in stool.
Hallert et al., (1991)	Psyllium	Group A: 20 g/day psyllium Group B: 1.5g/day mesalamine Group C: 20 g/day psyllium and 1.5 g/day mesalamine	105 patients with UC in remission	24 weeks	Decreased symptoms of UC, increase in *Bifidobacteria* and decrease in free water in the stool.
Hallert et al., (2003)	Oat bran	60 g/day	22 patients with UC in remission	12 weeks	Increased butyrate concentrations, significant improvement in symptoms.

2.4.7.2 Colon Cancer

Experimental observations have suggested that prebiotics may play a protective role in the development of colon cancer. Recent research in experimental animal models has revealed that inulin has significant anticarcinogenic properties (Pool-Zobel & Cherbut 2003; Marteau & Boutron-Ruault, 2002). Inulin acts chemopreventively by reducing the incidence of tumours in the colon (Pool-Zobel & Cherbut, 2003). The effects may be due to the stimulation of the *Bifidobacteria*, which themselves have been shown to be antigentoxic in the colon (Pool-Zobel & Cherbut, 2003). Fermentation

products, such as the short chain fatty acids may also contribute to the protective effects (Marteau & Boutron-Ruault, 2002). The experimental evidence from animal studies strongly supports the possibility that inulin will contribute to reducing colon cancer risk in humans (Pool-Zobel & Cherbut, 2003). Clinical and epidemiological studies in humans are currently in progress to evaluate the relevance of prebiotics in reducing colon cancer risk (Van Loo, Clune , Bennett, & Collins, 2005).

2.4.7.3 Bowel Habits

All carbohydrates that reach the large intestine have a mild laxative effect. Non-digestible carbohydrates with a low molecular weight exhibit a positive effect on intestinal transit in constipated patients (Marteau & Boutron-Ruault, 2002). The clearest demonstration of a mild laxative effect of prebiotics is in a controlled diet study which showed that 15 g of FOS increased stool output significantly from 136 to 154 g/day (Gibson, Beatty et al., 1995). In studies reporting only qualitative data, FOS or inulin 'improved constipation' in small groups of hospitalized patients (Kleesen et al., 1997; Hidaka & Hirayama, 1991). Inulin and FOS are likely laxative but because the effects are small, it is difficult to detect except in carefully controlled studies (Cummings & Macfarlane, 2002).

2.4.7.4 Traveller's Diarrhea

Traveller's diarrhea remains a common problem for those journeying overseas for either business of holidays. It is particularly frequent on visitors to Central America, the Far East, India and parts of Asia. Current estimates are that 60 million travellers from the West visit high-risk areas annually and of these, 30-50% had episodes of diarrhea (Cummings & Macfarlane, 2002). A diet controlled study showed that administering 10 g of FOS daily failed to prevent traveller's diarrhea in 244 healthy subjects travelling to high and medium risk destinations for traveller's diarrhea (Cummings, Christie & Cole, 2001). There are multiple causes for diarrhea in travellers, such as exposure to different foods, increased alcohol consumption and pathogens that affect the small bowel versus the large bowel (Cummings & Macfarlane, 2002). The wide variety of etiologies of traveller's diarrhea may be some of the reasons non-digestible carbohydrates failed to show beneficial effects in prevention of traveller's diarrhea.

2.4.7.5 Antibiotic-Associated Diarrhea (AAD)

The incidence of diarrhea following antibiotic use is particularly high with *Clostridium difficile* disease being most common (Aslam, Hamill & Musher, 2005). Antibiotic-Associated Diarrhea (AAD) is the result of disrupted microflora and most often occurs two to eight weeks after antibiotic use and is associated with age, health status, hospitalization and exposure to nosocomial pathogens (McFarlane, 1998). Oligofructose is metabolized by *Bifidobacteria* resulting in a marked increase in numbers of *Bifidobacteria* in healthy volunteers, thereby decreasing *Clostridia* species (Gibson, Beatty et al., 1995). Lewis et al. (2005) provided 12 g of oligofructose or placebo (sucrose) daily to 435 elderly patients along with their prescribed antibiotics. Stools were cultured for *Bifidobacteria*, *C. Difficile* and anaerobes. Oligofructose was well tolerated by the patients and concentration of fecal *Bifidobacteria* was significantly higher in the patients receiving oligofructose versus the placebo group ($p<0.001$). Stool form and consistency was not altered with the oligofructose supplementation nor was the incidence of diarrhea reduced. Lewis et al. (2005) concluded that oligofructose had no effect on the prevention AAD.

Lewis, Burmeister, & Brazier (2005) provided 142 inpatients diagnosed with *C. difficile* toxin-associated diarrhea with 12 g of oligofructose or placebo for 30 days in addition to antibiotic therapy. Compared to baseline stool culture, oligofructose resulted in increased fecal concentrations of *Bifidobacteria* ($p<0.0001$). Patients taking the oligofructose were less likely to develop a relapse of diarrhea than those taking the placebo (8.3% oligofructose vs. 34.3% of placebo; $p<0.001$). Lewis, Burmeister and Brazier (2005) concluded that oligofructose appears to be effective in preventing relapse of *C. difficile*-associated diarrhea.

2.4.7.6 Necrotizing Enterocolitis (NEC)

Necrotizing Enterocolitis (NEC) is an acquired disease, primarily of very low birth weight infants (<1500 g at birth), which is characterized by mucosal or intestinal necrosis in the terminal ileum, with the colon and the proximal small bowel involved less frequently (Beers & Berkow, 2005). NEC is a serious gastrointestinal disease of unknown etiology (Lin et al., 2005). Despite advances in neonatal practice, NEC

remains a major cause of gastrointestinal issues in premature infants and the first cause of death in extremely premature infants (Butel, Waligora-Dupreit & Szylit, 2002).

The pathophysiology of NEC still remains to be elucidated, but immaturity of the gut, enteral feeding and bacterial colonization are all thought to be involved (Butel et al., 2002). Butel et al. (2002) have suggested that a delayed colonization by *Bifidobacteria* promotes colonization of potentially pathogenic bacteria which may contribute to the development of NEC. It is now being postulated that modulating the microbiota of the intestine by prebiotics, may play a role in prevention of NEC. A study by Butel et al. (2002) using gnotobiotic quails as an experimental model of NEC revealed that oligofructose dietary supplementation was shown to increase *Bifidobacteria* level and decrease pathogenic bacteria. Oligofructose acted as an anti-infective agent and decreased the occurrence and severity of intestinal lesions associated with NEC. The researchers concluded that the addition of oligofructose to pre-term milk may maintain colonization of *Bifidobacteria* and play a role in the prevention of NEC. Studies in human neonates have yet to be completed.

2.5 PROBIOTICS

2.5.1 Definition

A probiotic has been defined as a preparation of a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial effects in the host (Schrezenmeir & De Vrese, 2001). In order for microorganisms to belong to the probiotic group they must meet several criteria. These criteria include: be able to withstand and survive the effect of gastric acid, biliary secretions and pancreatic secretions in order to reach the small and large intestines; be non-pathogenic and non-toxic; remain viable during transport and storage; exert beneficial effects on the host; stabilize the intestinal microflora; adhere to the intestinal epithelial cell lining; and produce antimicrobial substances towards pathogens (Tomasik & Tomasik, 2003).

2.5.2 Types of Probiotics

The most well-studied probiotic species belong to the *Lactobacillus* and *Bifidobacterium* species. Table 2.8 lists some of the commercially available probiotics that have been added to foods (e.g., yogurt or fermented milk products) or concentrated in supplements intended for human consumption.

Table 2.8 Summary of the Probiotic Species Studied for Clinical Use

***Lactobacillus* Species**	*L. acidophilus, L. bulgaricus, L. casei, L. fermentum, L. gasseri, L. johnsonii, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus, L. salivations*
***Bifidobacteria* Species**	*B. bifidum, B. breve, B. lactis, B. longum*
***Saccharomyces* Species**	*S. boulardii*
***Escherichia coli* Species**	*E. coli* Nissle 1917
***Enterococcus* Species**	*E. faecium*
***Streptococcus* Species**	*S. thermophilus*

Lactobacilli are non-spore-forming bacteria that ferment glucose into lactate, (Attisha & Clark, 1995). The most common application of *Lactobacillus* is industrial, specifically for the production of dairy products (Kopp-Hoolihan, 2001). Because of their ability to derive lactic acid from glucose, these bacteria create an acidic environment which inhibits growth of many bacterial species (Attisha & Clark, 1995). This genus also contains several bacteria that make up part of the natural flora of the human gastrointestinal tract (Linskens, Huijsdens, Savelkoul, Vandenbroucke-Grauls & Meuwissen, 2001). *Lactobacillus* is generally harmless to humans, rarely inciting harmful infections or diseases (Salminen & Donohue, 1996). *Lactobacillus GG (LGG)* is the most well studied probiotic (Salminen & Donohue, 1996). The clinical potential has been documented in several diarrheal diseases such as *C. difficile*-associated diarrhea, antibiotic-associated diarrhea, infectious diarrhea, atopic eczema and IBD (Schultz, Scholmerich & Rath, 2003).

Bifidobacteria are a major group of saccharolytic bacteria found in the large intestine (Teitelbaum & Walker, 2002). Members of the genus *Bifidobacterium* are

anaerobic, gram-positive bacilli rarely associated with infection (Makelainen, Tahvonen, Salminen & Ouwehand, 2003). *Bifidobacterium* accounts for 25% of the bacteria in the adult colon and 95% of the bacteria in the breastfed newborn (Teitelbaum & Walker, 2002).

Saccharomyces boulardii is a yeast preparation that has been shown to inhibit the growth of pathogenic bacteria both *in vitro* and *in vivo* (Teitelbaum & Walker, 2002). It lives at an optimum temperature of 37°C, and has been shown to resist digestion and thus reaches the colon in a viable state (Teitelbaum & Walker, 2002). *S. boulardii* has also been found to be unaffected by antibiotic therapy (Teitelbaum & Walker, 2002).

2.5.3 Efficacy of Probiotics

Studies on lactose intolerance, diarrhea and colon cancer show that a daily dose of 10^9 to 10^{10} (one to ten billion) colony forming units (cfu) of viable bacteria are needed for any measurable effect on health (Sanders, Walker, Walker, Aoyama & Klaenhammer, 1996). Effects of consuming lower levels have not been documented in research studies. It is believed that probiotics do not permanently adhere to the intestinal cells, but exert their effects as they metabolize and grow. Goldin et al. (1992) found that when *LGG* supplementation was stopped, the *LGG* disappeared from the feces of 67% of the volunteers within 7 days. Thus, daily consumption of probiotic is the best way to maintain the probiotic effects (Kopp-Hoolihan, 2001).

In the future, probiotics are to be regulated by the Natural Health Products Directorate (NHPD) under the direction of the Health Products and Food Branch of Health Canada. At the present time, the concentration of probiotics in food and supplements varies tremendously between products (Kopp-Hoolihan, 2001). It is up to manufacturers to disclose the probiotic content of their products. With the implementation of the NHPD requirements for probiotic concentration and viable counts, at the time of consumption, will become standardized for these products.

The shelf-life, for refrigerated products containing probiotics, ranges from 3-6 weeks (Kopp-Hoolihan, 2001). The shelf-life of dried supplements is about 12 months. However, the viable counts may drop off significantly during this time (Kopp-Hoolihan, 2001).

2.5.4 Safety of Probiotics

The majority of scientific studies completed to date have described a good tolerance to probiotic preparations and the absence of significant adverse effects. Although most commercially available probiotic strains are considered to be safe, there are concerns with their use in particular patient populations.

The most important area of concern is the risk of developing sepsis following supplementation with probiotics. Several reports have directly linked cases of sepsis to ingestion of probiotics (Boyle, Robins-Browne & Tang, 2006). Bacterial sepsis, endocarditis and liver abscess have been reported in both adults and children. Rautio et al. (1999) report a case of an elderly woman with Diabetes Mellitus (Type 2, non-insulin dependant) who developed liver abscess and pneumonia following daily supplementation with LGG. Endocarditis developed in a 67-year old-man with mitral regurgitation following a dental extraction who was taking the probiotic *L. rhamnosus* (Mackay, Taylor, Kibbler & Hamilton-Miller, 1999). Bacterial sepsis has been reported in three premature infants with short bowel syndrome taking LGG supplements (Kunz, Noel & Fairchok, 2004; De Groote, Frank, Dowell, Glode & Pace, 2005). *S. Boulardii* related fungemia is the most commonly described risk factor of probiotic therapy. Boyle et al. (2006) reviewed over 19 cases of *S. Boulardii* related fungemia in both adults and children with a variety of medical conditions.

Bifidobacteria are generally recognized as safe for human consumption, based on their wide use in fermented dairy products (Makelainen et al., 2003). Furthermore, *Bifidobacteria* are the major component of intestinal microbiota. To date, there have been no case reports in the literature linking *Bifidobacteria* to infectious disease. Makelainen et al. (2003) assessed the tolerance and safety of two *B. longum* strains in 39 healthy adults between the ages of 19 and 60. The subjects were randomly assigned into two groups, each of which received two capsules daily containing 10^9 cfu of *B. longum* strains (46 and 2C) or a placebo (maltodextrin) for three weeks. No side effects were reported and no undesirable changes in immune parameters were discovered. Makelainen et al. (2003) concluded that the two *B. longum* strains (46 and 2C) were well tolerated by the subjects and are safe for human consumption.

All cases of bacteremia and fungemia related to administration of probiotics have occurred in individuals who were immunocompromised or had an underlying chronic disease or debilitation (Boyle et al., 2006). Many cases of probiotic sepsis involved patients who had underlying intestinal pathology (e.g., short bowel syndrome or diarrhea) which increases the risk for bacterial translocation. Tube feeding or the presence of a central venous catheter is also a common finding in cases of probiotic sepsis. Premature infants are at increased risk for bacteremia. No reports of sepsis have occurred in healthy individuals. Boyle et al. (2006) propose that the following risk factors merit caution in using probiotics: 1) immune compromise or premature infants, 2) central venous catheter, 3) administration of probiotic via jejunostomy, 4) concomitant administration of broad spectrum antibiotics to which probiotic is resistant, 5) probiotics with properties of high mucosal adhesion or known pathogenicity and 6) cardiac valve disease. Thus, most *Lactobacilli* and *Bifidobacteria* used in the food industry and in clinical trials appear to be safe for the general adult and pediatric populations.

2.5.5 Mechanisms of Action of Probiotic Bacteria

Several mechanisms of action have been postulated to influence the effectiveness of probiotics in IBD. The mode of action of probiotic strains is likely to be multifactoral and strain specific. Some of the postulated mechanisms include suppression of growth or epithelial binding/invasion of pathogenic enteric bacteria, improvement of epithelial and mucous barrier function or engagement in immunomodulatory activities (Sartor, 2004).

The concept of microbiological balance existing in the intestine, involving competition between probiotic and pathogenic bacteria for specific binding sites on intestinal epithelial cells has been well established (Fedorak & Madsen, 2004). Probiotic bacteria have been shown to colonize the gut during therapy to induce specific pH and other chemical changes in the lumen, which may directly inhibit the growth of the pathogenic bacteria (Hart, Stagg & Kamm, 2003). Suppression of growth and function of pathogenic bacteria has been attributed to decreases in luminal pH via production of organic acids, secretion of bactericidal proteins and prevention of epithelial adherence (Sartor, 2004).

Intestinal epithelial cells produce mucin genes (i.e., MUC2 and MUC3) which bind to pathogens, inhibiting invasion into the epithelial cell (Chang et al., 1994). Mack et al. (1999) reported that patients with IBD have reduced numbers of goblet cells at the sites of inflammatory lesions. In addition, the mucins present at the inflamed sites have reduced ability to bind pathogenic bacteria. Mack et al (1999) demonstrated *in vitro* that probiotics *L. plantarum* and *L. rhamnosus* GG inhibit the adherence of pathogenic *E.coli* to intestinal epithelial receptors through the increased expression of intestinal mucin genes, MUC2 and MUC3.

Probiotics have the ability to produce antimicrobial compounds called bacteriocins which have an antagonistic effect against pathogens (Flynn et al., 2002). Bacteriocins are bioactive peptides synthesized by probiotic bacteria that have a bactericidal effect or bacteriostatic effect on other closely related species (Flynn et al., 2002). Bifidobacterium strains synthesize a broad range of bacteriocin-like inhibitory compounds that play a key role in the inhibition of pathogens (Collado, Hernandez & Sanz, 2005).

Probiotic bacteria have been shown to enhance gut epithelial and mucosal barrier function. Bacteria pathogens exert their detrimental effects by disrupting the barrier functions of mucosal cells, thereby increasing pathogen invasion (Madsen et al., 2001). Specifically, pathogenic bacteria disrupt the protein phosphorylation of claudin and occludin causing mucosal injury and subsequent pathogen invasion (Resta-Lenert & Barrett, 2003). When human intestinal cells are pretreated with two probiotic strains (e.g., *L. acidophilus* and *S. thermophilus*) invasion of the pathogenic bacteria enteroinvasive *Escherichia coli* was prevented (Resta-Lenert & Barrett, 2003). The mechanism of action is suggested to be that probiotics have the ability to modify tight junctions and the cytoskeleton in the epithelial cells, which interferes with pathogen adhesion and invasion.

Probiotic bacteria have been found to interact with epithelial and immune cells in the gastrointestinal tract altering immune function (Fedorak & Madsen, 2004). A variety of probiotic strains have the ability to block cytokine production by the intestinal epithelial cells (Fedorak & Madsen, 2004). The murine interleukin-10 (IL-10) deficient mouse colitis model has provided great insight into the role of probiotics as a treatment

modality for IBD. Mice that lack IL-10 develop lesions that closely mirror the lesions found in IBD when infected with pathogenic bacteria. Madsen et al. (2001) were the first to demonstrate that administration of *L. reuteri* into the lower gastrointestinal tracts of IL-10 deficient mice prevented the development of colitis in these animals. The mechanism of action is believed to be the replenishment of IL-10 by the probiotic bacteria, indicating the importance of IL-10 in controlling intestinal inflammation. Pena et al. (2005) pretreated IL-10 deficient mice with *L. reuteri* and *L. paracasei* and then challenged the mice with pathogenic bacteria (*Helicobacter hepaticus*). This group found that the pro-inflammatory cytokines (IL-12 and tumor necrosis factor-α) were lowered in the probiotic-treated mice, thus showing the important role probiotics play in immunomodulatory activities.

It remains to be established which particular mechanism of action will relate to the treatment of IBD. Significant advances continue to be made in the understanding of these complex mechanisms. However, future research will need to determine whether probiotic bacteria exert their beneficial effects through a single versus multiple mechanisms of action.

2.5.6 Probiotic Use in Gastrointestinal Diseases

The use of probiotics as therapeutic agents for gastrointestinal disease is rapidly moving into mainstream medical management. Probiotic therapy has been investigated for its effectiveness against a range of gastrointestinal disorders such as IBD (i.e., UC, Crohn's disease and pouchitis), diarrhea, irritable bowel syndrome (IBS), *H. pylori* infection, lactose intolerance and necrotizing enterocolitis. Most of the probiotic agents currently being examined appear to be safe, with no adverse effects noted in thousands of subjects involved in clinical research (Saavedra, 2001).

2.5.6.1 Ulcerative Colitis

Preliminary controlled clinical data suggests that *Escherichia coli* Nissle 1917, VSL#3®, *Bifidobacteria* and *S. boulardii* may be beneficial in the management of UC. The majority of clinical trials that have assessed the efficacy of probiotics in UC have focused on the maintenance of remission. Table 2.9 summarizes the randomized control trials that support the use of probiotics in UC.

Clinical studies have demonstrated that *Escherichia coli* Nissle 1917 is as effective as mesalazine in maintaining remission in UC. The first study completed by Kruis, Schulze, et al. (1997) studied 120 patients with UC in remission in a multicenter, double-blind, double-dummy study. For 12 weeks, patients received either 1.5 g of mesalazine daily or 5.0 x 10^{10} cfu/day of the viable *E.coli strain* Nissle 1917. Relapse rates were 11.3% for subjects receiving mesalazine and 16% for subjects receiving the *E.coli* Nissle 1917 (NS). Kruis, Fric, et al. (2004) completed a larger trial with 327 patients with UC in remission. The subjects were randomized to receive 5.0 x 10^{10} cfu/day of *E.coli* Nissle 1917 or 1.5 g/day of mesalazine for one year. Relapse rates were 36.4% patients in the *E.coli* Nissle 1917 group and 33.9% in the mesalazine group (p=0.003), which suggests equivalence between *E.coli* Nissle 1917 and mesalazine in maintaining remission in UC.

Rembacken, Snelling, Hawkey, Chalmers and Axon (1999) performed a single center, randomized, double dummy study in 120 patients with active UC. In the initial phase of the study, patients were randomized to receive 2.4 g daily of mesalazine or 1.00 x 10^{11} cfu/day of *E.coli* Nissle 1917 in attempt to induce remission. All patients received standard medical therapy (e.g., hydrocortisone enemas or oral Prednisone®) together with a one week course of oral gentamicin at entry into the study. Patients that were in remission within three months entered a follow-up phase of the study. The patients were randomized to receive 1200 mg/day of mesalazine or 5.0 x 10^{10}cfu/day of *E.coli* Nissle 1917 for one year. Initially, 75% of patients in the mesalazine group attained remission versus 68% in the *E.coli* group (p=0.0508). During the one year follow-up, relapse occurred in 73% of patients in the mesalazine group versus 67% in the *E. coli* group (NS). Rembacken et al. (1999) concluded that treatment with *E.coli* Nissle 1917 is equivalent to mesalazine in maintaining remission of UC.

Tursi et al. (2004) compared the efficacy and safety of a low dose of balsalazide (2.25 g/day) plus VSL#3® (9.0 x 10^{12} cfu/day) with balsalazide alone (4.5g/day) and with mesalazine (2.4 mg/day) in 90 patients with mild-moderate UC. All patients were randomly assigned to treatment groups with the duration of the treatment being eight weeks. The efficacy of the treatment was assessed by symptoms, endoscopic appearance and histological evaluation. The remission rate was 80% for the balsalazide/VSL#3®

group, 77% for the balsalazide group and 53% for the mesalazine group ($p<.02$). Balsalazide plus VSL#3® was found to be significantly superior to balsalazide and to mesalazine in obtaining remission, it had better tolerance and was also superior in improving the clinical parameters evaluated.

Twenty patients with UC in remission, who were intolerant or allergic to 5-ASA, were treated with two doses of 3 g of VSL#3® (3.0×10^{12} cfu/day) for one year (Venturi et al., 1999). After 12 months of treatment, 75% of the patients were still in remission. Fecal concentrations of *S. salivarius* ssp. *thermophilus, lactobacilli* and *Bifidobacteria* increased significantly from baseline level ($p<.05$) and fecal pH was significantly reduced ($p<.05$). These results indicate that VSL#3® was able to colonize the intestine and may be useful in maintaining remission in UC patients.

In an open-label study, 24 patients with mild to moderate UC completed a four week course of *S. boulardii* (750 mg/day) while receiving maintenance treatment with mesalazine (3 g/day) (Guslandi, Giollo & Testoni, 2003). During this four week pilot study, 71% of the patients attained clinical remission, which was confirmed endoscopically.

Ishikawa et al. (2003) provided 100 ml/day of a fermented milk product containing live strains of *B. bifidum*, *B. breve* and *L. acidophilus* (1.0×10^{10} cfu/100 ml) for one year to patients in an unblinded, randomized control study. Patients had been diagnosed with UC at least one year previously and were in remission. Standard treatment for UC was provided as usual using salazosulfapyridine, mesalazine and steroids. Twenty-one subjects were assigned to either the bifido-fermented milk group (n=11) or the control group (n=10). During the one year study duration, exacerbation of UC symptoms was seen in three out of the 11 subjects in the bifido-fermented group versus nine out of ten subjects in the control group. A significant reduction in cumulative exacerbation rates was seen in the group consuming the bifido-fermented milk in addition to the standard medical therapy ($p = 0.0184$).

Kato et al. (2004) conducted a randomized placebo controlled study in 20 subjects with mild to moderate, active UC. A fermented milk product containing live strains of *B. bifidum*, *B. breve* and *L. acidophilus* (1.0×10^{10} cfu/100 ml) was provided for 12 weeks along with conventional treatment (e.g., 5-ASA or sulfasalazine). The

subjects treated with bifido-fermented milk (BFM) had a 70% response to treatment with 40% achieving remission, where as 33% of the placebo group had a response to treatment with only 33% achieving clinical remission. Clinical activity index scores were significantly lower in the BFM group versus placebo ($p<0.05$). Increases in fecal butyrate, propionate and other short chain fatty acids were also noted to be higher in the BFM group. The researchers concluded that supplementation with BFM along with conventional treatment is a safe and effective way to manage active UC.

Bibiloni et al. (2005) recently published the results of an open-label trial in patients with active UC not responding to conventional therapy. Thirty-two patients completed six weeks of treatment with VSL#3® (3.6×10^{12} cfu/day). Intention to treat analysis revealed that VSL#3® resulted in combined induction remission/response rate in 77% of the patients. No adverse effects were reported.

Table 2.9 Efficacy of Probiotic Therapy in the Management of Ulcerative Colitis

Author	Probiotic	Dosage (cfu/day)	Mode of Study	Length of Treatment (weeks)	Evidence of Probiotic Effect
Bibiloni et al. (2005)	VSL#3®	3.6 x 10^{12}	32 patients with active UC	6 weeks	Combined induction of remission/response rate in 77% of patients, probiotic species reached target site.
Kato et al. (2004)	*Bifidobacteria*-fermented milk	1.0 x 10^{10}	20 patients with mild-moderate active UC	12 weeks	Improved clinical activity index scores, endoscopic activity index, histological scores and increases in fecal SCFA.
Tursi et al. (2004)	VSL#3®	9.0 x 10^{12}	90 patients with mild-moderate active UC	8 weeks	VSL#3®/balsalazide combination was superior to balsalazide or mesalazine alone in inducing remission.
Kruis et al. (2004)	*E. coli* Nissle 1917	5-50 x 10^{9}	327 patients with UC in remission	12 months	Maintenance of remission in UC (as effective as mesalazine).
Guslandi et al.(2003)	*S. boulardii*	750 mg/day	24 patients with mild moderate active UC	4 weeks	17/24 patients attained clinical remission, confirmed endoscopically.
Ishikawa et al. (2003)	*B. breve*, *B. bifidum* and *L. acidophilus*	1.0 x 10^{10}	21 subjects with UC in remission	12 months	Significant reduction in the exacerbation rates seen in the probiotic group.
Venturi et al. (1999)	VSL#3®	3.0 x 10^{12}	20 patients with UC in remission, intolerant to 5-ASA	12 months	VSL#3® colonized the intestine and fecal PH reduced, useful in maintaining remission in UC.
Rembacken et al. (1999)	*E. coli* Nissle 1917	Initial: 1.0 x 10^{11} Follow-up: 5 x 10^{10}	120 patients with active UC	Initial: 12 weeks Follow-up: 12 months	Maintenance of remission in UC (as effective as mesalazine).
Kruis et al. (1997)	*E. coli* Nissle 1917	5.0 x 10^{10}	120 patients with UC in remission	12 weeks	Maintenance of remission in UC (as effective as mesalazine)

2.5.6.2 Crohn's Disease

There are limited numbers of randomized control trials using probiotics in Crohn's disease. The studies completed to date have examined the effectiveness of LGG, *E. coli* Nissle 1917 and *S. boulardii* in the management of Crohn's disease.

In 1997, Malchow conducted a double-blind pilot study that tested the effects of *E. coli* Nissle 1917 on efficacy and tolerance in maintaining remission in 28 patients with active colonic Crohn's disease. All patients received 60 mg of Prednisolone® and then were randomized to receive either *E. coli* Nissle 1917 (5×10^{10} viable bacteria/capsule) or placebo for one year. In both groups, the Prednisolone® was tapered according to protocol. All patients receiving prednisolone and *E.coli* Nissle 1917 were able to discontinue steroids six months into the trial, where as only a few patients in the placebo group could stop the Prednisolone® completely. In the group receiving the *E.coli* Nissle 1917, 33.3% had a relapse over the one year of treatment period compared to 63.6% in the placebo group. Malchow (1997) concluded that the intake of Prednisolone® was reduced by the adjuvant application of the *E.coli* Nissle 1917 in patients with colonic Crohn's disease.

In an open trial conducted by Guslandi et al. (2000), 32 patients with Crohn's disease in clinical remission were randomly treated with mesalamine (3g/day) or mesalamine (2 g/day) plus *S. boulardii* (1 g/day) for six months. Clinical relapse was observed in 37.5% patients on mesalamine alone versus 6.25% patients receiving mesalamine plus *S. boulardii* ($p=0.04$). Relapse was assessed using the Crohn's Disease Activity Index (CDAI). All patients completed the study without reporting side effects.

Gupta et al. (2000) conducted a pilot trial in four children with active Crohn's disease to investigate the effect LGG supplementation on clinical outcome. The patients received LGG therapy (10^{10}cfu/ twice daily) in conjunction with their current therapy of Prednisone® and immunomodulatory agents. There was significant clinical improvement in all four subjects, as evidenced by reduced disease activity scores, improved intestinal permeability and a reduction in steroid dose.

Prantera et al. (2002) completed a single center, randomized, double-blind, and placebo-controlled trial in 45 patients. The study examined the effect of LGG in preventing postoperative endoscopic recurrence following intestinal resection in Crohn's

disease. Each patient was randomly allocated to receive 1.2 x 10^{10}cfu/day of LGG or a placebo for one year. After 52 weeks of treatment, 83.3% of patients receiving the LGG versus 89.4% of patients receiving the placebo remained in remission (p=0.894). Of the patients in remission, 60% of the patients in the probiotic treated group had recurrent endoscopic lesions compared with 35.3% in the placebo (p=0.0297). The patients receiving the LGG also had more severe endoscopic recurrence as compared to placebo. The authors concluded that LGG did not reduce the recurrence of lesions nor decrease the severity of recurrent lesions.

Shultz et al. (2004) enrolled 11 patients with moderate to active Crohn's disease in a randomized, double-blind, placebo controlled trial. All patients were started on oral antibiotic treatment for two weeks and started on a tapering steroid regime. Following week one, the patients were randomized to receive either LGG (2 x 10^9 cfu/day) or a placebo for six months. Five out of 11 patients completed the trial with two patients in each group sustaining remission. Four patients in the treatment group and five patients in the placebo group reached remission during the tapering period. The mean time to relapse was 16 ± 4 weeks in LGG group and 12 ± 4.3 weeks in the placebo group (p=0.05). The researchers could not demonstrate a benefit of LGG in inducing remission or maintaining remission in Crohn's disease.

Bousvaros et al. (2005) conducted a randomized, placebo controlled trial with LGG in 75 pediatric patients with Crohn's disease in remission. The probiotic therapy was provided in addition to standard medical therapy for two years. Median time to relapse in the LGG was 9.8 months and 11.0 months in the placebo group (p=0.24). The study was stopped early as the interim analysis showed lack of efficacy. Bousvaros et al. (2005) concluded that LGG does not prolong time to relapse over standard medical therapy.

2.5.6.3 Pouchitis

Pouchitis is a non-specific inflammation of the ileal reservoir following surgical creation of an ileoanal pouch anal anastomosis following resection of the colon (Sandborn, 1994). It is manifested clinically by abdominal pain, diarrhea, urgency, rectal bleeding and sometimes systemic features such as fever (Sandborn, 1994). The overall prevalence of pouchitis is approximately 50% at ten years follow-up (Tamboli,

Caucheteux, Cortot, Colombel & Desreumaux, 2003). The cause of pouchitis has not been clearly established, but the literature suggests that the intestinal flora may be disrupted. Pouchitis has been associated with reduced counts of *Lactobacilli* and *Bifidobacteria* found within the pouch (Gosselink et al., 2004).

A systematic review was completed by Sandborn et al. (1998) to determine the effectiveness of a variety of medical therapies for inducing or maintaining remission in pouchitis. The review included the oral probiotic therapy VSL#3® as a potential treatment for pouchitis. At that time, Sandborn et al. (1998) concluded that probiotic therapies appear to be efficacious, but selection, dose and duration of therapy remain empiric.

Gionchetti et al. (2000) conducted a randomized, double-blind, placebo controlled trial in 40 subjects with chronic active pouchitis, defined as having at least three relapses per year. The subjects were induced into clinical remission after one month of antibiotic treatment with ciprofloxacin and rifaximin and then randomized to receive VSL#3® (1.8×10^{13} cfu/day) or placebo for nine months. Of the 20 patients who received the placebo, 100% relapsed within the nine month period. Of the subjects that received the VSL#3®, 85% remained in remission for the nine months ($p<0.001$). This was the first control trial suggesting that probiotics may play a therapeutic role in the management of pouchitis.

Gionchetti et al. (2003) used VSL#3® (9.0×10^{11} cfu/day) to prevent pouchitis in another double-blind, randomized control trial. Following ileostomy closure, 40 patients were randomized to receive either VSL#3® or placebo for one year. Pouchitis occurred in 40% of the placebo group versus 10% of the VSL#3® group ($p<.05$). Health related quality of life was significantly improved in the probiotic group ($p< 0.001$).

In an open label study, Laake et al. (2003) provided a fermented milk product (Cultura®; 500ml) containing 10^8cfu/ml of *Lactobacilli* and *Bifidobacteria* to ten patients with IPAA. The primary aim of the study was to determine if inflammation of the pouch reservoir is affected by four weeks of treatment with probiotics. Seven out of ten patients had reduced macroscopic inflammation in the pouch mucosa after probiotic treatment, but histologically the mucosa did not change significantly.

Kuisma et al. (2003) completed a double-blind randomized control trial using *L. rhamnosus* GG to determine the efficacy of a single strain probiotic as a primary therapy for pouchitis and its effect on the microflora in the pouch. Twenty patients with history of pouchitis were randomized to receive supplements of *L. rhamnosus* GG (1.0-2.0 x 10^{10} cfu/day) or a placebo twice daily for three months. No differences in clinical, endoscopic or histological scores were found between baseline and at completion of the study following supplementation with *L. rhamnosus* GG ($p=0.97$). As a primary therapy, *L. rhamnosus* GG was not effective for the clinical improvement of pouch inflammation. *L. rhamnosus* GG was effective in increasing the ratio of total fecal lactobacilli to total fecal anaerobes ($p=0.03$), but only 40% of patients were colonized with *L. rhamnosus* GG.

A subsequent study completed by Gosselink et al. (2004) used a fermented dairy product containing *L. rhamnosus* GG (1.4 x 10^{10} cfu/day) to delay the onset of pouchitis. Episodes of pouchitis were observed less frequently in the patients treated with *L. rhamnosus* GG than in the control group ($p=0.011$). The first onset of pouchitis was also delayed up to three years when a daily dose of the *L. rhamnosus* GG was provided. The authors concluded that daily intake of *L. rhamnosus* GG when provided as part of a fermented product is not only effective in delaying episodes of pouchitis but it is less expensive than VSL#3®.

Mimura et al. (2004) published results of a multicenter, randomized, placebo-control trial using the probiotic mixture VSL#3® in 36 patients with recurrent or refractory pouchitis, defined as at least two relapses per year. Patients were induced into remission by providing four weeks of combined metronidazole and ciprofloxacin and then randomized to receive VSL#3® (6.0 x 10^{11} cfu/day) or placebo once daily for one year or until relapse. Remission was maintained at one year in 85% of patients receiving VSL#3® and in 6% patients receiving the placebo ($p<0.0001$). Quality of life scores remained high in the VSL#3® group ($p=0.3$), but deteriorated in the placebo group ($p<0.0005$).

Shen et al. (2005) studied 31 patients with antibiotic-dependent pouchitis were treated with VSL#3® (6.0 x 10^{11} cfu/day) for eight months. All patients received a two week course of ciproflaxicin or metronizazole prior to starting the VSL#3® therapy. At

eight months follow-up, six patients remained on VSL#3® therapy, where as 23 patients discontinued the VSL#3® prior to the eight months because of recurrence of symptoms. The endoscopic scores of the six patients that remained on the VSL#3® were not significantly different from baseline (p=0.27), suggesting that VSL#3® may reduce symptoms of pouchitis but improve endoscopic scores to a lesser degree.

2.5.6.4 Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) is a motility disorder involving the entire gastrointestinal tract, causing recurring upper and lower GI symptoms, including variable degrees of abdominal pain, constipation and/or diarrhea, and abdominal bloating (Beers & Berkow, 2005).

L. plantarum 299v (5×10^7 cfu/ml) provided in a fruit drink called Pro Viva® (Probi AB, Ltd. Sweden) has been studied in IBS with conflicting results (Sen et al., 2002; Niedzielin, Kordecki & Birkenfeld, 2001; Nobaek , Johansson, Molin, Ahrne & Jeppsson, 2000). Sen et al. (2002) administered *L. plantarum* 299v (125 ml/day) or placebo for four weeks in a crossover design. No improvement was noted in symptoms or colonic fermentation following administration of the probiotic. Nobaek et al. (2000) reported a significant reduction in flatulence and abdominal pain in the patients receiving the *L. plantarum* 299v (400 ml/day) compared with the placebo group. Similarly, Neidzielin et al. (2001) found patients treated with *L. plantarum* 299v (400 ml/day) had improvement in IBS symptoms and resolution of abdominal pain compared with placebo. A trend towards normalization of stool frequency was noted in patients with constipation treated with *L. plantarum* 299v.

VSL#3® has been used to improve specific symptoms of IBS in two open label and two randomized control trials. Kim et al. (2003) provided VSL#3® (9.0×10^{11} cfu/day) to patients with diarrhea-predominant IBS for eight weeks. An improvement was noted in abdominal bloating with VSL#3®, with no effect on other symptoms. A subsequent study examining patients with IBS with excess bloating, demonstrated that VSL#3® (9.0×10^{11} cfu/day) significantly reduced flatulence and delayed colonic transit, but had no effect on abdominal bloating (Kim et al., 2005). Two uncontrolled studies using VSL#3® noted clinical improvement related to changes in fecal bacterial flora and fecal biochemistry following probiotic administration in patients with diarrhea-

predominant IBS (Bazzocchi, Gionchetti, Almerigi, Amadini, & Campieri, 2002; Brigidi, Vitali, Swennen, Bazzocchi & Matteuzzi, 2001).

L. casei strain GG (LGG) has been tested in two randomized control trials in both adults and children with IBS (Bausserman & Michail, 2005; O'Sullivan & O'Morain, 2000). In the study involving adults, LGG (10^{10} cfu/day) did not improve symptoms of bloating, pain or urgency in IBS patients with bloating related symptoms (O'Sullivan & O'Morain, 2000). The study involving children with IBS showed similar results to the adult study. LGG (10^{10} cfu/day) did not improve abdominal pain or other gastrointestinal symptoms (Bausserman & Michail, 2005).

More recently, Niv et al. (2005) administered *L. reuteri* ATCC 55730 (2.0×10^8 cfu/day for six months) to patients with all types of IBS (constipation-predominant, diarrhea-predominant and mixed types) in a double-blind randomized control trial. Patients in both the placebo and treatment groups improved with no significant differences between the two groups, demonstrating a strong placebo effect. The authors concluded that *L. reuteri* 55730 did not show improvement in any IBS symptoms. The lack of uniformity in the subjects may have accounted for the lack of effect.

A long term, controlled trial using *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS ($8\text{-}9 \times 10^9$ cfu/day x 6 months) demonstrated that the probiotic mixture improved abdominal pain, distention, flatulence and borborygmi (Kajander, Hatakka, Poussa , Farkkila & Korpela, 2005). A trend toward increased stool frequency was noted in the probiotic group with IBS (constipation-predominant and mixed type).

Preliminary data using a combination of *L. plantarum* LP01 and *B. breve* BR0 (5×10^9 cfu/day) or *L. plantarum* LP01 and *L. acidophilus* LAO 2 (5×10^9 cfu/day) for four weeks in a randomized control trial showed both reduction in abdominal pain scores and severity of the pain in the probiotic treated groups versus placebo (Saggioro, 2004). Overall symptom scores (included constipation, diarrhea, abdominal pain, bloating, flatulence, nausea, dyspepsia and cephalae) also improved in IBS patients receiving the probiotics.

Seventy-five subjects with IBS (all types of IBS included) were randomized to receive a malted milk drink containing the probiotic preparations *L. salivarius*

UCC/1331 or *B. infantis* 35624 (1 x10^{10} cfu) or placebo for eight weeks (O'Mahony et al., 2005). Patients receiving the *B. infantis* 35624 experienced a greater reduction in symptoms (e.g., abdominal pain, bloating/distention and bowel movement difficulty) as compared to placebo and the *L. salivarius* UCC/1331 group. There was a trend towards improved quality of life scores in the patients receiving the probiotics.

2.5.6.5 Lactose Intolerance

Individuals with low levels of the lactase enzyme have limited ability to digest lactose, which can result in intestinal distress or lactose intolerance. Symptoms of lactose intolerance include loose stools, abdominal bloating, pain, flatulence and nausea after eating a lactose containing food (Haubrich, Schaffner & Berk, 1995). Probiotics are believed to adhere to the intestinal lining and digest dietary lactose, alleviating the symptoms of lactose intolerance (Levri, Ketvertis, Deramo, Merenstein & D'Amico, 2005).

A recent systematic review investigating the effectiveness of probiotics in reducing symptoms of lactose intolerance (Levri et al., 2005), included seven randomized placebo controlled studies with the majority of studies using the probiotic strain *L. acidophilus.* Five of seven studies yielded negative results, one positive results and one reported both positive and negative outcomes. The authors concluded that probiotics do not reduce lactose intolerance. However some individuals will have symptoms eliminated for unknown reasons. A small pilot study using the multi-strain probiotic VSL#3® in ten patients with lactose intolerance, failed to demonstrate improvement in clinical symptoms and H_2 excretion (Yesovitch, Cohen & Szilagyi, 2004).

2.5.6.6 Diarrhea

The strongest evidence for the use of probiotics in gastrointestinal illness has been in the treatment of diarrhea. The efficacy of the probiotic against diarrheal illness depends on the type of diarrhea and differs between infectious, antibiotic-associated, *C. difficile*-associated and traveller's diarrhea.

2.5.6.6.1 Acute Infectious Diarrhea in Children

The most well established benefit of using probiotics has been in the management of acute pediatric diarrheal disease. A number of randomized, placebo-

controlled trials have been completed in both the United States and Europe, which have evaluated several strains of probiotics in the treatment of acute diarrhea in children. Two meta-analyses of published controlled clinical trials of probiotic therapy in children with acute-onset diarrhea have been completed to date (Huang, Bousvarous, Lee, Diaz & Davidson, 2002; Van Niel, Feudtner, Garrison;Christakis, 2002). Huang et al. (2002) included 18 randomized control trials of probiotic therapy in healthy children < 5 years of age with acute onset diarrhea. Co-administration of probiotics with oral rehydration therapy was found to reduce the duration of diarrhea by approximately one day in favor of the probiotic-treated subjects (95% confidence interval -1.1 days to -0.6 days, $p<0.001$). Further subanalyses limited to hospitalized children, double-blind trials and studies using *Lactobacilli* resulted in similar pooled estimates (-0.6 to -1.2 days, $p<0.001$). Van Niel et al. (2002) completed meta-analyses of nine randomized, placebo-controlled studies examining the effect of *Lactobacillus* on the duration of diarrhea and diarrhea frequency. All subjects in the nine studies received oral rehydration therapy in addition to the *Lactobacillus* therapy. The *Lactobacillus* therapy reduced the duration of the diarrhea by 0.7 days (95% confidence interval: -0.3 to 1.2 days) compared with controls. *Lactobacillus GG* therapy appeared to have a greater reduction in the duration of diarrhea (a reduction of 1.2 days; 95% confidence interval: -1.6 to -0.08 days, $p<0.001$). A reduction in diarrhea frequency by 1.6 stools on the second day of treatment with *Lactobacillus* was also noted (95% confidence interval: 0.7-2.6 fewer stools).

2.5.6.6.2 *Clostridium Difficile*-Associated Diarrhea

Clostridium difficile is a gram-positive, anaerobic bacillus that colonizes the human large intestine and produces at least two exotoxins: Toxin A (endotoxin) and Toxin B (cytotoxin) (Beers & Berkow, 2005). Colonization by this organism and subsequent infection occur in response to disruption of the stability of the microflora, usually following antibiotic therapy (Plummer, Weaver, Harris, Dee & Hunter, 2004). *Clostridium difficile*-associated diarrhea is one of the most common causes of infectious diarrhea in hospitalized patients (Plummer et al., 2004).

A recent meta-analysis examined the effectiveness of probiotics in the treatment of *Clostridium difficile*-associated diarrhea (McFarland, 2006). McFarland (2006) included six randomized control trials which used a variety of probiotic preparations (*L.*

rhamnosus GG, *L. plantarum* 299v, *S. boulardii* or LABB (*L. acidophilus* and *B. bifidum*), dosages (mean 5 x 10^{10} cfu/day) and duration (median 3 weeks). From the six randomized control trials examined, probiotics showed significant efficacy for reducing recurrences of *Clostridium difficile*-associated diarrhea (RR=0.59, 95% confidence interval: 0.41, 0.85, p<0.005). Two trials reported a 33% reduction in *Clostridium difficile*-associated diarrhea recurrences in the probiotic treated group compared with the placebo group. Five of the six randomized control trials used standard antibiotics (e.g., vancomycin or metronidazole) in combination with probiotic therapy. *S. boulardii* was the only probiotic preparation that showed significant efficacy in reducing the recurrences of *Clostridium difficile*-associated diarrhea. Further studies need to focus on expanding the types and dosages of probiotic, as well as provide sufficient power to detect a significant.

2.5.6.6.3 Antibiotic-Associated Diarrhea (AAD)

Six meta-analyses have evaluated the efficacy of probiotics in prevention of AAD (Cremonini et al., 2002; D'Souza, Rajkumar, Cooke & Bulpitt, 2002; Szajewska & Mrukowicz, 2005; McFarland, 2006; Sazawal, Hiremath, Shingra, Malik, Deb & Black, 2006; Johnston, Supina & Vohra, 2006). The meta-analyses included studies completed in both children and adults.

Cremonini et al. (2002) analyzed seven randomized, placebo-controlled trials, with a minimum of two weeks of follow-up with administration of either *Lactobacillus* ssp. or *S. boulardii*. A total of 881 patients were included in the seven trials. Cremoni et al. (2002) found that supplementation with probiotic therapy resulted in a combined relative risk reduction of 0.3966 (95% confidence interval: 0.27, 0.57).

D'Souza et al. (2002) examined nine randomized, double-blind, placebo-controlled trials that looked at prevention of AAD. The study regimens used probiotics combined with one antibiotic or a variety of antibiotics. The probiotic strains used in the trials were *S. boulardii*, *Lactobacilli* spp., *Bifidobacteria* spp., *E. faecium* or a combination of probiotic strains. The odds ratio in favor of the probiotic treatment in preventing AAD was 0.37 (95% confidence interval: 0.26, 0.53). Six studies showed a significant benefit of probiotic treatment compared with placebo (p<0.05). The authors

concluded that *Lactobacilli* spp.and *S. boulardii* have the greatest potential to prevent AAD.

Szajewska and Mrukowicz (2005) evaluated the effectiveness of *S. boulardii* in preventing AAD in children and adults. Five randomized control trials (1076 participants) were analyzed and found that treatment with *S. boulardii* compared with placebo reduced the risk of AAD from 17.2% to 6.7% (RR: 0.43; 95% CI: 0.23-0.78). The number needed to treat to prevent one case of antibiotic-associated diarrhea was 10 (95% CI: 7-16).

The largest meta-analysis completed to date, examining the efficacy of probiotics in the prevention of AAD involved 25 randomized control trials (2,810 patients) (McFarland, 2006). Of the 16 trials that involved adults, 44% (7) showed significant efficacy for probiotics and 67% (6) of the trials that involved children had significant efficacy. The combined efficacy shows that probiotics have a significant protective effect for AAD. The relative risk reduction for AAD was 0.43 (95% confidence interval: 0.31, 0.58 $p<0.001$). A variety of probiotic strains were assessed in this meta-analysis, but two single probiotic strains (*S. boulardii* and *L. rhamnosus* GG), as well as mixtures of two different strains of probiotics significantly reduced the development of AAD. Daily dosages ranged from 10^7 to 10^{11} and duration of treatment ranged from five days to eight weeks. High daily dosages of probiotic ($\geq 10^{10}$/day) were associated with significant efficacy for reducing the incidence of AAD; where as duration of probiotic administered did not significantly differ between the trials.

Sazawal et al. (2006) pooled 19 trials with data on AAD and found similar results to previously published meta-analyses. Probiotics reduced the incidence of AAD by 52% (95% confidence interval, 35, 65% $p<0.001$).

Johnston et al. (2006) evaluated the potential effectiveness of probiotics in preventing AAD in pediatric patients less than 19 years of age (n=707 patients). Pooled results yielded favorable results for the efficacy of probiotics (RR: 0.43; 95% CI: 0.25-0.75); treatment of six patients should prevent one case of diarrhea. These findings are consistent with D' Souza et al. (2002) and Cremonini et al. (2002).

2.5.6.6.4 Traveller's Diarrhea

Probiotics have been used as prophylaxis in traveller's diarrhea in an attempt to colonize the gut with beneficial bacteria that compete with pathogenic bacteria for nutrients and binding sites in the gastrointestinal tract.

Sazawal et al. (2006) completed a meta-analysis of six randomized control trials which examined the efficacy of probiotics in the prevention of traveller's diarrhea. The probiotics used in these trials included *L. rhamnosus* GG*, S. boulardii, L. fermentum* KLD and *L. acidophilus.* Of the six trials, three had positive point estimates but did not achieve statistical significance. Probiotics reduced the incidence of traveller's diarrhea by 8% (95% confidence interval: -6%, 21%; p=0.235).

2.5.6.7 Necrotizing Enterocolitis (NEC)

The pathogenesis of NEC may be related to the colonization of pathogenic bacteria in the bowel leading to development of an abnormal fecal flora, which in turn causes mucosal injury or bowel necrosis (Bin-Nun et al., 2005). Probiotics may protect against NEC by altering the intestinal microbial flora from one that contains harmful bacteria to one that is beneficial to the host (Lin et al., 2005). *Bifidobacteria* and *Lactobacilli* are the two most common species of probiotic bacteria found in the intestinal lumen of healthy infants, therefore have been the most studied in NEC (Dani, Biadaioli, Bertini, Martelli, & Rubaltelli, 2002, Hoyas, 1999, Lin et al., 2005; Bin Nun et al., 2005).

In an open label study, Hoyos (1999) provided daily doses of 2.5 x 10^8 live *L. acidophilus* and 2.5 x 10^8 live *B. infantis* to 1237 newborns admitted to a neonatal intensive care unit over a one-year period. The newborns received the probiotics during their entire length of stay; 1282 patients hospitalized in the previous year were used as the control group. In the control group there were 85 cases of NEC compared to 34 cases in the probiotic fed group ($p<.0002$). There were 35 NEC associated fatalities in the control group compared to 14 NEC associated fatalities in the probiotic fed group ($p<0.005$). The results showed a significant reduction in the incidence of NEC and NEC associated deaths in the infants treated with probiotics versus the controls.

A multicenter, double-blind, prospective study randomized 585 patients born <33 weeks gestation or at a birth weight of < 1500 g to receive *Lactobacillus* GG (6 x 10^9

cfu/day) or placebo once daily until discharge (Dani et al., 2002). The investigators were evaluating the effectiveness of *Lactobacillus GG* supplementation in reducing the incidence of NEC. The incidence of NEC was 1.4% in the probiotic fed group versus 2.8% in the placebo, these difference were not significant. Dani et al. (2002) concluded that *Lactobacillus* GG did not reduce the incidence of NEC.

A prospective, masked, randomized control trial of 367 very low birth weight (VLBW) infants who survived greater than seven days were randomized to receive either breastmilk supplemented with probiotics (*L. acidophilus* and *B. infantis*; dosage 2.0×10^6 cfu/day) or breastmilk alone from the start of feeds until the time of discharge (Lin et al., 2005). The incidence of death or NEC was significantly lower in the probiotic fed group (5%) versus the control group (12.8%) ($p=0.009$). The incidence of NEC was lower in the probiotic fed group (1.1%) versus the control group (5.3%) ($p<0.05$). There were six cases of severe NEC in the control group, but no cases in the probiotic fed group. Lin et al. (2005) concluded that *L. acidophilus* and *B. infantis* reduces the severity and incidence of NEC in VLBW infants.

In a double-blind study, 145 VLBW infants were randomized to receive either placebo or a probiotic mixture of *B. infantis, S. thermophilus* and *B. Bifidus* (10^9 cfu/day) from the initiation of feeds until discharge (Bin-nun et al., 2005). The incidence of NEC was significantly reduced in the probiotic supplemented group (4%) versus the control group (16.4%) ($p<0.05$). NEC was also less severe in the probiotic supplemented group. All NEC related deaths occurred in the control group, where as there was no NEC related deaths in the probiotic supplemented group. A trend towards improved weight gain was noted in the probiotic supplemented group but the results were not significant.

2.5.6.8 *Helicobacter pylori* Gastritis

Scientific studies have suggested a role for probiotics in the treatment and prevention of *Helicobacter pylori*. *Helicobacter pylori* is a gram-negative bacteria that specifically colonizes the gastric mucosa, inducing a chronic and, in most people, asymptomatic gastritis (Marshall, 1994). It is currently recognized as an etiologic agent of gastroduodenal ulcer and a risk factor for the development of gastric lymphoma or adenocarcinoma (Marshall, 1994). Some strains of *Lactobacillus* and *Bifidobacterium* have been shown to exert bacteriostatic or bactericidal effects against *H. pylori* through

the release of bacteriocins or organic acids in both *in vitro* and *in vivo* models (Gotteland, Brunser & Cruchet, 2006; Cruchet, Obregon, Salazar, Diaz & Gotteland, 2003). Probiotics also have a possible role in stabilization of the gastric barrier function and decreasing mucosal inflammation in the gastric mucosa (Gotteland et al., 2006).

Gotteland et al. (2006) recently conducted a systematic review of the clinical trials using probiotics in adults and children colonized with *H. pylori*. The authors concluded that probiotics as a single therapy do not appear to eradicate *H. pylori* but may maintain lower levels of this pathogen (Gotteland et al., 2006). The probiotic strains *L. johnsonii* (Pantoflickova et al., 2003*), L. gasseri* (Sakamoto et al., 2001), *L. casei* (Tursi, Brandimarte, Giorgetti & Modeo, 2004) and *Clostridium butyricum* (Shimbo et al., 2005) appear to be most promising in reducing the density of colonization of *H. pylori*. When probiotics are administered in combination with antibiotics, probiotics may increase eradication rate and decrease adverse effects associated with antibiotic therapy (Gotteland et al., 2006). The probiotic strains *L. acidophilus* alone (Canducci et al., 2000) and the mixture of *L. acidophilus* and *B. lactis* (Sheu et al., 2002) when provided in combination with antibiotic therapy appear to improve eradication of *H. pylori*.

2.6 SYNBIOTIC

2.6.1 Definition

Synbiotic refers to a mixture of prebiotics and probiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare (Gibson & Roberfroid, 1995). Synbiotic therapy aims to generate synergistic effects by combining prebiotic and probiotic treatments and is predicted to be effective against diseases caused by abnormal gastrointestinal flora (Kanamori et al., 2004). A product containing the prebiotic inulin and the probiotic *Bifidobacteria* fulfill the synbiotic definition (Schrezenmeir & De Vrese, 2001).

2.6.2 Synbiotic Therapy in Gastrointestinal Disease

Synbiotic therapy is a potent new strategy used to treat various intestinal diseases such as IBD, diarrhea, short bowel syndrome, malignant colonic disease and viral and bacterial infections.

2.6.2.1 Ulcerative Colitis

Furrie et al. (2005) developed a synbiotic therapy to treat UC patients with active disease. The probiotic *B. longum* (2×10^{11} cfu/day) and a prebiotic (Synergy 1), made up of inulin-oligofructose (12 g/day), were administered to 18 patients with active UC for a period of one month. The study was a double-blind, randomized control trial. Furrie et al. (2005) demonstrated that the synbiotic therapy resulted in improvements in inflammatory markers, reduced inflammation in the rectal mucosa and regeneration of epithelial tissue in patients with active UC. Furrie et al. (2005) concluded that the short term synbiotic therapy for active UC resulted in improvement of the full clinical appearance of chronic inflammation.

2.6.2.2 Diarrhea

The concurrent administration of *L. rhamnosus*, dietary fibre and micronutrients has shown significant effects on duration of diarrhea (Ahmad, Widjala, Firmansyah, Gliwitzki & Suhardjo, 2000). In a double-blind, randomized control trial, 58 children with acute gastroenteritis were randomized to receive a low lactose formula with or without the *L. rhamnosus* and oligofructose. Ahmad et al. (2000) reported that the duration of diarrhea was significantly reduced in the supplemented group (1.63 versus 2.45 days). A trend toward a reduction in stool output was noted in the supplemented group but the results were not statistically significant.

2.6.2.3 Short Bowel Syndrome

Short bowel syndrome refers to patients that have undergone massive bowel resection. An inadequate absorptive surface often results in severe malnutrition and bacterial overgrowth of the intestine (Beers & Berkow, 2005). Regulation of intestinal bacterial overgrowth in patients with short bowel syndrome is necessary to maximize the absorptive capacity of the intestine (Kanamori, Hashizume, Sugiyama, Morotomi & Yuki, 2001). Typically patients with bacterial overgrowth are treated with antibiotics, but an alternative strategy of applying prebiotics and probiotics has been reported.

Kanamori et al. (2001) administered synbiotic therapy to a four year old girl suffering from bacterial growth as a result of short bowel syndrome. The synbiotic therapy *L. casei, B. breve* and galactooligosaccharides and were administered for two years. In this case report, the patient's intestinal absorptive capacity and motility improved. After two years, the patient's fecal bacterial flora was anaerobic dominant and the pathogenic bacteria (e.g., *E. coli* and *Candida*) were suppressed.

Refractory enterocolitis is a serious problem in pediatric surgical patients suffering from short bowel syndrome (Kanamori et al., 2004). Large amounts of pathogenic bacteria have been found in the bacterial flora of patients with refractory enterocolitis (Kanamori et al., 2004). Seven malnourished patients with chronic refractory enterocolitis, as a result of short bowel syndrome, received synbiotic therapy (e.g., *B. breve, L. casei* and galactooligosaccharides) for over one year (Kanamori et al., 2004). The synbiotic therapy improved the intestinal bacterial flora by inducing the domination of anaerobic bacteria and suppressed the growth of pathogenic bacteria. Short chain fatty acids were also significantly increased in the feces.

2.6.2.4 Colon Cancer

There are currently no published human clinical trials that examine the efficacy of probiotic preparations on colon cancer risk or colon cancer suppression. Evidence is primarily from animal and *in vitro* studies. However, there is an ongoing clinical trial involving eight research centers in Europe called the SYNCAN project. The SYNCAN project will examine the effects of a synbiotic preparation on colon cancer risk biomarkers in humans (Van Loo et al., 2005) This study is a 12 week randomized, double-blind, placebo-controlled trial of a food supplement containing *Lactobacillus GG*, *B.* Bb-12 and Raftilose Synergy 1 in adenoma patients. Colonic mucosal markers, fecal water markers and immunological markers are being measured. To date, results from the human intervention study have not been published.

2.6.2.5 *Helicobacter Pylori* Gastritis

One randomized control trial has examined the effect of a synbiotic on *H. pylori* eradication in children (Gotteland, Poliak, Cruchet, & Brunser, 2005). *L. acidophilus LB* or *S. Boulardii* plus inulin were compared with standard antibiotic treatment in182 children colonized with *H. pylori*. Gotteland et al. (2005) reported that *H. pylori* was

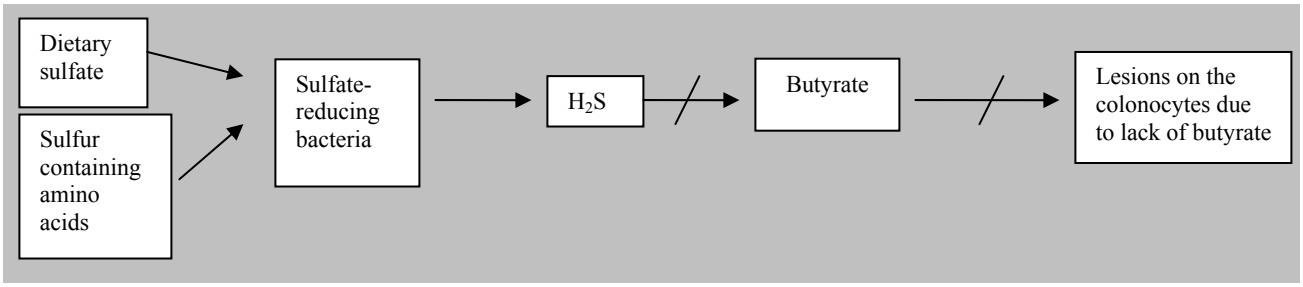
eradicated in 66% receiving antibiotics, 12% receiving the synbiotic and 6.5% of the children receiving the *L. acidophilus LB.*

2.7 HYDROGEN SULFIDE

2.7.1 Introduction

There is growing evidence to suggest that hydrogen sulfide may play a role in initiation and perpetuation of mucosal damage in the colon (Figure 2.2) (Roediger, Moore & Babidge, 1997). Hydrogen sulfide is produced in the colonic contents by the action of sulfate-reducing bacteria (SRB) on dietary sulfate and sulfur amino acids (Jorgensen & Mortensen, 2001). SRB use sulfate as terminal electron acceptor in the electron transport chain for the dissimilation of short chain fatty acids or molecular hydrogen which are produced during colonic fermentation (Gibson, 1990). Hydrogen sulfide is the end product of bacterial sulfate respiration. It has been shown that patients with UC have significantly higher concentrations of sulfate reducing bacteria (Roediger et al., 1997) and luminal concentrations of luminal hydrogen sulfide (Pitcher, Beatty & Cummings, 2000) as compared to healthy controls. Thus, a role for colonic sulfide in the pathogenesis and treatment of UC has emerged (Roediger et al., 1997).

Figure 2.2 Possible Mechanism for Pathogenesis of Hydrogen Sulfide in UC



2.7.2 Impaired Colonic Epithelial Cell Metabolism in UC

Roediger (1980) investigated the metabolism of butyrate in isolated colonic epithelial cells in a group of patients with both active and quiescent UC. Roediger (1980) found that in both acute and quiescent UC, oxidation of butyrate was significantly lower in UC tissues than in the control tissues, and the decrease correlated

with the state of the disease. The diminished oxidation of butyrate was suggested to be due to a metabolic defect in the mucosa which explained the characteristic distribution of colitis in the colon of patients with UC (Roediger, 1980). The most compelling evidence to support this theory is derived from animal models. Rectal instillation of sodium 2-bromo-octanoate in rats, a potent inhibitor of butyrate oxidation, produces histological, clinical and biochemical lesions of acute UC similar to those found in UC in man (Roediger & Nance, 1986). In animal models, fecal concentrations of SCFAs in the colon parallel the severity of the lesions in UC (Stonerook, Tefend, Sharma, Peck, & Wood, 1996). Stonerook et al. (1996) found that in the cotton-top tamarin model of colitis, tamarins with moderate or severe colitis had significantly reduced levels of fecal SCFAs and progressive inflammation in a pattern similar to human colitis (Stonerook et al., 1996). Observations in vivo, in patients with active UC, show significantly lower butyrate oxidation than healthy controls (Den Hond et al., 1998). Den Hond et al. (1998) found that oxidation of butyrate was not decreased in most of the patients with UC in remission. Data (both *in vivo* and *in vitro*) supports the role of impaired colonic epithelial cell metabolism in the pathogenesis of UC.

2.7.3 Hydrogen Sulfide and Colonic Epithelial Cell Metabolism

Although some sulfur containing compounds are required for the production of butyrate, higher concentrations result in toxic effects. There is increasing evidence to suggest that excess hydrogen sulfide impairs the oxidation of butyrate in colonocytes (Roediger, Duncan, Kanpaniris, & Milliard, 1993). Roediger et al. (1993) demonstrated that when human colonocytes are exposed to hydrogen sulfide, fatty acid oxidation was impaired leading to similar metabolic abnormalities observed in active UC. Ohge et al. (2005) demonstrated that patients with ongoing pouchitis released significantly more hydrogen sulfide in the pouch contents than patients with inactive disease. Hydrogen sulfide in the feces of UC patients has been found to be elevated 3-4 fold compared to feces of healthy controls (Levine, Ellis, Furne, Springfield, & Levitt, 1998). Patients with active UC have also been found to have higher amounts of SRB with clinically active disease compared with patients in remission (Ohge et al., 2005; Pitcher et al., 2000). Pitcher et al. (2000) demonstrated that SRB were almost three log values higher

in feces of active UC patients as compared to quiescent UC and total viable counts correlated with clinical severity grade. 5-aminosalicylic acid (5-ASA), a common drug used to treat UC, has been shown to significantly suppress hydrogen sulfide production, by reducing the fermentative production of sulfide from sulfur amino acids (Pitcher et al, 2000). Fecal sulfide levels of UC patients receiving 5-ASA have been found to be significantly lower than those patients not receiving 5-ASA (Pitcher et al., 2000).

The finding that the feces of patients with UC tend to have higher SRB and higher levels of hydrogen sulfide may provide valuable insight into the pathogenesis of UC.

2.7.4 Nutrition Sources of Sulfate

Intestinal sulfate is derived from exogenous sources, namely sulfate in drinking water and sulfur-containing foods (Roediger et al., 1997). Much of the sulfate derived from foods is from preservatives. Diets rich in dried fruits, Brassica vegetables, sausages and beer contain substantial sulfate concentrations (Florin et al., 1993). Sulfate is also derived from the sulfur containing amino acids. Neither an Estimated Average Requirement (and thus a Recommended Dietary Allowance) nor an Adequate Intake for sulfate has been established (Institute of Medicine, 2004).

Sulfate is also commonly found in drinking water. Health concerns regarding sulfate in drinking water have been raised because of reports that diarrhea may be associated with the ingestion of water containing high levels of sulfate (Government of Saskatchewan [GOS], 2003 & United States Environmental Protection Agency, 1994). The laxative effect is often observed in water containing over 500 mg/L of sulfate (GOS, 2003). Sulfate levels in central Canada are particularly high, out of 428 sampling locations across Alberta and Saskatchewan, 13% had sulfate concentrations of greater than 500 mg/L (Health Canada, 1994). In Saskatchewan, median levels of sulfate concentrations of drinking water range from 3 to 2170 mg/L when derived from private wells (Health Canada, 1994). The Saskatchewan Drinking Water Quality Standards and Objectives has established an aesthetic objective of less than 500 mg/L of sulfate in drinking water based on the potential for laxative effects (GOS, 2003).

2.8 MALTODEXTRINS

2.8.1 Introduction

Maltodextrins used commercially, are derived from corn, potato and rice starches. They represent a mixture of saccharides with varying molecular weights between oligosaccharides and polysaccharides. Maltodextrins are available as dried white powders or in concentrated liquid forms. Maltodextrins are water soluble and have very little taste.

2.8.2 Properties of Maltodextrins

Maltodextrins are defined as hydrolysis products of starches with a dextrose equivalent (DE) of less than 20 (Wang & Wang, 2000). DE value is a measure of the total reducing power of all sugars present relative to glucose which has a DE of 100 (Wang & Wang, 2000). A high DE value means that the product has been subjected to a greater degree of hydrolysis. Variations in DE values results in maltodextrins with varying physiochemical properties (Chronakis, 1998). Hygroscopicity, solubility, osmolality and their effectiveness to reduce the freezing point increase with increasing DE. Viscosity and cohesiveness increase as the DE decreases (Chronakis, 1998). Hydrolysis products with a DE greater than 20 are considered syrups.

Maltodextrins contain linear amylase and branched amylopectin degradation products (Chronakis, 1998). α-Amylase employed in starch hydrolysis cleaves the α-1-4 linkages of the starch molecule at random with little to no effect on the α-1-6 linkages (Wang & Wang, 2000). Thus, maltodextrin contains α-D-glucopyranosyl residues joined by α-1-4 linkages to give linear chains with a degree of α-1-4 with α-1-6 linked or α-1-6 linked branch points (Chronakis, 1998). They have the same general formula as other carbohydrates but are of shorter chain length. Maltodextrins are easily digestible, being absorbed as rapidly as glucose.

Resistant maltodextrins (RMD) are produced from hydrolysis and subsequent enzymatic treatment of starch to purposefully convert a portion of the normal α-1-4 glucose linkages to random 1-2, 1-3, and 1-4 alpha or beta linkages (Matsutani America, 2006). The human digestive system effectively digests only α-1-4 linkages; therefore the other linkages render the molecules resistant to digestion.

2.8.3 Food Applications of Maltodextrins

Maltodextrins are used in the food industry as food additives. They have the ability to absorb waters and form gels, therefore are used as texture modifiers and as fat substitutes. (Chronakis, 1998). Some of the main food applications of maltodextrins are in salad dressings, rehydration drinks, dairy products, baked goods and in confectioneries (Chronakis, 1998). One of the most novel applications of maltodextrin is its recent use as a dietary fibre. Fibersol-2® is a maltodextrin, resistant to human digestion (i.e., resistant maltodextrin) which is currently being added to a variety of food products in Europe, Japan and the USA to increase dietary fibre content (Matsutani America, 2006) and for its demonstrated prebiotic effects.

2.9 SUMMARY OF THE LITERATURE

Human studies support the idea that prebiotics and probiotics modify disease states, such as UC by altering bacterial composition, immune status and inflammation. Until recently, prebiotic and probiotics were considered alternative medicine, but there is now increasing scientific evidence to support the use of both prebiotics and probiotics in the management of UC.

Four randomized control trials and one open label trial have demonstrated that when probiotics are provided in addition to standard medications, remission was not only achieved faster, but endoscopic and histological scores were significantly improved. A variety of probiotic preparations and dosages have been studied, with limited adverse effects reported. More research is needed to determine the optimal strain and dosage of probiotics needed for both active UC and UC in remission.

Prebiotics offer an exciting potential treatment for patients with UC. The rationale for stimulating the growth of *Bifidobacteria* and *Lactobacillus* is uncontested. To date there have been no published studies that have used inulin or FOS in maintaining or inducing remission in UC. Administration of prebiotics seems to be a safe, physiological and could be considered as prophylactic therapy for those individuals living with bowel diseases. Although this therapy appears promising, more clinical trials

need to be completed to firmly establish the efficacy of prebiotics in the management of UC.

Synbiotic therapy is the most recent novel idea used to induce favorable intestinal environments in the host (Gibson & Roberfroid, 1995). Scientific research in the area of synbiotic therapy and UC is limited to one open label trial, but it appears to be a promising supportive therapy. More long-term, large scale clinical trials are needed using synbiotics.

CHAPTER 3
RESEARCH METHODS

3.1 Study Design

The study was initially approved as a single center, randomized, double-blind, placebo controlled study examining the effect of *B. longum R0175* and inulin on maintaining remission in pediatric UC. The study was to be completed over a period of 12 months. Ten months into the study, a novel report by Furrie et al. (2005) was published. Furrie et al. (2005) indicated that treatment with synbiotic therapy using both *B. longum* and inulin resulted in clinical improvement of chronic inflammation in adults with active UC. This finding, along with the observation that some subjects in our study were clinically well and some subjects were doing quite poorly (as indicated by low quality of life scores and the presence of severe symptoms), the latter potentially receiving the placebo, prompted us to ask the Biomedical Research Ethics Board at the University of Saskatchewan for guidance. The Biomedical Research Ethics Board indicated that the study must be unblinded. The remainder of the study was conducted as an open label trial. The single center, randomized, double-blind, placebo controlled study is called Phase I – Pediatric Pilot Study. Phase II – Pediatric Case Series is the open label portion of the study. Phase III - Adult Case Series was completed as an open label study.

3.2 Recruitment

Pediatric subjects were recruited through a pediatrician in the Saskatoon Health Region that specializes in gastroenterology. The pediatrician provided the names of potential subjects to the research coordinator. Chart reviews were completed to determine the subjects that were eligible for the study. The parent or caregiver of each eligible subject was contacted by telephone where a brief description of the study was provided (Appendix 1.1). If the parent or caregiver and eligible subject showed interest in participating in the study, a meeting was organized to discuss the study in further detail.

In order to increase the sample size, pediatricians from three pediatric hospitals in Western Canada (Winnipeg Children's Hospital, Stollery Children's Hospital and Regina General Hospital) were asked to participate in the study. A letter describing the study was sent to each of the pediatricians (Appendix 1.2). A follow-up phone call or e-mail was completed within two weeks of sending the initial letter to ensure the pediatricians received the letter and to discuss potential participation in the study. The pediatric gastroenterologists from Winnipeg Children's Hospital and Stollery Children's Hospital reported being unable to participate; in one case, a group was currently running a probiotic trial in UC subjects and could not run another trial concurrently. A pediatrician at Regina General Hospital was contacted and expressed interest in participating in the trial. A meeting was organized to discuss further details of the study. The pediatrician had two pediatric patients that were eligible to participate in the study. One of the two patients was already involved in the study. The pediatrician indicated that he would discuss the study with the remaining patient and provide the family with study coordinator's contact information. The family did not contact the research coordinator.

Due to the lack of success in recruiting pediatric subjects, the decision was made to expand the recruitment demographic to include adult subjects between the ages of 19 to 40 years from the Saskatoon Health Region. Three adult gastroenterologists and one surgeon (involved with patients with UC) from the Saskatoon Health Region were contacted by letter to participate in the study (Appendix 1.3). All four physicians expressed interest in participating in the study. Meetings were held with all four physicians. Two of the four physicians decided to participate in the study. The physicians forwarded the names of the eligible subjects to the research coordinator.

3.3 Phase I – Pediatric Pilot Study

3.3.1 Participants

Eligible subjects were between the ages of 8 and 18 years of age and had been diagnosed with UC in remission, defined as the absence of visible blood in the stools and less than two bowel movements per day. The initial diagnosis of UC had to have been confirmed by endoscopic and histological examination. Corticosteroid therapy was

allowed up to two weeks prior to study initiation. Subjects were excluded if they had a non-intact colon (colectomy) or were considered to be immunocompromised by the attending physician.

Approval for the study was granted by the Biomedical Research Ethics Board of the University of Saskatchewan, Saskatoon, Saskatchewan (Appendix 2.1 and 2.2). Written and informed consent was obtained from both the caregiver and the child before participation (Appendix 3.1.1 and 3.1.2).

3.3.2 Study Medication

Institut Rosell (Montreal, QC, Canada) provided the placebo capsule (maltodextrin and ascorbic acid) and the probiotic (*B. longum* R0175 in maltodextrin and ascorbic acid, 2.0 x 10^{10} cfu/capsule). The placebo capsule and probiotic capsule were packaged in a laminate foil envelope (45 capsules per envelope). Although the probiotic was a freeze-dried formulation, it was advised by the Institut Rosell to keep the probiotic refrigerated. All stock was stored at four degrees and all subjects were instructed to store the laminate foil envelope at refrigeration temperature.

Sensus America LLC (Monmouth Junction, NJ, USA) supplied the inulin (Fruitafit® CLR). Fruitafit® CLR is a mixture of both short and long chain oligosaccharides. Fifteen grams of inulin was weighed out on an electronic scale and placed in a sachet. Corn Products International, Inc. (Casco Incorporated, Etobicoke, Ontario, Canada) provided the maltodextrin (Casco, Globe Plus, 18 DE - Maltodextrin). Fifteen grams of maltodextrin was weighed out on an electronic scale and placed in a sachet. The weighing of the maltodextrin and the inulin was completed by a summer student. Taste and smell of the active treatment and placebo were not readily identifiable.

Every three months, the study medications were delivered via courier to the homes of the study participants in a cooled container for both the placebo and treatment groups.

3.3.3 Randomization of Subjects

Phase I of the trial was designed as a randomized double-blind, placebo control trial. Eligible subjects were randomly allocated to the control group (15 g/day of non-resistant maltodextrin, and a control capsule of maltodextrin and ascorbic acid) or the treatment group [15 g/day of inulin (Fruitafit® CLR, Sensus America LLC., Monmouth Junction, NJ, USA) and *B. longum* R0175 (Institut Rosell, Montreal, QC, Canada) at a dosage of 2.0×10^{10} cfu/capsule] for ten months or until relapse. Treatment allocation was performed by a staff member of the Department of Applied Microbiology and Food Science, University of Saskatchewan. The group assignment was not revealed to the research coordinator, physician, subjects or families.

3.3.4 Study Protocol

At study entry, subjects and families were counseled regarding the study protocol. Subjects were instructed to consume one capsule containing the probiotic supplement (*B. longum* R0175 and 15 g of powdered inulin) or the placebo capsule and 15 g of maltodextrin daily. The powder was to be mixed with hot or cold beverages. The capsules were to be swallowed.

Standard pharmacologic therapy for UC continued to be provided as per the attending physician. No dietary alterations were recommended except that all subjects were encouraged to avoid high sulfate water sources and bottled water use was encouraged. All subjects were instructed to avoid additional probiotic supplements during the study period.

Subjects were provided with a study diary and asked to keep a daily record of clinical symptoms. Clinical symptoms included stool consistency (loose or formed), stool frequency (number in 24 hours), blood or mucous (present or not), abdominal pain (present or not) and overall feeling (well or not) for the duration of the study (Appendix 4). The study diary also recorded whether the subjects consumed the pill, powder and used bottled water.

Health-related quality of life was assessed using the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) (Irvine et al., 1996) at study entry and every two months for ten months or until relapse (Appendix 5). The SIBDQ is a validated

questionnaire for use in IBD that considers bowel, systemic and emotional symptoms, as well as social function. The score ranges from 10 (worst quality of life) to 70 (best quality of life). The SIBDQ was administered either in person or over the telephone. Prior to administering the SIBDQ to the child, verbal consent was requested from the parents (Appendix 3.2).

Chart reviews were undertaken at study initiation, at six months and at the end of the study. The following data was collected: current age, sex, age at diagnosis, age at randomization, location of disease, medical history, current medications, current status, date of last remission, length of last remission, birth place, parent's birth place, current dwelling (urban/rural) and drinking water source (Appendix 6).

An exacerbation of UC was defined as a drop in SIBDQ score of ≥11.8 points (Jowett et al. 2001), with the presence of blood or mucous in the stool or admission to hospital for management of UC.

Compliance to the study protocol was assessed by examining the study diary, as well as questioning the subjects.

3.4 Phase II – Pediatric Case Series

Following completion of Phase I of the study, all subjects involved in the pilot study were provided the synbiotic therapy (*B. longum* R0175 and 15 g of powdered inulin). All subjects followed the study protocol as described in Section 3.3.4 for Phase I – Pediatric Pilot Study.

Ethical approval to extend the study beyond one year was completed and approved by the Biomedical Research Ethics Board of the University of Saskatchewan (Appendix 2.3).

3.5 Phase III – Adult Case Series

Eligible subjects were between the ages of 19 and 40 years of age. Eligibility criteria were a firmly established diagnosis of UC obtained by endoscopic and histological examination. Subjects could be in remission or have active UC. Exclusion criteria were extensive bowel surgery, non-intact colon (colectomy), existing or intended pregnancy, existing cardiac, renal or hepatic disease and diabetes. Immunocompromised

subjects were excluded from the study. Corticosteroids were allowed up to two weeks prior to the study. Concurrent pharmacologic treatment continued as per the attending physician.

Ethical approval to include the extended age group and subjects with active UC was completed and approved by the Biomedical Research Ethics Board of the University of Saskatchewan (Appendix 2.4).

3.5.1 Study Design

Phase III was designed as an open label case series, where all subjects received the treatment (15 g/day of inulin and *B. longum* R0175 - 2.0 x 10^{10} cfu/capsule) for up to 12 months or until relapse. All subjects followed the study protocol as described in Section 3.3.4 for Phase I – Pediatric Pilot Study. In addition, the SIBDQ was administered on two occasions (over a period of one month) prior to starting the synbiotic therapy in order to obtain baseline quality of life data.

3.6 Safety Assessment

Subjects recorded all adverse symptoms (e.g., flatulence, bloating, etc.) related to the study medication in the study diary. Severe adverse events (e.g., sepsis, etc.) related to the study medication were to be reported to the research coordinator as soon as possible.

3.7 Data Analysis

3.7.1 Sample Size Calculation

In studies of IBD, clinical investigators suggest a 25% response in the placebo group and 75% response in the treatment group, and such a difference is relevant from a clinical point of view (Gionchetti et al., 2000). For $\alpha = 0.05$ (two tailed test) and $\beta = 0.20$, a sample size of more than 19 patients per group is required in order to achieve a normal distribution.

3.7.2 Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS© Version 14.0 for Windows, Chicago Illinois). Since the results of the

study do not follow a normal distribution, non-parametric tests were used to analyze the data. A *P*-value of <0.05 (two sided) was considered to be statistically significant.

3.7.3 Demographic Data

Demographic and clinical characteristics for both the placebo and synbiotic groups (Phase I subjects) were calculated as median values with a range following randomization. Sex of the subjects (male versus female) was tabulated. Comparisons between the placebo and synbiotic group were made using the Mann-Whitney U test and Chi-squared test (for nominal data).

The demographic and clinical characteristics are profiled for each subject that participated in Phase II of the study.

3.7.4 Quality of Life Scores

The SIBDQ consists of ten questions with responses to each question graded on a seven point Likert scale. Each question scores between a range of one (represents the "worst" aspect) to seven (representing the "best" aspect). The response category "All of the time" was assigned a score of one, "Most of the time" a score of two, "A good bit of the time" as score of three, "Some of the time" a score of four, "A little of the time" a score of five, "Hardly any of time" a score of six and "None of the time" as score of seven. The sum score was totaled for all the individual questions contained in the SIBDQ (higher scores indicate better quality of life). The SIBDQ has its own standardized scoring system which is based on the IBDQ-36.

The ten questions on the SIBDQ are clustered into four dimensions of quality of life: systemic domain (two questions), emotional domain (three questions), bowel domain (three questions) and social domain (two questions). The dimensional score was the sum of the scores of the items included in each domain.

Comparison of the total SIBDQ scores at baseline with month ten or at relapse were made between the placebo group and synbiotic group using the Mann-Whitney U test. The Wilcoxon signed-rank test was used to determine differences between SIBDQ scores within the placebo and synbiotic groups. Dimensional SIBDQ scores are presented as median values. Differences were assessed using the Mann-Whitney U test,

when more than two groups were compared the Kruskal-Wallis test was used; if statistically significant, the variable that differed from the others was identified using the Bonferroni test.

Comparisons of the total SIBDQ scores pre-treatment and post-treatment for Phase II were completed using the Wilcoxon signed-rank test. Comparisons of the SIBDQ scores within subjects were completed using the Friedman Test.

3.7.5 Symptoms

Stool consistency (loose or formed), stool frequency (number in 24 hours), blood or mucus (present or not), abdominal pain (present or not) and overall feeling (well or not) were labeled, coded and entered into a spreadsheet. The mean values with the standard deviation were derived for both the synbiotic and placebo groups. Comparisons between the placebo and synbiotic group were made using the Mann-Whitney U test.

Phase II symptom data were presented as the mean value. Symptom scores pre-treatment and post-treatment were compared using the Wilcoxon signed-rank test.

3.7.6 Phase III – Adult Case Series Analyses

Phase III subjects are described in this thesis as case reports.

CHAPTER 4
RESULTS

4.1 PEDIATRIC PILOT STUDY

4.1.1 Baseline Characteristics of the Subjects

Of the 14 subjects invited to participate in the study, consent was obtained from 10 subjects. The reasons for refusal to participate were as follows: three subjects were not interested in participating in the trial and one subject did not show up for the study meeting on two separate occasions. In addition to the 14 subjects initially contacted to participate in the study, an eight year old child with moderately severe UC unable to be weaned off Prednisone® consented to participate in the study. Due to the severity of her disease and steroid-dependency, she was included as an open-label case study.

Two subjects enrolled in the study, but discontinued within two weeks of orientation to the study. One subject withdrew following the orientation to the study due to worsening of symptoms and a reason for the second subject's withdrawal from the study was not provided. Both subjects withdrew from the study prior to starting the study medications. Appendix 7 depicts a visual schematic of the recruitment process for the study.

The baseline characteristics of the subjects are shown in Table 4.1. There were no significant differences observed between the two groups at study entry.

Table 4.1 Baseline Characteristics of the Pediatric Study Subjects (Phase I – Pediatric Pilot Study)

	Synbiotic (n=4)[A]	Placebo (n=5)	*P**
Median age (years)	12.6 (8.9-17)	12.6 (11.4-18.4)	0.539
Gender			
Male	1	3	
Female	3	2	0.520
Age at diagnosis (years)	10.9 (7.9-12.4)	11.7 (10.7-15.4)	0.140
Duration of disease (months)	25 (4-65)	20 (9-36)	0.712
Time since last relapse (months)	6 (1-64)	8 (5-36)	0.537
Disease extent			
Total/subtotal	3	2	
Left-sided	1	1	
Proctosigmoid	0	2	
Medications†			
Prednisone®	1	0	
Pentasa®	1	1	
Salofalk®	0	1	
Sulfasalazine®	3	2	
None	0	1	

[A]Open label case included
†UC related drugs only

P values were determined using the Mann-Whitney U test or Chi-square test. $P < 0.05$ were considered to be significant.
Values are presented as median with the range.

4.1.2 Clinical Course

As shown in Appendix 8.1, three out of four subjects (cases 6, 7 and 9) in the synbiotic group were still in remission at ten months. The subjects in the synbiotic group did not experience severe symptoms. Severe symptoms were defined as the presence of blood or mucous in the stool or presence of abdominal pain. Case 9 (open-label case study) was a nine year old female with moderate to severe UC who was corticosteroid-dependant (40 mg/day). Prior attempts at weaning corticosteroids resulted in return of severe symptoms. Following 43 days of synbiotic therapy, Case 9 was able to wean off corticosteroids and remain in remission. Case 8 dropped out of the study after two months without a reason being provided.

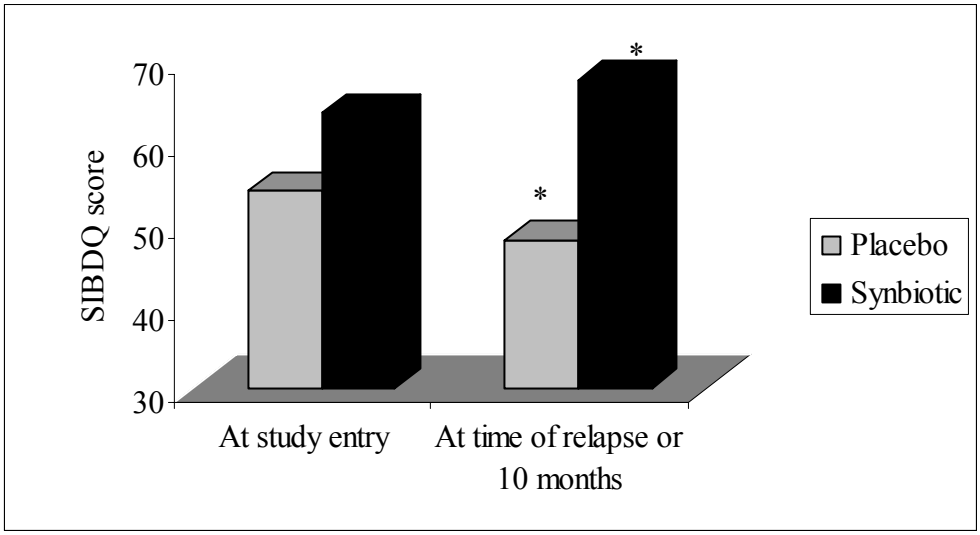
Of the five subjects receiving the placebo, three subjects experienced a severe relapse of UC (Cases 1, 2 and 5). Case 5 was admitted to hospital within 55 days of

starting the study with severe diarrhea, rectal bleeding and abdominal pain. Case 2 did not report severe symptoms in the study diary, but relapsed and was admitted to hospital within 134 days of study initiation. By the end of ten months, Case 1 experienced blood or mucous in the stool on a daily basis and quality of life scores dropped 23 points. Four out of five subjects in the placebo group reported the presence of severe symptoms in their study diaries (Cases 1, 3, 4 and 5).

4.1.3 Quality of Life

As presented in Figure 4.2, in the four subjects receiving the synbiotic therapy, SIBDQ scores were high at study entry (median 63.5 points) and at ten months increased even further (median 67.5 points); this difference was not statistically significant (p = 0.109). In the five subjects receiving the placebo, the SIBDQ scores dropped from a median of 54 points to a median of 48 points at the time of relapse or ten months (p = 0.345). Comparing the two groups, there was no significant differences at study entry (synbiotic *v* placebo: median 63.5 points *v* 54 points; p = 0.140) but the groups differed significantly at the time of relapse or ten months (median 67.5 *v* 48; p = 0.014).

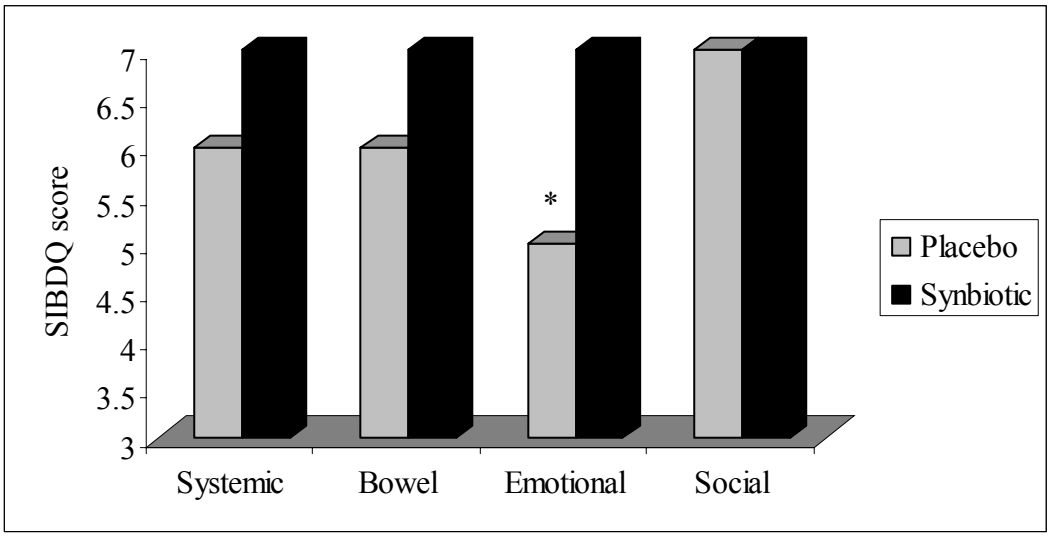
Figure 4.1 SIBDQ Scores at Study Entry and at Ten Months or Time of Relapse for Synbiotic Therapy (n = 4) and Placebo Groups (n = 5) (*p = 0.014)



***P* values were determined using the Mann-Whitney U test or Wilcoxon-signed rank test. *P* <0.05 were considered to be significant.

The results of the dimensional scores of the SIBDQ at ten months or time of relapse for the synbiotic and placebo groups are shown in Figure 4.3. Distribution of the dimensions for the synbiotic and placebo groups differed significantly from each other (p = 0.011). There was a greater preservation of the social dimension as compared to the bowel, systemic and emotional dimensions (p = 0.016). In the placebo group, the lowest dimension of the SIBDQ was the emotional impact (p=0.018). The latter finding suggests that the emotional dimension is the most affected in UC.

Figure 4.2 Median Values of SIBDQ Scores by Dimension at Ten Months or Time of Relapse for Synbiotic and Placebo Groups



*****P values were determined using the Kruskal-Wallis test. P <0.05 were considered to be significant.

4.1.4 Symptoms

Stool frequency, days with formed stool and days without abdominal pain were not significantly different between the two groups (Table 4.2). There was a significant difference in days without blood or mucous in the stool (p = 0.032) and days feeling well (p = 0.014) in the subjects receiving the synbiotic therapy versus the placebo.

Table 4.2 Comparison of Bowel Function and Symptom Scores of Synbiotic Group and Placebo Group at the End of Phase I - Pediatric Pilot Study

	Between groups (post-treatment)		
	Synbiotic Therapy (n=4)[A]	**Placebo (n=5)**	***P****
Stool frequency (#/day)	1.5 ± 0.4	2.2 ± 2.4	0.624
Days with formed stool (%)	61.4 ± 46.5	61.3 ± 38.0	0.806
Days without abdominal pain (%)	97.3 ± 4.9	89.1 ± 14.9	0.213
Days without blood or mucous in the stool (%)	100 ± 0	71.6 ± 41.9	0.032*
Days feeling well (%)	99.3 ± 0.9	63.4 ± 34.4	0.014*

[A]Open label case included in synbiotic group.

***P values were determined using the Mann-Whitney U test. P <0.05 were considered to be significant. Values are presented as mean ± standard deviation.

4.1.5 Compliance

The mean compliance rate in the synbiotic group was 93.1% for the probiotic capsules and 92.2% for the inulin. The mean compliance rate in the placebo group was 90.4% for the placebo capsule and 84.3% for the maltodextrin powder. There were no significant differences detected between the two groups in consumption of powder (p = 0.389) or the capsules (p = 0.431). Some subjects disliked the taste of the beverages when mixed with the inulin or maltodextrin.

Low sulfate water was consumed 98.4% of the time in the synbiotic group and 79.9% of the time in the placebo group. The large difference in consumption of low sulfate water is because one subject in the placebo group did not use low sulfate water on a regular basis. The two groups were not significantly different from each other (p = 0.110).

4.1.6 Safety

No side effects were reported in the subjects receiving neither the treatment nor the placebo. No adverse effects occurred as a result of the study medications.

4.2 PEDIATRIC CASE SERIES

4.2.1 Subject Characteristics

Following completion of Phase 1- Pediatric Pilot Study, subjects in both the placebo and synbiotic groups were asked to continue with the study. Three subjects in the placebo group (Cases 1, 3 and 4), one subject (Case 2) that had relapsed (initially in placebo group), two subjects (Cases 6 and 7) in the synbiotic group and the open label case (Case 9) agreed to continue participation in the study. One subject (Case 10) with active UC was newly recruited. Appendix 7 provides a schematic of recruitment.

The demographic and clinical characteristics of the eight subjects are shown in Table 4.3. Six patients are females and two are male. The age of the subjects ranges from 9 years, 9 months to 19 years, 3 months. All subjects were initially diagnosed with UC through endoscopic and histological evaluation. Six subjects (Cases 2, 4, 6, 7, 9 and 10) had total/subtotal UC, one subject (Case 1) had proctosigmoid UC and one subject (Case 3) had left-sided UC. Six subjects (Cases 2, 3, 4, 6, 7 and 9) were in remission at the start of Phase II and two subjects (Cases 1 and 10) had active UC. Cases 6, 7 and 9 had been receiving the synbiotic therapy for ten months.

Table 4.3 Demographic and Clinical Characteristics of Pediatric Study Subjects (Phase II – Pediatric Case Series)

Case No.	Age	Gender	Location of Disease	Medications	Remission	Months on Synbiotic Therapy
Case 1	17 yrs, 5 mo	F	Proctosigmoid	Pentasa®	N	0
Case 2	13 yrs, 5 mo	M	Total/Subtotal	Prednisone®, Salofalk®	Y	0
Case 3	19 yrs, 3 mo	M	Left-sided	Sulfasalazine®	Y	0
Case 4	13 yrs, 2 mo	F	Total/Subtotal	Sulfasalazine®	Y	0
Case 6	13 yrs, 8 mo	F	Total/Subtotal	None	Y	10
Case 7	17 yrs, 6 mo	F	Total/Subtotal	Sulfasalazine®	Y	10
Case 9[A]	9 yrs, 9 mo	F	Total/Subtotal	Pentasa®	Y	10
Case 10	12 yrs, 8 mo	F	Total/Subtotal	Salofalk®, Salofalk® enemas	N	0

[A]Open label case.

4.2.2 Clinical Course

The clinical course of each subject is presented in Appendix 8.2. Case 1 received the synbiotic therapy for a total of 60 days and continued to experience blood and mucous in the stool. Case 1 decided to discontinue the synbiotic therapy as it did not improve symptoms. Case 2 received the synbiotic therapy for 66 days and subsequently went on to have another exacerbation within six weeks of weaning off Prednisone®. The synbiotic therapy was discontinued. Case 2 was prescribed Immuran® (100 mg/day) with Prednisone® (60 mg/day) to control the UC. He entered remission with this medication regime and was able to wean off Prednisone®. Case 2 continues to receive Salofalk® and Immuran® to control the UC.

Case 3 received the synbiotic therapy for 60 days and then decided to discontinue the study as the original 12 month commitment was completed. Case 3 did not experience severe symptoms while receiving the synbiotic therapy. Within 45 days of discontinuation of the study, Case 3 contacted the study coordinator and requested to reenter the study, as he was experiencing intermittent abdominal pain and blood in the

stool. Case 3 received the treatment for an additional four months. Following the four months, the research coordinator was unable to contact Case 3 and the subject was subsequently lost to follow-up.

Case 4, 6, 7 and 9 reported no severe symptoms of UC while receiving synbiotic therapy. Case 6 and 7 participated in the study for approximately 12 months and then decided to withdraw from the study. Case 6 and 7 fulfilled the original 12 month commitment of the study. The reason the subjects withdrew from the study was not requested, as the ethics agreement indicated the subjects could withdraw from the study at any point without a reason being provided.

Case 4 was diagnosed with UC at 10 years, 8 months. Case 4 had never had a formed stool since diagnosis and was poorly controlled with standard medical therapy. She had trialed Pentasa®, Sulfalsalazine® and Salofalk® but continued to experience intermittent symptoms despite the medical therapy. Case 4 entered the study in September 2004. One week prior to study initiation, Case 4 had been weaned off Prednisone®. Case 4 received the placebo for ten months and the synbiotic therapy for 14 months. Case 4 has remained in remission for 24 months. Case 9 (open label case) received the synbiotic therapy for 26 months and continues to remain symptom-free, steroid free and in remission.

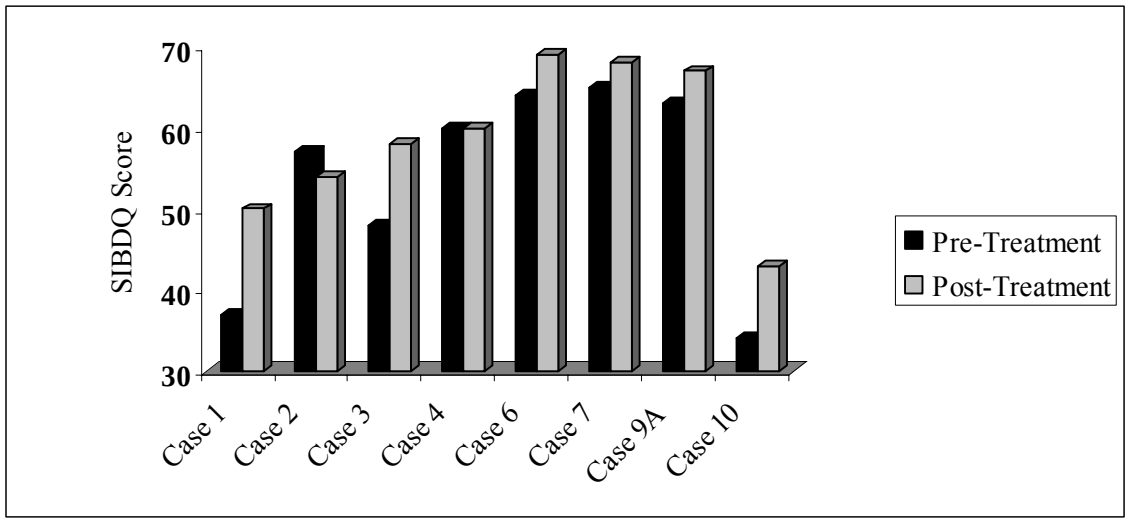
Case 10 entered the study with active UC at the end of March 2006. She was experiencing tenesmus, joint pain, four stools during the day plus one at night, all stools contained blood and mucous. Medications included Salofalk® (2500 mg/day) and Salofalk® enemas (2 g/day). Case 10 returned for a follow-up appointment in April 2006 and reported 3-4 stools/day, no stools at night and less blood present in the stool and less urgency. Case 10 was prescribed 60 mg Prednisone® in attempt to induce remission in May 2006. During the month of May 2006, Case 10 was admitted to hospital with epigastric pain, diagnosed with esophagitis and treated with Pantaloc®. In June 2006, Case 10 was instructed by the pediatrician to begin to wean off Prednisone® and start Salofalk®. Case 10 successfully weaned off the Prednisone® and was diagnosed as being remission. Case 10 continues to experience blood in her stools despite being in remission. Tenesmus and joint pain are no longer present.

4.2.3 Quality of Life

The baseline SIBDQ scores ranged from 37 to 65 points (median 58.5 points). Following treatment with synbiotic therapy, SIBDQ scores ranged from 43 to 69 points (median 59 points). Figure 4.4 shows the changes in total SIBDQ scores for each subject treated with synbiotic therapy. Significant differences were found in the SIBDQ scores pre-treatment and post-treatment with synbiotic therapy (p= 0.034).

Six of the eight subjects SIBDQ scores improved post-treatment with synbiotic therapy. The SIBDQ score dropped in one subject (Case 2) while receiving synbiotic therapy, as the subject reported decreased quality of life related to side effects from Prednisone®. The SIBDQ score for Case 4 remained the same (60 points).

Figure 4.3 Phase II SIBDQ Scores Pre-Treatment and Post-Treatment With Synbiotic Therapy (n=8) (p = 0.034)



[A]Open label case included in synbiotic group.

4.2.4 Symptoms

Stool frequency, days with formed stool, days without abdominal pain, days without blood or mucous and days feeling well were analyzed for Cases 1, 2 and 4 pre and post-treatment with synbiotic therapy (Table 4.4). Pre-treatment was defined as the period of time the subjects were on the placebo. The results were not statistically significant (p = 0.564). Case 3 was not included in the analysis as the study diary data post-treatment was lost by the subject.

Cases 6, 7 and 9 remained well and experienced no severe symptoms of UC throughout the study period. Loose stools were reported by Case 6 and 7 in their study diaries.

Case 10 experienced more symptoms of UC (e.g., increased stools, blood, mucous, abdominal pain and increased loose stools) than the other subjects. The synbiotic therapy did not improve the symptoms Case 10 was experiencing, thus she remained unwell. Case 10 did not enter remission on synbiotic therapy.

Although the results are not statistically significant, they are clinically significant especially for Case 4 and 9. Case 4 experienced less stools, less blood and mucous, more formed stools, and felt better while receiving the synbiotic therapy (14 months) as compared to when she was receiving the placebo. Case 4 has been in remission for a total of 24 months; previous remission length was six months. Case 9 showed the most dramatic improvements in bowel function and symptoms, as Case 9 had remained symptom free for 26 months. The longest period Case 9 was symptom free before the synbiotic therapy was 4.5 months.

Table 4.4 Comparison of Bowel Function and Symptom Scores For Each Pediatric Subject (Phase II – Pediatric Case Series)

	Case Number							
	1	**2**	**3**	**4**	**6**	**7**	**9[A]**	**10**
Stool frequency (#/day)								
Pre-treatment	0.9	1.0	1.0	2.5	---	---	---	---
Post-treatment	0.8	1.1	†	1.8	1.4	1.0	1.5	3.1
Days with formed stool (%)								
Pre-treatment	60.3	99.0	85.9	61.8	---	---	---	---
Post-treatment	64.7	93.8	†	88.0	49.1	2.8	99.9	45.8
Days without abdominal pain (%)								
Pre-treatment								
Post-treatment	64.0	96.0	87.1	98.6	---	---	---	---
	59.6	98.5	†	98.0	91.5	100	99.1	80.7
Days without blood or mucous in the stool (%)								
Pre-treatment	68.4	100	93.7	95.8	---	---	---	---
Post-treatment	22.8	100	†	98.7	100	100	100	40.4
Days feeling well (%)								
Pre-treatment	12.8	97.0	70.6	89.9	---	---	---	---
Post-treatment	24.6	98.5	†	97.4	97.6	98.3	99.2	85.7

Values are presented as mean.
[A]Open label case included in synbiotic group.
[†]Study diary not available.
*****P values were determined using the Wilcoxon signed rank test. P <0.05 were considered to be significant.

4.2.5 Compliance

All subjects showed good compliance to the medication. The mean compliance rate was 92% for the probiotic capsules and 77.8% for the inulin. Some subjects disliked the taste of the beverages when mixed with the inulin.

4.2.6 Safety

Two subjects reported increased flatulence which was attributed to the inulin. In no cases was the gas severe enough to stop the study medication. There were no reports of severe adverse events by any of the subjects.

4.3 ADULT CASE SERIES

4.3.1 Subjects

Ten adult subjects were referred by three gastroenterologists from the Saskatoon Health Region. Three subjects with active UC were deemed eligible to participate in the study following extensive chart reviews. The subjects deemed ineligible to participate in the study had bowel resections or were on immunosuppressive therapy (e.g., Immuran® or Remicade®).

4.3.2 Adult UC - Case Reports

4.3.2.1 Case A

Case A was a 31 year old male with moderate to severe UC. A colonic biopsy in March 2005 revealed active UC throughout the colon (total/subtotal UC). He was placed on Ascacol® (3.2 g/day) and Prednisone® (30 mg/day) which improved the symptoms (diarrhea, blood in stools, abdominal cramping, tenesmus). On attempts at weaning the Prednisone® the symptoms returned. The gastroenterologist subsequently prescribed Immuran® in October 2005, as Case A had become steroid dependant. Case A trialed the Immuran® but developed more diarrhea with its use, so he subsequently discontinued the medication. In November 2005, the gastroenterologist suggested that Case A try Remicade® to induce remission, but Case A wanted to trial the synbiotic therapy. The gastroenterologist referred Case A for entry into the study.

Synbiotic therapy was provided in addition to the Prednisone® (40 mg/day) in attempt to induce clinical remission. *B. longum* R0175 and inulin Frutafit®CLR was used as the synbiotic therapy. A dosage of 2.0 x 10^{10} cfu/day of probiotic bacteria and 15 g/day of the prebiotic were administered. The short inflammatory bowel disease questionnaire (SIBDQ) was performed at baseline and every two months. Case A kept a daily record of symptoms (stool consistency, stool frequency, blood and mucous and abdominal pain). Case A was asked to report any adverse effects, deterioration of well-being, increased stool frequency, rectal bleeding or mucous in the stool to the research coordinator.

The initial SIBDQ score for Case A was 41. A follow-up phone call was placed 12 days following initiation of synbiotic therapy. Case A reported that his stools had increased to between 11 and 24 daily with blood and mucous present. He was experiencing severe abdominal pain and felt very unwell. As a result, the research coordinator recommended discontinuation of the study medication immediately and suggested that the subject seek medical advice as soon as possible. The research coordinator also notified the referring physician of the situation. Subsequently, Case A went on to receive immunomodulatory therapy (e.g., Remicade®).

4.3.2.2 Case B

Case B was a 28 year old female diagnosed with moderate to severe UC. A colonic biopsy in September 2005 revealed moderately active UC in the right, transverse and sigmoid colon. Initial symptoms included weight loss, tenesmus, 5-30 loose stools daily with blood and mucous present. She was treated with Prednisone® (40 mg/day) and the symptoms resolved until she began tapering the dosage. She was subsequently admitted to hospital in January 2006 with 4-10 bloody stools daily, severe abdominal pain, tenesmus, hypoalbuminemia and iron deficiency anemia. In hospital she was treated with IV Solumedrol® which induced remission and subsequently was started on 40 mg/day of Prednisone®.

Cased B was referred to the research coordinator and a SIBDQ was completed. The score was only 16. The research coordinator, the physician and Case B decided it would be prudent to wait to start the synbiotic therapy as Case B was on a high dosage

of Prednisone® (40mg/day). In March 2006, Case B contacted the research coordinator, as she wanted to enroll in the study. Case B had recently discontinued Prednisone® and was symptom free. A SIBDQ was completed and the score was 33, still indicating poor quality of life. The subject was very depressed, but was regularly seeing a counselor.

B. longum R0175 and inulin Frutafit®CLR was used as the synbiotic therapy. A dosage of 2.0 x 10^{10} cfu/day of probiotic bacteria and 15 g/day of the prebiotic were administered. Standard medications as prescribed by the physician continued (e.g., Asacol® 3.6g/day). The SIBDQ was performed on two occasions prior to starting the synbiotic therapy in order to obtain baseline data and every two months thereafter. Case B kept a daily record of symptoms (stool consistency, stool frequency, blood and mucous and abdominal pain). Case B was asked to report any adverse effects, deterioration of well-being, increased stool frequency, rectal bleeding or mucous in the stool to the research coordinator.

After one month on the synbiotic therapy, Case B's symptoms returned and she experienced another UC exacerbation. Stool frequency ranged from 5-15 times per day, with bleeding, urgency, abdominal cramping and severe flatulence. The flatulence was precipitated by the inulin. The physician restarted Prednisone® (30 mg/day) and started Case B on Immuran®. Due to the risks of an adverse event occurring with immunosuppressive therapy, the research coordinator advised Case B to discontinue the study.

4.3.2.3 Case C

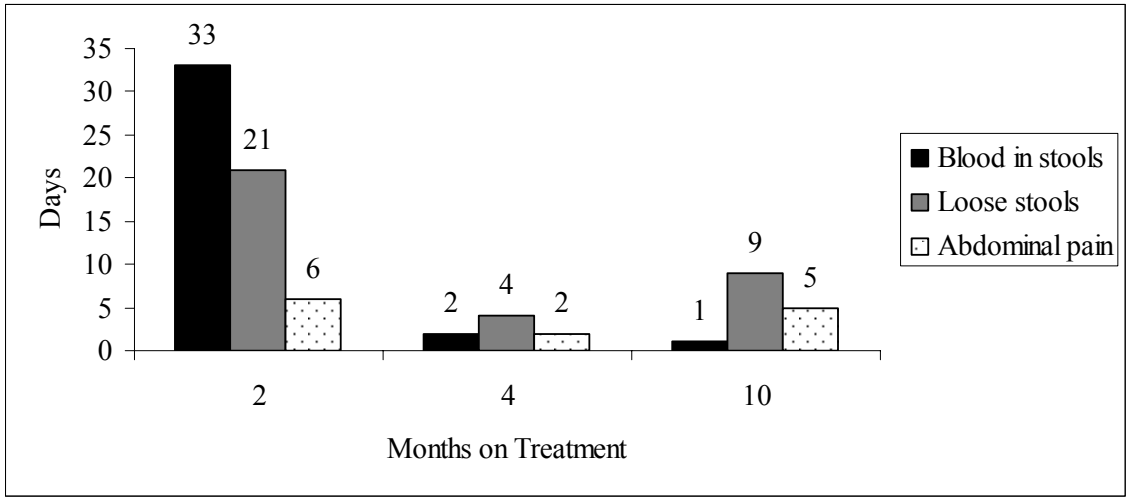
Case C was a 26 year old male diagnosed with distal colitis by histologic and endoscopic evaluation in November 2000. The UC was controlled with Pentasa® (2g/day) for approximately four and a half years. During remission, Case C experienced intermittent symptoms (i.e., stools that contained blood and mucous and increased frequency of stools). In June 2005, Case C presented with large amounts of blood in the stool despite being on the Pentasa®. Colonoscopy revealed active UC in the distal colon, he was prescribed hydrocortisone enemas for six weeks and the symptoms disappeared. In October 2005, the symptoms returned and the gastroenterologist referred Case C for entry into the study.

Synbiotic therapy was prescribed in addition to Pentasa® (1000 mg po bid) in October 2005. *B. longum* R0175 and inulin Frutafit®CLR was used as the synbiotic therapy. A dosage of 2.0 x 10^{10} cfu/day of probiotic bacteria and 15 g/day of the prebiotic were administered. The SIBDQ was performed on two occasions prior to starting the synbiotic therapy in order to obtain baseline data and every two months thereafter. Case C kept a daily record of symptoms (stool consistency, stool frequency, blood and mucous and abdominal pain). Case C was asked to report any adverse effects, deterioration of well-being, increased stool frequency, rectal bleeding or mucous in the stool to the research coordinator.

Case C returned for a follow-up appointment with the gastroenterologist in April 2006. Medications included the synbiotic therapy and Pentasa® (2g/day). Case C complained of blood in the stools for approximately two weeks prior to the appointment. The blood in the stool reoccurred following a bout of influenza. The gastroenterologist prescribed Cortifoam® enemas twice daily for one week and the symptoms disappeared. In May 2006, Case C reported the presence of a small amount of blood upon evacuation of his bowels; he was diagnosed with an anal fissure.

Figure 4.4 represents the available study diary data for Case C. The blood in the stools decreased after 30 days of treatment with synbiotic therapy. The study diary indicated that a small amount of blood was present in the stool on three occasions, but lasted only 1-2 days since initiation of synbiotic therapy. Stools average 1.3 per day. Abdominal pain was absent 94% of the time. The *B. longum* and inulin were consumed 98% of the time.

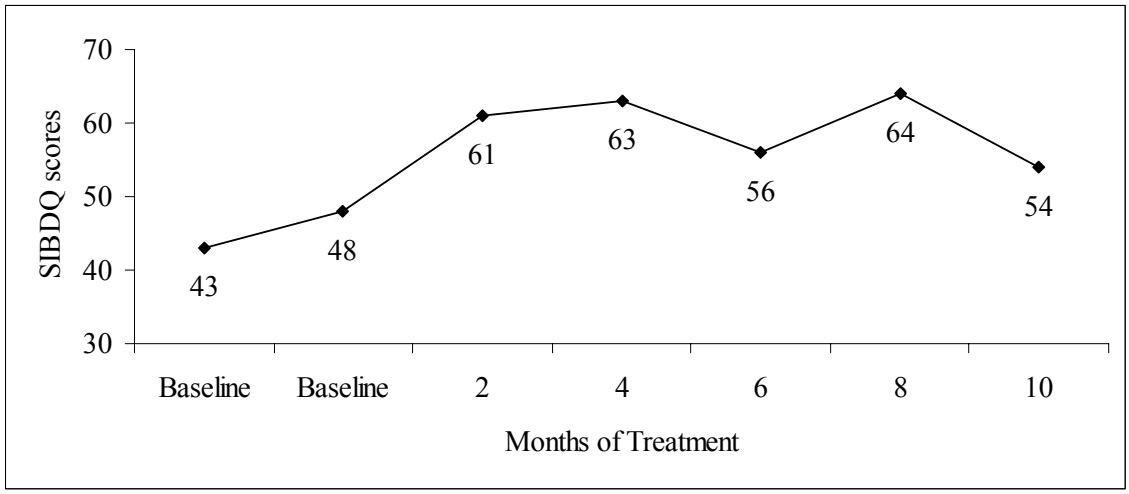
Figure 4.4 Study diary data for Case C



* No data available for month 6 and 8 as Case C lost diary.

Figure 4.5 shows the SIBDQ scores of Case C over the course of the study period. The baseline SIBDQ scores for Case C were 43 and 48 (completed two weeks after first SIBDQ). The SIBDQ scores range from 54 to 64 over the ten months on synbiotic therapy (70 indicates best health). No adverse effects were reported.

Figure 4.5 SIBDQ Scores for Case C While Treated With Synbiotic Therapy



CHAPTER 5
DISCUSSION

5.1 Discussion

The findings of the current study were fourfold. First, the study demonstrated that synbiotic therapy is a safe, adjunct treatment for UC. Second, synbiotic therapy with concomitant medical therapy resulted in significant improvements in HRQOL, a reduction in severe symptoms (blood and mucous) and reduced relapse rate in pediatric subjects with UC in remission. Third, synbiotic therapy provided at the current dosages was incapable of inducing remission in the pediatric and adult subjects that entered the study with active UC. Fourth, compliance to taking the synbiotic therapy was not an issue for the subjects.

The results of this intervention are unique because this was the first time synbiotic therapy has been investigated for use in pediatric UC. To date, there have been no published studies using prebiotic, probiotic or synbiotic therapy for the management of pediatric UC. However, a number of studies in adults using probiotics have been carried out. One study using synbiotic therapy (*B. longum* and FOS/inulin mix) was carried out by Furrie et al. in 2005, in adults with active UC. These authors reported reduced mucosal inflammatory markers (TNF-α and IL-1) and a reduction in colitis at both the macroscopic and microscopic level. Previous probiotic studies conducted in adults with UC have reported that *E. coli Nissle* 1917 is as effective as mesalamine for maintaining a relapse (Kruis et al., 1997; Kruis et al., 2004). *Bifidobacteria* fermented milk prevented exacerbation of UC symptoms in 73% of subjects versus 10% of subjects in the controls (Ishikawa et al., 2003). VSL#3® resulted in a remission rate of 75% in subjects that were intolerant or allergic to 5-ASA (Venturi et al., 1999). These studies suggest that probiotic preparations have a role in treating quiescent UC and preventing a relapse of UC.

There are even fewer studies looking at the effects of inulin on UC. Inulin prevented mucosal inflammation, as demonstrated by lower colonic lesion scores and the release of fewer inflammatory mediators in test rats with distal UC (Videla et al.,

2001). Supplementation with 24 g of inulin in patients with ileo-anal pouch anastomosis resulted in increased butyrate concentrations, lowered pH, decreased numbers of *Bacteriodes fragilis*, and diminished concentrations of secondary bile acids in feces (Welters et al., 2002). Thus, the role of inulin in the management of UC still remains very novel.

The efficacy of the synbiotic therapy may be related to the increased colonization of the protective bacteria, *B. longum* in the colon. Although not measured in the current study, a number of successful clinical trials have demonstrated that *Bifidobacteria* show a high survival rate through the gastrointestinal tract and exhibit probiotic properties in the colon (Picard et al., 2005). In addition, *B. longum* R0175 is resistant to gastric acidity, is not degraded by bile salts and colonizes the colon (Institut Rosell's Probiotic Strains, 2002). If the theory that subjects with UC suffer from abnormalities in intestinal bacterial content and host-bacterial interaction (Penner et al., 2005) is true, then the administration of *B. longum,* would have helped to normalize the intestinal flora of the UC subjects. Supplementation with the inulin likely contributed to this effect, as inulin is not only metabolized by the *Bifidobacteria*, but it supports the growth of the subjects' own resident *Bifidobacteria*. Thus, increased colonization of probiotic bacteria in the colon may be responsible for the improvement in symptoms and HRQOL in the pediatric UC subjects.

The efficacy for the synbiotic therapy may also be related to increased concentrations of short chain fatty acids (SCFA) in the colon as a result of prebiotic fermentation. Although not measured in the study, several well-designed studies have confirmed that inulin and FOS are highly fermented in the colon, thus produce SCFA (Cherbut, 2002). Inulin supplementation is reported to increase fecal butyrate concentrations and lower fecal pH in ulcerative colitis subjects with ileal pouch-anal anastomosis (Welters et al., 2002). Welters et al. (2002) demonstrated a reduction in inflammation of the mucosa of the ileal reservoirs of UC subjects which was confirmed endoscopically and histologically. It is also largely accepted that butyrate plays an essential role in maintaining colonic mucosa integrity by acting on epithelial cell metabolism, proliferation and differentiation (Cherbut, 2002). Therefore, enhancement

of butyrate production via supplementation with inulin may be related to the significant improvements noted in the subjects described in this study.

Endoscopy with histological analysis is the gold standard for the diagnosis of clinical activity and assessment of the treatment response in UC (Bremner, Griffiths & Beattie, 2004; Chudleigh & Hunter, 2003). The challenge with this procedure is that it is invasive and not always readily available in the outpatient setting. In the Saskatoon Health Region, endoscopy is completed during exacerbations of UC, and rarely if a child is in remission as endoscopy in children involves sedation and admission to hospital. A weakness of the current study is that endoscopic and histological analysis was not completed due to the nature of the procedure.

With the absence of the use of endoscopy and histological analysis, and the lack clearly defined definition of relapse provided in the scientific literature, relapse was defined in the current study as a drop in SIBDQ score by ≥11.8 points (Jowett et al. 2001) or the presence of blood or mucous in the stool or admission to hospital for management of UC. This definition was problematic, as not all subjects that had a relapse of the UC were admitted to hospital or experienced a drop in the SIBDQ scores. There were also subjects that had blood present in the stool but were not having a relapse. A clearer definition of relapse is required.

Disease activity indices have been developed to evaluate therapeutic interventions for UC (Higgins, Schwartz, Mapili & Krokos, et al., 2005), which all do not require endoscopic and histological analysis. The Simple Clinical Colitis Activity Index (SCCAI) is one example (Table 2.5) (Walmsley et al., 1998). The SCCAI has been validated against the Powell-Tuck and is designed specifically for use in the outpatient setting. The advantage to using a disease activity index, such as the SCCAI, in the current study is that it would provide a more objective definition of relapse with high specificity and sensitivity (Jowett et al., 2003).

While a limitation of the present study was the absence of a clinical disease activity tool, the strength was the inclusion of an instrument that measured quality of life. Health related quality of life (HRQOL) is an integral part of the management of UC in children (Bremner et al., 2004), as HRQOL is significantly altered when the disease is active and when it is in clinical remission and laboratory and endoscopic findings are

essentially normal (Martin et al., 1995). Traditional medical management and follow-up of subjects with UC fails to recognize the subjective experiences of the subjects (Martin, Leone, Fries & Naccarato, 1995). Chronic and unpredictable gastrointestinal symptoms and required treatments impose psychological and social stresses on young patients (Griffiths et al., 1999).

The SIBDQ was specifically designed to measure HRQOL in adult patients with IBD (Irvine et al., 1994). Although the SIBDQ was not originally designed for the pediatric population, the questionnaire appeared to be sensitive enough to detect changes in disease activity. When subjects were experiencing symptoms such as rectal bleeding, abdominal pain and increased stool frequency there was a subsequent deterioration in SIBDQ scores. The SIBDQ scores were high when the subjects were in remission. As patients began to relapse the SIBDQ began to decrease. An additional advantage of the SIBDQ was that it was quick and inexpensive to administer.

An interesting discovery was how UC impacted the different dimensions of HRQOL. Our results suggest that the social dimension was least affected by UC as compared to the bowel, systemic and emotional dimensions. Other studies have found similar results (Casellas et al., 2000). This observation suggests that patients are able to participate in social activities, despite their disease state. An unexpected finding was that the emotional dimension scored most poorly on the SIBDQ. This finding suggests that UC my cause major psychological distress.

The questions on the SIBDQ were easily answered by the pediatric subjects. The readability of the questionnaire was at a grade eight reading level. The majority of the subjects in this study were older than grade eight so this was not an issue. Subjects younger than grade eight did not appear have any problems interpreting the questions either. The question “Overall, in the last two weeks, how much of a problem have you had maintaining or getting to the weight you would like to be?” was problematic. The question was designed as a systemic measure of HRQOL, where if a patient has active disease, they would most likely experience weight loss. In this study, a female teenager scored poorly on this question, but when asked if she had lost weight in the past two weeks, she indicated she had not but she just wanted to weigh less. As teenagers often have body image issues, the way the question is currently worded may not be

appropriate for this population. The question could be reworded to "Overall, in the last two weeks, how much of a problem have you had stopping your weight from dropping?"

The IMPACT is a HRQOL tool, designed for use in the pediatric IBD population (Otley et al., 2002). The current study was initiated in 2003, prior to publication of the validated questionnaire in 2005(Shepanski et al., 2005). The advantage to using the IMPACT is that it incorporates body image issues into the questionnaire, which was a limitation of the SIBDQ. The disadvantage to using the IMPACT is that has 36 questions which is much lengthier than the SIBDQ which has ten questions. It would be interesting to assess the reliability and validity of the SIBDQ as disease specific measure of HRQOL in pediatric IBD against the IMPACT questionnaire which is specifically designed to measure HRQOL in pediatric IBD.

A variety of probiotic strains have successfully induced remission in adults with active UC (Rembacken et al., 1999, Guslandi et al., 2003, Furrie et al., 2005, Bibiloni et al., 2005 & Tursi et al., 2004). In the current study, the adult subjects with active UC did not enter remission. Although Case C did not enter remission and have complete resolution of symptoms, improved quality of life and reduced symptoms were noted. In studies where remission was attained successfully, the dosage of the probiotic ranged from 10^{11} - 10^{12} cfu/day, where as the current study provided 10^{10} cfu/day. There is a possibility that if the *B. longum* RO175 were provided at a higher dosage (>10^{11} cfu/day) remission could be attained in these subjects. Furrie et al. (2005) used the *B. longum* (2.0 x 10^{11} cfu/day) and 12 g/day of an inulin/FOS mixture and demonstrated improved sigmoidscopy scores and reduced inflammation at both the microscopic and macroscopic level in the colonic tissue using this higher dosage.

At the present time, no scientific studies have been completed in children that use probiotics, prebiotics or synbiotics to induce remission in UC. The current study was the first of its kind. The subjects that entered the study with active UC did not enter remission while receiving the synbiotic therapy, which was comparable to the adult subjects. Despite not attaining full remission, all the pediatric subjects experienced improved quality of life and an improvement in symptoms. Again, there is a possibility that if a higher dosage of the *B. longum* was provided to the pediatric subjects, remission could be induced.

Experimental studies completed in healthy individuals have shown significant increases in *Bifidobacteria* through supplementation of the diet with 5 to 40 g/day of inulin and FOS (Kruse et al., 1999, Bouhnik et al., 1999, Gibson et al., 1995, Klessen et al., 1997, Tuohy et al., 2001 and Buddington et al., 1996). The values that are recommended as formulation doses for inulin, based on both tests with volunteers and the experience of the food industry are 10 g/day (Roberfroid et al., 1993). The daily dosage of inulin (15g/day) provided in the current study was higher than recommended. The premise for using 15 g/day was based on the belief that compliance may be a problem with children especially with a study of such long duration. It was thought that even if the subjects were to consume the inulin every second day or half of a package daily, the subjects would still receive enough prebiotic to promote the growth of the *Bifidobacteria*. Compliance to taking the inulin proved not to be an issue with the subjects.

Inulin is also known for its fecal bulking effect and its ability to stimulate bowel movements in healthy subjects (Gibson et al., 1995, Kleesen et al., 1997 and Dahl et al., 2005). In the current study, stool frequency and stool consistency remained unchanged in the subjects. Interestingly, Lewis et al. (2005) found similar results when 12 g/day of oligosaccharides were provided to elderly individuals that were at risk for AAD. Inulin and FOS are mild laxatives but the effect is often small and difficult to detect unless diet is controlled. In the current study diet was not controlled, therefore the fecal bulking effect and laxation effect may have gone undetected.

Roberfroid et al. (1993) suggested that individuals can consume up to 10 g/day of a non-digestible fermentable carbohydrate without noticeable side effects. There was concern that providing over 10 g/day of inulin would cause tolerance issues (e.g., increased flatulence or diarrhea) in the subjects. This study was able to demonstrate that 15 g/day of inulin was safe to administer to children between the ages of 8 -18 years, as there were no reports of serious adverse reactions in this population. Two subjects reported some flatulence, but after a few days the flatulence subsided, no subjects reported diarrhea as a result of the inulin.

Subjects were recruited through a pediatrician that serves as the gastroenterology specialist for Saskatchewan. Attempts at recruiting out of province were unsuccessful as

the pediatric gastroenterologists approached declined participation. Due to the lack of success in recruiting pediatric subjects, we decided to recruit additional subjects as adults. The physician that referred the majority of potential subjects tends to manage the most difficult cases of UC in the Health Region. These subjects were not responsive to traditional pharmacological therapies used for UC and went on to require immunomodulatory agents such as Immuran® or Remicade® in order to induce remission. It is not clear in the scientific literature at this time, if subjects with severe UC will respond to synbiotic therapy.

Overall, it was also difficult to convince the physicians to partake in the study despite the potential benefits of prebiotic and probiotic therapy to UC patients. It could be that synbiotic therapy is an alternative therapy, and therefore is not part of the mainstream medical management of patients with UC. Physicians must practice evidence based medicine and unfortunately at this time the area of prebiotics and probiotics is still quite novel.

Although the pediatric subjects were using concomitant medications (e.g., Prednisone®, Pentasa®, Salofalk®, Sulfasalazine®) it is unlikely that these medications played a significant role in the positive treatment response. The subjects had been on these medications for long periods of time prior to starting the study. Intermittent symptoms were experienced by the subjects receiving the placebo despite the use of standard medications for UC. Case 9 had steroid dependant UC and with the synbiotic therapy was able to wean off the Prednisone® successfully. The pediatrician managing this case had been considering either immunomodulatory therapy such as Immuran® or colectomy. It is not known whether synbiotic therapy will work without concomitant medical therapy for UC.

An important advantage of the synbiotic therapy when provided in combination with standard medical therapy is its lack of side effects. No severe adverse effects were observed over the course of the study. Two subjects in the current study have received the synbiotic therapy in addition to conventional treatment for over two years with the absence of any side effects. Furrie et al. (2005) also reported no adverse reactions using the *B. longum* and a FOS/inulin mix in adult UC subjects with active disease. The safety

of *Bifidobacteria* is supported by the long historical use in fermented milks and its GRAS status.

There are two major limitations to the current study. First, conducting the study open-label for Phase II and III of the study is a major limitation. Without a control group, it is difficult to rule out whether a "treatment effect" did occur or rule out a placebo response. The second limitation is the small sample size, thus caution must be exercised when interpreting the results. Due to the small sample the results cannot be generalized to the UC population. Despite this limitation, the study was able to fulfill the objectives for the study, therefore providing valuable insight for future research direction.

5.2 Conclusion

In conclusion, this long term study has provided the first evidence that synbiotic therapy may improve HRQOL and symptoms in pediatric subjects with UC in remission. In addition, this study demonstrated that synbiotic therapy is also a safe adjunctive therapy for the long-term management of UC. Synbiotics have the potential to be developed into therapies to help patients suffering from UC.

5.3 Future Research

The preliminary results of the current study encourage the development of a large randomized control trial with more pediatric subjects to further confirm our results. The following changes should be made to enhance a future study:

- Inclusion of the Simple Colitis Clinical Activity Index to provide a clearer and more specific definition of relapse and disease activity.
- Reduce the inulin to 10 g/day since compliance in the current study was better than expected, therefore the higher dosage of 15 g/day is not required.
- To monitor HRQOL, use the IMPACT questionnaire as it is validated for use in pediatric IBD.

Future research will also need to determine if synbiotic therapy will be efficacious without concomitant medical therapy for UC. The potential mechanism of action of

Bifidobacteria longum R0175 and inulin in maintaining remission in UC will also need to be investigated.

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APPENDIX 1

Recruitment

1.1 Telephone Script for Recruitment Purposes

Hello, may I speak to the parent or guardian of ***(insert child's name here).***

My name is Natasha Haskey; I am a Pediatric Dietitian that works at Royal University Hospital with Doctor ***(insert doctor's name here)****. Doctor* ***(insert doctor's name here)****, researchers and myself are doing a research study on how changing the diet may effect the symptoms of Ulcerative Colitis. Your child is being invited to participate in this research study. Your child's participation is entirely voluntary, so it is up to you and your child to decide whether or not to take part in this study. If you wish to participate, we can set up a time to meet at* ***(insert meeting place)*** *to discuss the details of the study. If you do not wish to participate, you do not have to provide any reason for your decision nor will it affect the medical care of your child now or in the future.*

Would you be interested in setting up a time to meet at ***(insert meeting place)*** *to discuss the research study in further detail?*

1.2 Letter of Recruitment to the Pediatricians

Food and Nutrition Services
Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

Date

Dear ***(insert doctor's name here),***

I am coordinating a clinical trial with Dr. Garth Bruce MD, Wendy Dahl RD PhD and Susan Whiting PhD investigating the effectiveness of low sulfate water, prebiotics and probiotics on the length of remission and quality of life in pediatric ulcerative colitis patients.

Our research team is currently recruiting pediatric ulcerative colitis patients in clinical remission, between the ages of 8-18 years (both male and female), to participate in our clinical trial. Patients with non-intact colons and those considered to be immuno-compromised are excluded from participation.

I am requesting your participation in this clinical trial by providing a confidential list of eligible client names, along with their date of birth and health services number, that meet the criteria as outlined in the previous paragraph. As the study coordinator, I will contact the potential study volunteers to discuss their participation in the study.

The results of this study will be published and if you agree to participate, your name will be acknowledged.

A summary of the clinical trial is attached to this letter.

The University of Saskatchewan Biomedical Research Ethics Board has provided ethical approval for this study.

I look forward to speaking with you in the near future. If you have any questions, please call me at (306) 655-6512.

Sincerely,

Natasha Haskey RD, Masters student
Pediatric Dietitian

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8
(306) 655-6512
natasha.haskey@saskatoonhealthregion.ca

Trial Summary

An investigation into the effectiveness of low sulfate water, prebiotics and probiotics in increasing length of remission and quality of life in pediatric ulcerative colitis patients.

Summary: A clinical research study for pediatric ulcerative colitis patients that are in clinical remission.

Purpose: This is a research study to determine if low sulfate water, prebiotics and probiotics will increase the length of remission and improve quality of life in pediatric ulcerative colitis patients.

Description: This is a 12 month study for pediatric clients between the ages of 8-18 years of age (male and female), diagnosed with ulcerative colitis, who are in clinical remission. Clients that are immuno-suppressed or have non-intact colons cannot participate in the study. Each study volunteer will be randomly assigned into a control or treatment group. A capsule and a powder will be provided to both the treatment and control groups to be administered on a daily basis. During the 12 months, study volunteers will be contacted by telephone interview and monitored bimonthly for symptomology by the study coordinator. In addition, study volunteers will be asked to keep a diary of their symptoms. The study coordinator will travel to complete chart reviews at the initial visit, 6 month and 1 year follow-up visits. All study volunteers, family members, researchers and study coordinator will be blinded to the treatment and placebo.

Supporting Evidence for the Clinical Trial: Colonic microflora is altered in active ulcerative colitis (Fabia et al, 1993), and reduced numbers of *Bifidobacteria* have been found (Edman, 2000). In one uncontrolled study, probiotics maintained clinical remission in 75% of patients with mesalamine-intolerant ulcerative colitis (Venturi et al, 1999). Probiotics have recently been used to successfully prevent the recurrence of chronic relapsing pouchitis in patients who have undergone ileoanal pouch anal anastomosis due to ulcerative colitis (Gionchetti, 2000). Remission of pouchitis has been induced by the administration of certain *Lactobacillus* species (Kuisma et al, 2003). While these studies support the efficacy of probiotics for pouchitis, much more evidence is required support the efficacy of probiotic therapy for ulcerative colitis. It is not clear whether multiple bacterial strains are required to induce or maintain remission or if combination therapy will provide synergistic effect. It is not know if the provision of *Bifidobacteria* will result in improved colonic health and extend the clinical remission of ulcerative colitis.

Pediatric ulcerative colitis results in poor intake, growth delay and morbidity, therefore, aggressive; combination therapy may be indicated to circumvent such drastic treatment options as colonic resection. Current nutritional management of ulcerative colitis has limited success in maintaining remission (Saskatoon Health Region, unpublished). Supplementation of inulin and *Bifidobacteria*, and sulfate reduction pose no known serious risks. We hypothesize that the reduction in sulfate and supplementation with the prebiotic, inulin and probiotic, *Bifidobacteria* will result in a significant increase in remission length in pediatric ulcerative colitis patients.

Contact Information:

Natasha Haskey RD, Masters student
Pediatric Dietitian
Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8
(306) 655-6512
natasha.haskey@saskatoonhealthregion.ca

References:

Edman JS. Williams WH. Atkins RC. Nutritional therapies for ulcerative colitis: literature review, chart review study, and future research. *Alternative Therapies in Health & Medicine. 6(1):55-63, 2000 Jan.*

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Venturi A. Gionchetti P. Rizzello F. Johansson R. Zucconi E. Brigidi P. Matteuzzi D. Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Alimentary Pharmacology & Therapeutics. 13(8):1103-8, 1999 Aug.*

1.3 Letter of Recruitment to Adult Gastroenterologists

Food and Nutrition Services
Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

Date

Dear ***(insert doctor's name here),***

I am coordinating a clinical trial with Dr. Garth Bruce MD, Wendy Dahl RD PhD and Susan Whiting PhD investigating the effectiveness of low sulfate water, prebiotics and probiotics on the length of remission and quality of life in ulcerative colitis patients.

Our research team is currently recruiting ulcerative colitis patients in clinical remission, under the age of 40 years to participate in our clinical trial. Patients with non-intact colons and those considered to be immuno-compromised are excluded from participation.

I am requesting your participation in this clinical trial by providing a confidential list of eligible client names, along with their date of birth and health services number, that meet the criteria as outlined in the previous paragraph. As the study coordinator, I will contact the potential study volunteers to discuss their participation in the study.

The results of this study will be published and if you agree to participate, your name will be acknowledged. A summary of the clinical trial is attached to this letter. The University of Saskatchewan Biomedical Research Ethics Board has provided ethical approval for this study.

I look forward to speaking with you in the near future. If you have any questions, please call me at (306) 655-6512.

Sincerely,

Natasha Haskey RD, Masters student
Pediatric Dietitian
Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8
(306) 655-6512
natasha.haskey@saskatoonhealthregion.ca

APPENDIX 2

Confirmation of Ethical Approval

2.1 Ethical Approval for Phase I – Pediatric Pilot Study

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

15-Mar-2004

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Natasha Haskey	Food and Nutrition Services	03-1195

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

SUB-INVESTIGATOR(S)

Chris Arnold
Garth A. Bruce
Wendy J. Dahl
Susan J. Whiting

SPONSORING AGENCIES

ROYAL UNIVERSITY HOSPITAL FOUNDATION

TITLE:

An Investigation Into the Effects of Low Sulfate Water and Prebiotic and Probiotic-Enhanced Diet on Symptoms of Pediatric Ulcerative Colitis: A Pilot Project

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
23-Jan-2004		Parent/Guardina Information and Consent Form (02 Mar 04) Participant Information and Assent Form (08 Mar 04) Telephone Script for Research Purposes Telephone Script for Administration of Questonnaire (SIBDQ) Appendix I - the SIBDQ Study Diary Page

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

Please send all correspondence to:

Office of Research Services, University of Saskatchewan
Room 3403, 110 Gymnasium Place
Box 5000 RPO University
Saskatoon, SK S7N 4J8
Phone: (306) 966-4053 Fax: (306) 966-2069

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

09-Aug-2004

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Natasha Haskey	Food and Nutrition Services	03-1195

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

SUB-INVESTIGATOR(S)

Chris Arnold
Garth A. Bruce
Wendy J. Dahl
Susan J. Whiting

SPONSORING AGENCIES

ROYAL UNIVERSITY HOSPITAL FOUNDATION

TITLE:

Protocol An Investigation Into the Effects of Low Sulfate Water and Prebiotic and Probiotic-Enhanced Diet on Symptoms of Pediatric Ulcerative Colitis: A Pilot Project

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
23-Jan-2004	01-Jan-2005

CERTIFICATION UPDATE	APPROVED ON
Protocol Amendment 1 Child Assent Form v.1	09-Aug-2004

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio- REB) has reviewed the above-named research project including the protocol and consent form, where applicable. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility of ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENT(S) / REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED

BM McKay for:

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

Please send all correspondence to:

Office of Research Services, University of Saskatchewan
Room 3403, 110 Gymnasium Place
Box 5000 RPO University
Saskatoon, SK S7N 4J8
Phone: (306) 966-4053 Fax: (306) 966-2069

2.2 Ethical Approval for Inclusion of Additional Sites

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

19-Jan-2005

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Natasha Haskey	Food and Nutrition Services	03-1195

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

Regina General Hospital
1440-14th Ave.
Regina SK S4P 0W5

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

Stollery Children's Hospital
Edmonton AB

Winnipeg Children's Hospital
Winnipeg MB

SUB-INVESTIGATOR(S)

Chris Arnold
Garth A. Bruce
Wendy J. Dahl
Susan J. Whiting

SPONSORING AGENCIES

INSTITUT ROSELL INC.

TITLE:

Protocol An Investigation Into the Effects of Low Sulfate Water and Prebiotic and Probiotic-Enhanced Diet on Symptoms of Pediatric Ulcerative Colitis: A Pilot Project

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
23-Jan-2004	01-Jan-2006

CERTIFICATION UPDATE	APPROVED ON
Addition of Study Sites Change in Sponsor	19-Jan-2005

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio- REB) has reviewed the above-named research project including the protocol and consent form, where applicable. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility of ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENT(S) / REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

Please send all correspondence to:

Office of Research Services, University of Saskatchewan
Room 3403, 110 Gymnasium Place
Box 5000 RPO University
Saskatoon, SK S7N 4J8
Phone: (306) 966-4053 Fax: (306) 966-2069

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Natasha Haskey	Food and Nutrition Services	03-1195

APPROVED

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

Please send all correspondence to:

Office of Research Services, University of Saskatchewan
Room 3403, 110 Gymnasium Place
Box 5000 RPO University
Saskatoon, SK S7N 4J8
Phone: (306) 966-4053 Fax: (306) 966-2069

2.3 Ethical Approval to Increase Recruitment and Increase Treatment Period

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

05-Aug-2005

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Wendy J. Dahl	Pharmacy and Nutrition	03-1195

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

Regina General Hospital
1440-14th Ave.
Regina SK S4P 0W5

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

Stollery Children's Hospital
Edmonton AB

Winnipeg Children's Hospital
Winnipeg MB

SUB-INVESTIGATOR(S)

Chris Arnold
Garth A. Bruce
Wendy J. Dahl
Susan J. Whiting

SPONSORING AGENCIES

INSTITUT ROSELL INC.

TITLE:

Protocol An Investigation Into the Effects of Low Sulfate Water and Prebiotic and Probiotic-Enhanced Diet on Symptoms of Pediatric Ulcerative Colitis: A Pilot Project

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
23-Jan-2004	01-Jan-2006

CERTIFICATION UPDATE	APPROVED ON
Increased Recruitment by 20 subjects Increased Treatment period by 1 year	05-Aug-2005

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio- REB) has reviewed the above-named research project including the protocol and consent form, where applicable. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility of ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENT(S) / REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon, SK S7N 5C8
Phone: (306) 966-4053 Fax: (306) 966-2069

2.4 Ethical Approval to Increase Recruitment to Include Adult Subjects

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

31-Jan-2006

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio#
Wendy J. Dahl	Pharmacy and Nutrition	03-1195

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

Regina General Hospital
1440-14th Ave.
Regina SK S4P 0W5

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

Stollery Children's Hospital
Edmonton AB T6G 2B7

Winnipeg Children's Hospital
Winnipeg MB

SUB-INVESTIGATOR(S)
Wendy J. Dahl, Susan J. Whiting, Chris Arnold, Garth A. Bruce

SPONSORING AGENCIES
INSTITUT ROSELL INC.

TITLE:
Protocol An Investigation Into the Effects of Low Sulfate Water and Prebiotic and Probiotic-Enhanced Diet on Symptoms of Pediatric Ulcerative Colitis: A Pilot Project

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
23-Jan-2004	01-Jan-2007

CERTIFICATION UPDATE	APPROVED ON
Revised subject recruitment strategy (01-16-2006)	31-Jan-2006

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio- REB) has reviewed the above-named research project including the protocol and consent form, where applicable. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility of ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENT(S) / REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED

Michel Desautels, Ph.D.
University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305, Kirk Hall, 117 Science Place
Saskatoon, SK S7N 5C8
Phone: (306) 966-4053 Fax: (306) 966-2069

APPENDIX 3

Consent Forms

3.1 Written Consent

3.1.1 Parent/Guardian Information and Consent Form

PARENT/GUARDIAN INFORMATION AND CONSENT FORM

Natasha Haskey, Pediatric Dietitian, Doctor **(insert name here)** and researchers in the College of Pharmacy and Nutrition, University of Saskatchewan are investigating the effect of "health-promoting" bacteria on ulcerative colitis.

Study Title: An investigation into the effectiveness of low sulfate water, prebiotic and probiotics on length of remission and quality of life in pediatric ulcerative colitis patients.

Benefits: There may be no direct benefit to your child for participating in this study. Your child is being asked to participate in this study because your child has been diagnosed with ulcerative colitis.

Procedures:

1. Your child will be provided with and asked to consume inulin (or placebo) and health-enhancing bifidobacterium (or placebo) for 12 months. You and your child will not be told whether you received the placebo or the treatment until the conclusion of the study.

2. Your child will be expected to consume one capsule of probiotic (or placebo)/day and 15 grams of inulin (dissolved in usual beverages) or placebo each day for up to one year.

3. Every second month you will be called to gain consent to speak with your child. If your and your child's consent is gained, your child will be interviewed regarding their health using the SIBBQ questionnaire.

4. The information regarding whether or not your child received the placebo or the treatment is available in the case of an emergency.

Confidentiality:

While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name and your child's name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team.

Participation is Voluntary:

The participation of your child in this study is entirely voluntary. You and your child have the right to refuse to participate and to withdraw from the study at any time, for any reason. Early withdrawal from the study will not result in the loss of health care services or result in any sort of penalty. For the telephone survey, your child does not have to answer any question that he/she does not want to.

In the event that any new information is available that may affect your decision to allow your child's participation, the researchers will advise you.

Potential Risks:

The prebiotic and probiotics to be given in this study may result in changes in bowel symptoms including gas and bloating. There is the possibility of unforeseen risks during and following the study.

Some of the questions on the SIBBQ telephone survey may be potentially upsetting. If responses to the telephone survey indicate that your child is experiencing serious difficulties with his/her physical or mental health, the clinical dietitian conducting the survey will forward these concerns to the attending physician.

Research Related Injury:

There will be no costs to you for your child's participation in this study. You will not be charged for any research procedures. In the event that your child becomes ill or injured as a result of participating in this study, necessary medical treatment will be made available at no cost to you.

Dissemination of Results:

Data resulting from this information will be submitted for publication in a medical or nutrition journal. If you wish, a report of the results of this study will be forwarded to you.

Compensation:

You will not receive an honorarium for your child's participation in this study.

Contacts:

If you have any questions with regards to this research project, please do not hesitate to contact the researchers below:

Natasha Haskey	306 655 6512
Wendy Dahl	306 655 1310
Chris Arnold	306 655 5045

If you have any questions about your rights as a research subject, you may contact the chair of the Biomedical Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966 4053.

The contents of this consent form have been explained to me. I have been able to ask questions about the study and these questions have been answered to my satisfaction. I have received a copy of the consent form for my own records.

Signatures:

Parent/Guardian: _____ Date: _____

Research Coordinator: _____ Date: _____

Witness: _____

3.1.2 Participant Information and Assent Form

PARTICIPANT INFORMATION AND ASSENT FORM

Title of study: An investigation into the effectiveness of low sulfate water, prebiotic and probiotics on length of remission and quality of life in pediatric ulcerative colitis patients.

You are invited to be a volunteer in a project to study how "good bacteria" affect the health of people with ulcerative colitis. You are being asked to take part in this study because you have ulcerative colitis.

Taking part in this study is not part of your schoolwork. This study is not part of your medical treatment. You do not have to take part in this study. If you decide to take part, you can quit at any time for any reason. Quitting the study will not result in any penalty or loss of health care services.

If you volunteer for this study,

1. You will be asked to take a capsule of "good bacteria" everyday for up to one year.
2. You will be asked to eat 15 grams (about one heaped tablespoon) of inulin (a sugary powder made from a root vegetable) each day. The inulin can be mixed with juice, milk, pop or foods.
3. Taking your capsule and inulin will take about 5 minutes each day.
4. You will be phoned by a dietitian about every two months and asked about your how you are feeling. These calls will take about 15 minutes each time. There will be 6 phone calls in one year.

In this study, some of the volunteers will receive "placebos". The placebo capsules and inulin will look like the real "good bacteria" capsules and inulin, but will **not** be the good bacteria or the inulin. The placebos will be only a sugar and starch filler. You might be chosen for the placebo group. You will not know which group you were in until the end of the study.

After eating good bacteria and inulin, you may notice changes in your bowel function. You may notice more or less gas and bloating. You may notice changes in your stools. You may notice changes in how often you need to pass stools.

Being part of this study will be kept private. No one other than the research people will know that you are taking part in this study. No one at your school will be told about the study. What you tell the dietitian will also be private. She will not use your name to tell the other researchers about how you are feeling.

The information we find out from this study will be used to write a paper for a medical or nutrition journal. No names of study participants will be used.

This study has been approved by the Biomedical Research Ethics Board (Bio-REB) of the University of Saskatchewan.

If you have any questions about this research project, please call:

Natasha Haskey	306 655 6512
Wendy Dahl	306 655 1310
Chris Arnold	306 655 5045

If you have any questions about your rights as a research subject, you may contact the chair of the Biomedical Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966 4053.

The contents of this consent form have been explained to me. I have been able to ask questions about the study and these questions have been answered to my satisfaction. I have received a copy of the consent form for my own records.

Signatures:

Study Volunteer: _____ Date: _____

Research Coordinator: _____ Date: _____

Witness:_____

3.2 Verbal Consent to Administer the SIBDQ to Study Participants

TELEPHONE SCRIPT FOR ADMINSTRATION OF SIBDQ TO CHILD

Section A

Hello, may I speak to the parent or guardian of ***(insert child's name here)****.*

My name is Natasha Haskey; I am a Pediatric Dietitian that works at Royal University Hospital with Doctor ***(insert doctors name here)****. I am calling in regards to the research study that Doctor* ***(insert doctors name here,*** *researchers and myself are completing on how changing the diet may affect the symptoms of Ulcerative Colitis. We are asking for consent for your child to participate in a 15-minute telephone questionnaire. The questionnaire has been designed to find out how your child has been feeling in the last two months as a result of Ulcerative Colitis. Your child will be asked some questions about their symptoms related to Ulcerative Colitis, the way they have been feeling in general and how their mood has been.*

Some of the questions may be potentially upsetting or embarrassing; your child is under no obligation to answer these questions. Completion of the telephone questionnaire is voluntary and your child may withdraw from the survey at any time without any explanation or effect on health care services. Every effort will be made to ensure that the information collected today remains confidential. Your child's name will not be linked to any information, nor mentioned in any study report, nor be made available to anyone except the research team.

If you have any questions with regards to this project, you may contact

Natasha Haskey	*655-6512*
Wendy Dahl	*655-1310*
Chris Arnold	*655-5045*

If you have any questions about your child's rights as a research subject, you may contact the chair of the Biomedical Ethics Board, c/o the Office of Research Services, University of Saskatchewan at 966-4053.

Do I have your verbal consent for your child to participate in the telephone questionnaire?

If consent provided by parent or guardian, continue to section B.

Section B

*Hi, **(insert child's name here)** my name is Natasha Haskey; I am a Pediatric Dietitian that works at Royal University Hospital with Doctor **(insert doctor's name here)**. I was wondering if I could ask you some questions about how you have been feeling in the last 2 months as a result of Ulcerative Colitis. It will take about 15 minutes of your time. Some of the questions may be upsetting or embarrassing to you. If any of the questions make you feel that way; you do not have to answer them. If at any time you do not want to continue with the questions, let me know and our conversation can end. You do not have to give me any reason for why you do not want to continue. If you decide to stop, I will not be angry or upset with you and you will not be penalized in any way. The information I collect today will remain confidential. It will not be shared with other people in the study, parents or teachers. Do you have any questions? Do I have your consent to ask some questions?*

APPENDIX 4

Study Diary

My Study Diary

Name _____

Date							
Medications – checkmark the days you take your medicine							
Probiotic							
Inulin							
Water – checkmark the days you use bottled water							
Bottled Water							
Stools – describe stools when present							
Frequency (# of stools/day)							
Consistency (loose or formed)							
Presence of blood or mucous							
Overall Feeling 4							
Well							
Terrible							
Abdominal Pain							

APPENDIX 5

The Short Inflammatory Bowel Disease Questionnaire (SIBDQ)

SHORT INFLAMMATORY BOWEL DISEASE QUESTIONNAIRE

This questionnaire is designed to find out how you have been feeling during the last two weeks. You will be asked about symptoms you are having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been.

1. How often has the feeling of fatigue or of being tired and worn out been a problem for you during the last two weeks? Please indicate how often the feeling of fatigue or tiredness has been a problem for you during the last two weeks by picking one option from the following: (SYSTEMIC)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

2. How often during the last two weeks have you had to delay or cancel a social engagement because of your bowel problem? Please choose an option from the following: (SOCIAL)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

3. How much of the time during the last two weeks have you been troubled by a feeling of having to go to the toilet even though your bowels were empty? Please choose an option from the following: (BOWEL)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

4. How much of the time during the last two weeks have you felt angry as a result of your bowel problem? Please choose an option from the following: (EMOTIONAL)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

5. Overall, in the last two weeks, how much of a problem have you had passing large amounts of gas? Please choose an option from the following: (BOWEL)

❍ A major problem
❍ A big problem
❍ A significant problem
❍ Some trouble
❍ A little trouble
❍ Hardly any trouble
❍ No trouble

6. Overall, in the last two weeks, how much of a problem have you had maintaining or getting to the weight you would like to be? Please choose an option from the following: (SYSTEMIC)

❍ A major problem
❍ A big problem
❍ A significant problem
❍ Some trouble
❍ A little trouble
❍ Hardly any trouble
❍ No trouble

7. How often during the last two weeks have you felt relaxed and free of tension? Please choose an option from the following: (EMOTIONAL)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

8. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have liked to have done over the last two weeks? Please choose an option from the following: (SOCIAL)

❍ A great deal of difficulty, activities made impossible
❍ A lot of difficulty
❍ A fair bit of difficulty
❍ Some difficulty
❍ A little difficulty
❍ Hardly any difficulty
❍ No difficulty; the bowel problems did not limit sports or leisure activities

9. How often during the last two weeks have you been troubled by pain in the abdomen? Please choose an option from the following: (BOWEL)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

10. How often during the last two weeks have you felt depressed or discouraged? Please choose an option from the following: (EMOTIONAL)

❍ All of the time
❍ Most of the time
❍ A good bit of the time
❍ Some of the time
❍ A little of the time
❍ Hardly any of the time
❍ None of the time

APPENDIX 6

Chart Review Data Collection Sheet

CHART REVIEW FORM

Pediatric Ulcerative Colitis Patients	Name: DOB:
Date Began Study	
Age at Randomization (years/months)	
Sex	
Age at Diagnosis (years/months) Date	
Location of the Disease	
Medical Management History	
Current Status	
Current Medication	
Date of Last Remission	
Length of Last Remission	
Birth Place	
Parent's Birth Place	
Current Dwelling (urban/rural)	
Drinking Water Source	

APPENDIX 7

Schematic of Recruitment

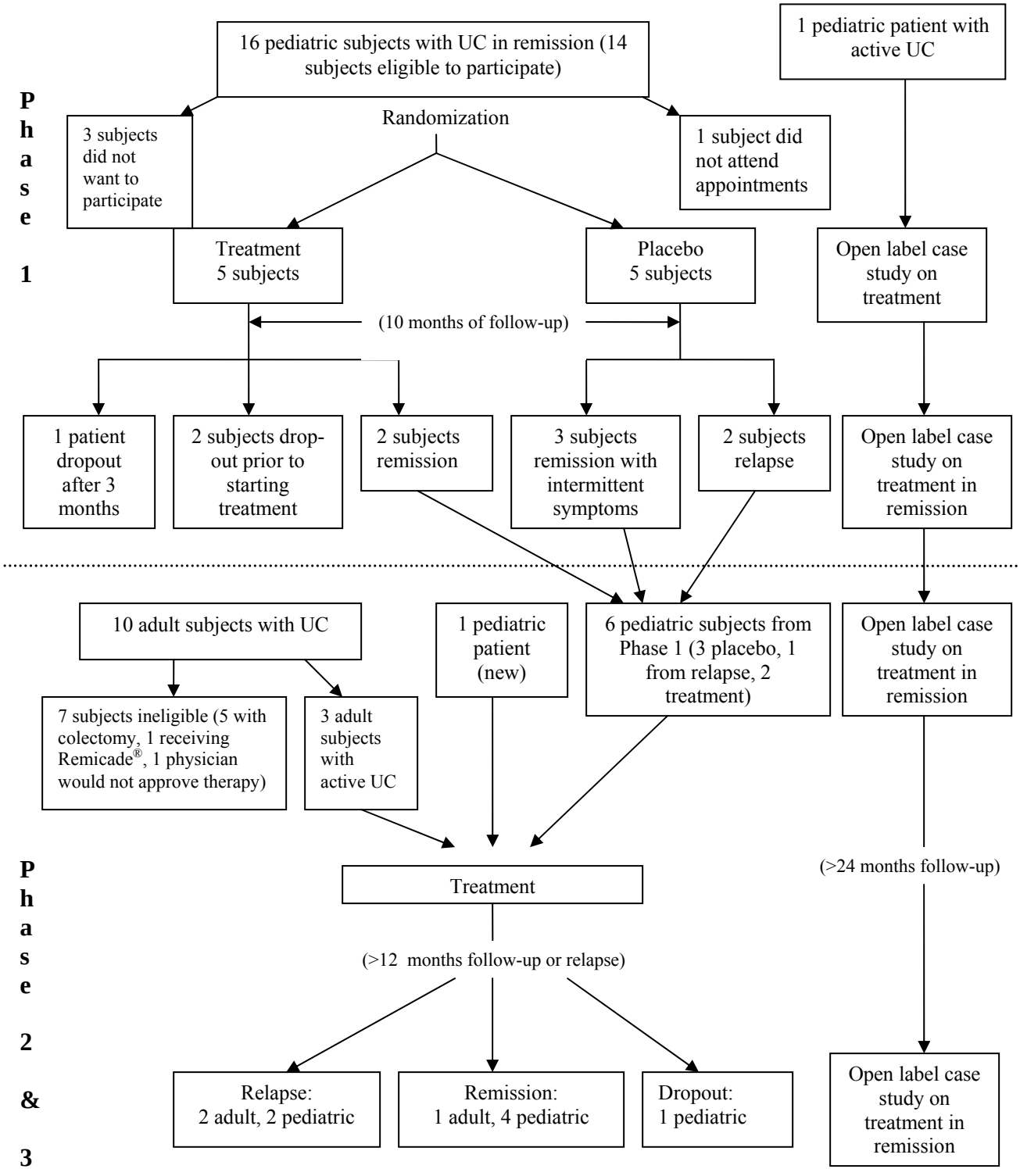
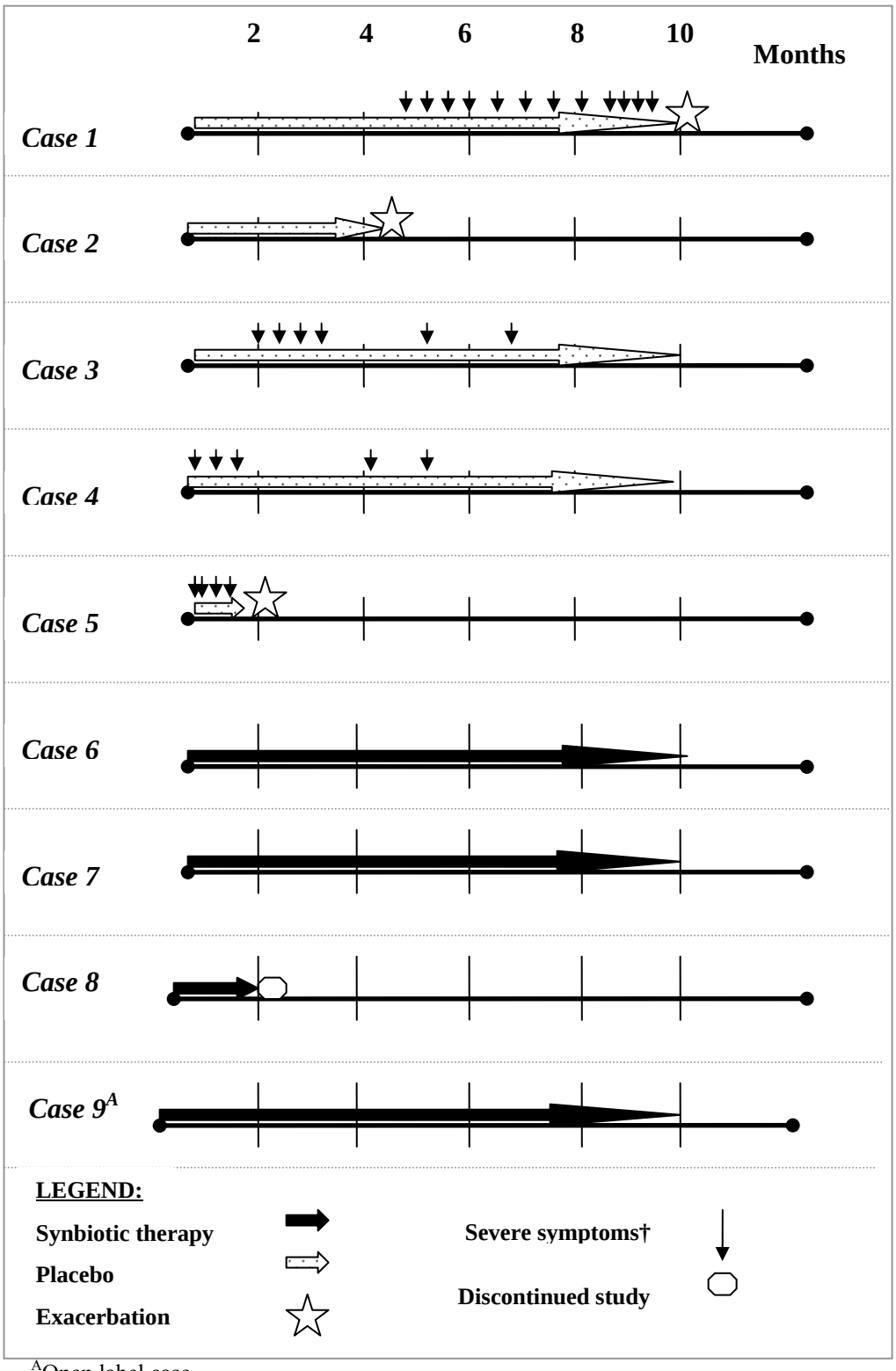


Figure 3.1 Results and Study Design of Phase 1 and 2 of the trial. Phase 1 was a double blind placebo controlled randomized trial. Phase 2 & 3 was an open label trial.

APPENDIX 8

Clinical Course

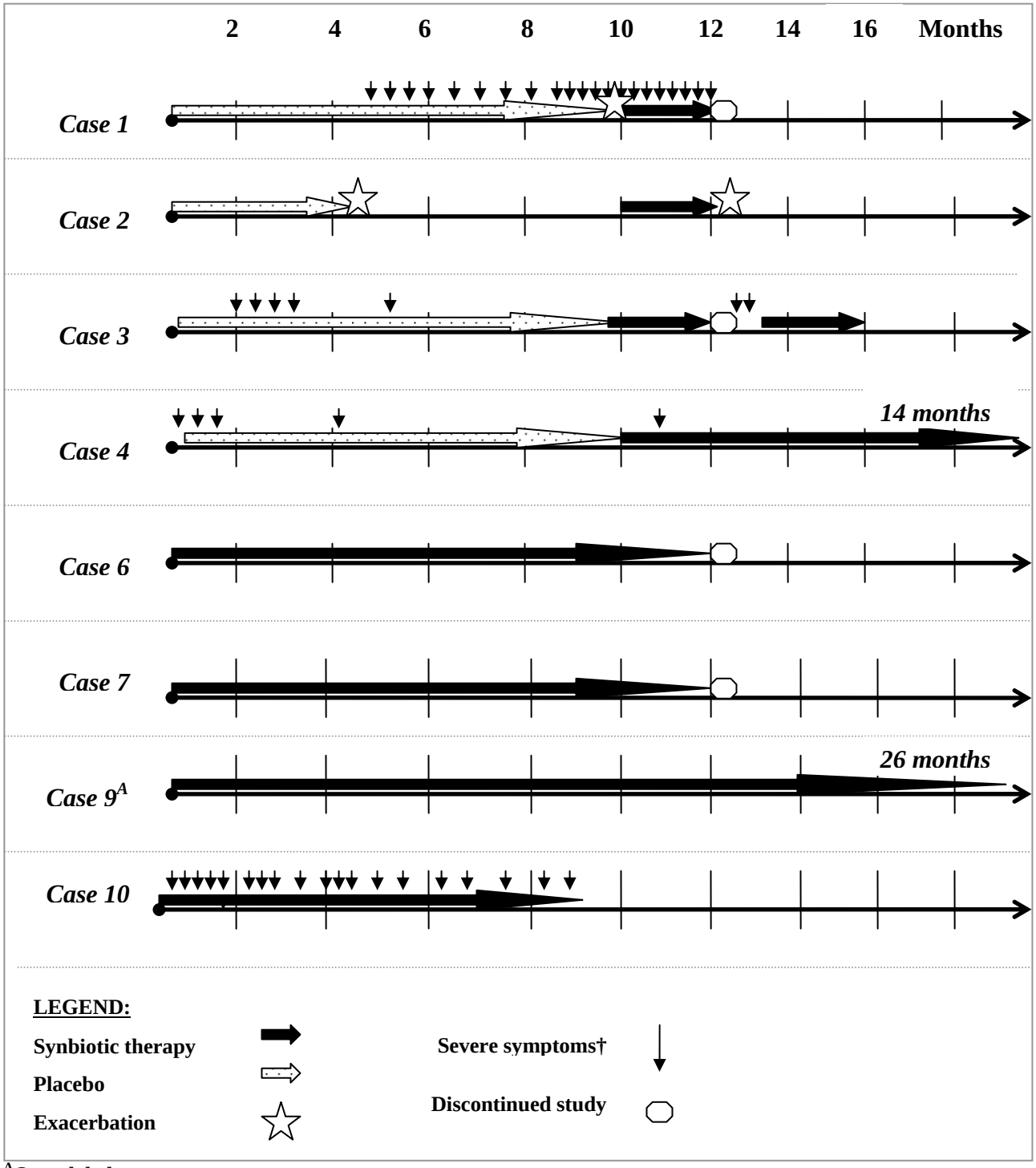
Appendix 8.1 Clinical Courses in Placebo and Treatment Subjects (Phase I – Pediatric Pilot Study)



[A]Open label case

[†]Severe symptoms = presence of blood or mucous in the stool or presence of abdominal pain

Appendix 8.2 Clinical Course of Phase II Pediatric Subjects.



[A]Open label case

†Severe symptoms = presence of blood or mucous in the stool or presence of abdominal pain.