Nutrient intake, gastrointestinal microbiota and the effect of *Lactobacillus plantarum* 299v in irritable bowel syndrome patients

by
Cheryl Stevenson

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Promoter: Prof Saartjie Roux
Co-promoters: Prof Renée Blauuw and Mrs Janicke Visser

Faculty of Medicine and Health Sciences
Department of Interdisciplinary Health Sciences
Division of Human Nutrition

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes three original papers in peer-reviewed journals and two unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

Contributions by principal researcher and fellow researchers

The principal researcher, Cheryl Stevenson, developed the idea and the protocol. The principal researcher planned the study, undertook data collection (with a research assistant), captured the data for analyses, analysed the data with the assistance of a statistician (Prof DG Nel), interpreted the data and drafted the thesis. Profs Saartjie Roux and Renée Blaauw and Mrs Janicke Visser (Promoters) provided input at all stages and revised the protocol and thesis.

Summary

Background: Irritable bowel syndrome (IBS) is a common gastrointestinal (GI) disorder. GI symptoms and impaired quality of life affect between 10-20% of all adults, corresponding to about 25-50% of all patients who visit a gastroenterologist's clinic. In recent years, several novel mechanisms of IBS that likely relate to previously established theories have been identified. Inflammation, postinfectious low-grade inflammation, immunological and genetic predisposition along with altered microbiota are critical in IBS development, while several dietary factors may also play a role in this syndrome. However, none of these factors accounts for the full repertoire of IBS symptoms, and the pathophysiology of this condition is not fully understood. The overarching aim of this study was to investigate the nutrient intakes, GI microbiota and the effect of *Lactobacillus plantarum (L.plantarum)* 299v in IBS patients.

Sub-aims: 1) Update healthcare professionals on current probiotic information and provide an overview of probiotic treatment approaches, with special emphasis on IBS, 2) conduct a well designed randomised, double blind, placebo-controlled trial (RCT) with *L. plantarum* 299v as part of an intervention and establish whether a course of probiotics may alleviate undesirable symptoms of IBS and improve quality of life, 3) assess nutrient intake in patients with irritable bowel syndrome (IBS) compared to dietary recommendations, 4) validate and assess the reproducibility of food records and 5) identify possible nutrient risk components for establishing GI microbiota involved in IBS and as part of an intervention, determine whether a course of probiotics may alter stool microbiota.

Results: 1) A review article published by the author provides an overview of current probiotic treatment options to health care professionals and indicates certain probiotics are a promising therapeutic treatment option for management of IBS symtpoms, 2) the effects of the single strain

probiotic, *L. plantarum* 299v, supplementation was evaluated in a RCT. Compared to placebo, the probiotic supplementation showed no significant reduction in GI symptom severity scores, particularly abdominal pain relief. Quality of life was also not improved in the treatment versus control group. Both the treatment and placebo groups improved significantly over the trial period, indicating a large placebo effect, 3) nutrient intakes of the IBS patients compared to current dietary reference recommendations indicates that this group of patients are at risk for nutrient inadequacies in key macro and micronutrients, 4) the validity and reliability of the dietary data showed good reliability but poor validity as measured by plasma fatty acids and 5) the GI microbiota composition in the phenotypically different diarrhoea-predominant IBS (D-IBS) vs. constipation-predominant IBS (C-IBS) showed that D-IBS patients had significantly lower counts of *Lactobacillus plantarum* compared to C-IBS patients. The probiotic had no significant effects on the GI microbiota as measured by quantitative polymerase chain reaction (qPCR). It was found that nutrient intakes had a significant impact on the microbiota. Lower fibre intakes were associated with higher *Bacteroides* spp., lower *Bifidobacteria bifidum* and *Lactobacillus plantarum* counts in both IBS groups.

Conclusion: Taken together, *L.plantarum* 299v did not alleviate the GI symptoms of IBS, nor was it associated with significant changes in the GI microbiota. IBS patients may be at risk of key nutrient inadequacies. The influence of nutrient intakes on the GI microbiota provides an attractive explanation as a potential pathophysiological factor for IBS.

Opsomming

Agtergrond: Prikkelbare derm-sindroom (PDS) is ‘n algemene gastro-intestinale (GI) stoornis. GI simptome affekteer die lewenskwaliteit van 10-20% van alle volwassenes. Dit stem ooreen met ongeveer 25-50% van alle pasiënte wat ‘n gastroënteroloog konsulteer. Verskeie oorspronklike meganismes vir die ontwikkeling van PDS is onlangs identifiseer. Inflammasie, post-infektiewe lae-graadse inflammasie, immunologiese en genetiese vatbaarheid tesame met veranderde mikrobiota is krities vir die ontwikkeling van PDS. Sekere dieetfaktore mag ook bydraend wees tot hierdie sindroom. Geen van hierdie faktore is egter verantwoordelik vir die volle spektrum van PDS simptome nie en die patofisiologie van die toestand word ook nog nie ten volle verstaan nie. Die oorkoepelende doel van hierdie studie is om nutriëntinname, GI mikrobiota en die uitwerking van *L.plantarum* 299v in PDS pasiënte bepaal.

Sub-doelwitte: 1) Om gesondheidswerkers in te lig aangaande die nuutste inligting oor probiotika en om ‘n oorsig van probiotika behandelingsopsies te verskaf, met spesiale klem op PDS, 2) om ‘n goed beplande ewekansige, dubbel-blinde, plasebo-beheerde kliniese studie met *L.plantarum* 299v as deel van die intervensie uit te voer om sodoende te bepaal of ‘n kursus probiotika ongewensde simptome van PDS kan verbeter en lewenskwaliteit sodoende verhoog, 3) om nutriëntinname in pasiënte met PDS te bepaal vergeleke met dieet aanbevelings, 4) om die geldigheid en herhaalbaarheid van voedselrekords te bepaal en 5) om moontlike nutriënt risikokomponente vir die ontwikkeling van GI mikrobiota betrokke in PDS te identifiseer en om as deel van ‘n intervensie te bepaal of ‘n kursus probiotika stoelgang mikrobiota patrone verander.

Resultate: 1) ‘n Oorsigartikel gepubliseer deur die kandidaat dui probiotika aan as ‘n belowende terapeutiese opsie in die behandeling van PDS simptome, 2) die effek van ‘n enkelstam

probiotikum, *L.plantarum* 299v, is evalueer deur ‘n ewekansige, dubbel-blinde, plasebo-beheerde kliniese studie. Vergeleke met die plasebo, het probiotiese aanvulling geen betekenisvolle vermindering in die GI simptome in PDS pasiënte tot gevolg gehad nie. Lewenskwaliteit het ook nie verbeter in die behandelde versus die kontrole groep nie. Beide die behandelde en plasebo groepe het aansienlik verbeter oor die studietydperk, wat ‘n groot plasebo effek aandui, 3) nutriëntinname van die PDS groep vergeleke met huidige dieetaanbevelings, dui daarop dat hierdie groep pasiënte ‘n risiko het vir die ontwikkeling van kern nutriënttekorte (makro- en mikronutriënte), 4) die geldigheid en betroubaarheid van die dieetdata dui op goeie betroubaarheid, maar swak geldigheid soos bepaal deur plasma vetsure en 5) die dermkanaal mikrobiotiese samestelling in die verskillende fenotipes, diarree-oorheersende PDS (D-PDS) vs. konstipasie-oorheersende PDS (K-PDS) dui daarop dat D-PDS pasiënte aansienlike minder *Lactobacillus plantarum* gehad het vergeleke met K-PDS pasiënte. Die probiotikum het geen beduidende uitwerking op die oorheersende mikrobiota gehad nie, soos gemeet deur kwantitatiewe polimerase kettingreaksie (kPKR). Daar is gevind dat dieet ‘n beduidende impak op die mikrobiota gehad het. Daar is ‘n verband tussen laer vesel inname en hoër *Bacteroides* spp. en laer *Bifidobacteria bididum* en *Lactobacillus plantarum* tellings gevind in beide PDS groepe.

Gevolgtrekking: Die *L.plantarum* 299v enkelstam probiotikum het nie die gastrointestinale simptome van PDS pasiënte verlig nie en daar is ook geen beduidende veranderinge in die mikrobiota gevind nie. PDS pasiënte mag ‘n verhoogde risiko toon vir kern nutriënttekorte. Die invloed van nutriëntinname op GI mikrobiota verskaf ‘n belowende verduideliking as ‘n potensiële patofisiologiese faktor in PDS.

A good set of bowels is worth more to a man than any quantity of brains.

Josh Billings (Henry Wheeler Shaw), AD 1818 - 1885

Abbreviations

Abbreviations used in the introduction and discussion sections only

5-HIAA	5-hydroxyindoleactic acid
5-HT	5-hydroxytryptamine
5-HT3	5-hydroxytrptamine receptor 3
5-HT4	5-hydroxytrptamine receptor 4
5-HT5	5-hydroxytrptamine receptor 5
5-HTT LPR	Serotonin transporter-linked polymorphic region
AI	Adequate intake
A-IBS	Alternating irritable bowel syndrome
AMDR	Acceptable macronutrient distribution ranges
CFTR	Cystic fibrosis transport regulator
cfu	Colony forming unit
CI	Confidence interval
C-IBS	Constipation predominant irritable bowel syndrome
CIC-2	Chloride channel protein 2
CNS	Central nervous system

D-IBS	Diarrhoea predominant irritable bowel syndrome
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intake
EPS	Exopolysaccharides
FA	Fatty acid
FAME	Fatty acid methyl esters
FBD	Functional bowel disorder
FGID	Functional gastrointestinal disorder
FISH	Fluorescent *in situ* hybridisation
FODMAP	Fermentable Oligo-Di-and Mono-saccharides and Polyols
GC – C	Guanylate cyclase - C
GC-MS	Gas chromatography and mass spectroscopy
GI	Gastrointestinal
GIT	Gastrointestinal tract
GNß3	ß3 subunit of the G protein
HITChip	Human Intestinal Tract Chip
HREC	Health Research Ethics Committee

IBS	Irritable bowel syndrome
IBS-DGDG	Irritable bowel syndrome Dietetic Guideline Development Group
IBS-QOL	Irritable bowel syndrome quality-of-life
IL-10	Interleukin – 10
IOM	Institute of Medicine
MCID	Minimally clinically important difference
M-IBS	Mixed irritable bowel syndrome
mRNA	Messenger ribonucleic acid
NNT	Number needed to treat
NSP	Non-starch polysaccharide
PI-IBS	Post infectious irritable bowel syndrome
QoL	Quality of Life
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
SACN	Scientific Advisory Committee on Nutrition
SCFA	Short chain fatty acids
SERT	Serotonin transporter gene

SGA	Subjective Global Assessment
SIBO	Small intestinal bacterial overgrowth
SIGN	Scottish Intercollegiate Guidelines Referral
Spp.	Sub species
USA	United States of America
VAS	Visual analogue score
vs.	Versus

Tables and Figures

Tables and figures used in the introduction and discussion sections only

Introduction

Discussion

Acknowledgements

A man's heart plans his way, But the LORD directs his steps (Proverbs 16:9)

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Contents

Chapter 1

Introduction

Literature overview

1. Irritable bowel syndrome

1.1. Definition and diagnosis

Irritable bowel syndrome (IBS) belongs to a group of functional bowel disorders (FBD), which also includes functional bloating, functional constipation, functional diarrhoea and unspecified bowel disorder.[1] The main feature of IBS is recurrent abdominal pain or discomfort that is associated with disordered defecation and changes in bowel habit.[2] Other symptoms characteristic for IBS and classified as supportive symptoms include: abnormal stool frequency (≤3 stools/week or > 3/day), abnormal stool form, defecation straining, urgency, incomplete bowel movements, mucus and bloating. Based on the supportive symptoms, IBS can be subdivided into diarrhoea predominant IBS (D-IBS), constipation predominant IBS (C-IBS) and a mixed or alternating IBS (A-IBS). The syndrome is chronic in nature, but associated with a good prognosis and no increased mortality in the long-term follow-up.[3]

The diagnosis of IBS is based on the identification of symptoms consistent with the condition and on case-by-case evaluated exclusion of other diseases with similar clinical features.[2] The first symptom-based diagnostic criteria were published by Manning in 1978,[4] and have been widely utilised in epidemiological and clinical studies. The multinational Rome working committee published its first criteria for IBS in 1989[5] and has since then regularly published updated versions.[6,7] The most current version, the Rome III criteria, was issued in 2006.[2] To date there is only one validation study for the Rome III criteria;[8] this highlights the need for further validation studies with the criteria.[9] The Rome I, II and III criteria appear in **Table 1**. Given the

lack of definitive *sine qua non* biomarkers that firmly indicate a diagnosis of IBS, it remains a clinical diagnosis that is largely based on symptom-based criteria.

Table 1. The Rome I, II and III criteria for IBS

Rome 1 criteria[6]
At least three months of continuous or recurrent symptoms of: 1. Abdominal pain or discomfort which is: Relieved with defecation and/or associated with a change in frequency of stool and/or associated with a change in consistency of stool, and 2. Two or more of the following, at least a quarter of occasions or days: altered stool frequency; altered stool form (lumpy/hard or loose/watery stool); altered stool passage (straining, urgency or feeling of incomplete evacuation); passing of mucus; and bloating or the feeling of abdominal distension.
Rome II criteria[7]
At least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two or three features: 1. Relieved with defecation; and/or 2. Onset associated with a change in frequency of stool; and/or 3. Onset associated with a change of form (appearance) of stool The following symptoms cumulatively support the diagnosis of IBS: abnormal stool frequency; abnormal stool form (lumpy/hard or loose/watery stool); abnormal stool passage (straining, urgency of feeling or incomplete evacuation); passing of mucus; and bloating or feeling of abdominal distension.
Rome III criteria[*2]
Recurrent abdominal pain or discomfort[**] at least 3 days per month in the last 3 months associated with 2 or more of the following: 1. Improvement with defecation 2. Onset associated with a change in frequency of stool 3. Onset associated with a change in form (appearance) of stool

[*] Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

[**] Discomfort means an uncomfortable sensation not described as pain. In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days a week during screening evaluation is necessary for subject eligibility.

1.2. Epidemiology

In a recent meta-analysis by Lovell *et al*. the global pooled prevalence of IBS was 11,2%. The prevalence varied according to country (from 1,1% to 45,0%) and the criteria used to define IBS.[10] Though the majority of prevalence findings are from Western populations, increasing data reveal that the syndrome is at least as prevalent in non-Western societies such as China, South Korea, India and Malaysia.[11-14] There is little data available on IBS prevalence in Africa, however data from Kenya and Nigeria shows prevalence between 8 – 33%.[15-17] A study conducted in South Africa almost 30 years ago indicated that the prevalence in the black population group was 8,1%.[18] The prevalence of IBS in the general South African population is unknown. However, the progressive westernisation of diets and lifestyles of less-privileged populations is likely to be associated with an increased incidence of bowel disease and IBS. IBS patients can account for up to 30-50% of gastroenterology clinic visits.[19] In general, there is a clear female predominance among IBS patients. IBS can affect people at any age, but the condition is most commonly diagnosed between ages 20 – 40, whereas organic gastrointestinal predominates in the over 60 years.[20]

1.3. Social and economic impact

Although IBS is not life-threatening, it is a painful, bothersome and distressing condition that interferes with daily life and places a considerable economic burden on a country's health care system. This is seen in both direct and indirect medical costs such as lost productivity and absenteeism, in addition to the less quantifiable costs of a diminished quality of life. In 2004,

annual IBS medical care in the United Sates was believed to be between $1.7 - $10 billion in direct costs and $20 billion for indirect costs.[21]

1.4. Pathophysiology

The discovery of a single unifying pathway of pathogenesis for IBS has been elusive, and there are no biochemical or physiologic markers.[9] However, one such pathway may not exist for IBS since IBS is likely multi-faceted in its aetiology and is seen as a complex biopsychosocial condition in which a number of major mechanisms at the central and peripheral level interact.[1] These include environmental factors (psychological disturbances and stress), previous infection, low grade mucosal inflammation, altered bowel motility and/or secretion, visceral hypersensitivity, genetic links (select gene polymorphisms, including those in interleukin-10, G-protein GNb3, alpha-adrenoreceptor and serotonin reuptake transporter), small GI bacterial overgrowth, food intolerance, altered central nervous system sensory processing, disturbed autonomic nervous system regulation and serotonin dysregulation.[22,23] The contribution of the GI microbiota and diet/nutrient intakes to the pathophysiology of IBS will be discussed separately under sections 2.2 and 3.2 respectively. The prominence of any particular factor may vary from patient to patient. **Figure 1** illustrates the interaction between the various mechanisms involved in the pathophysiology.

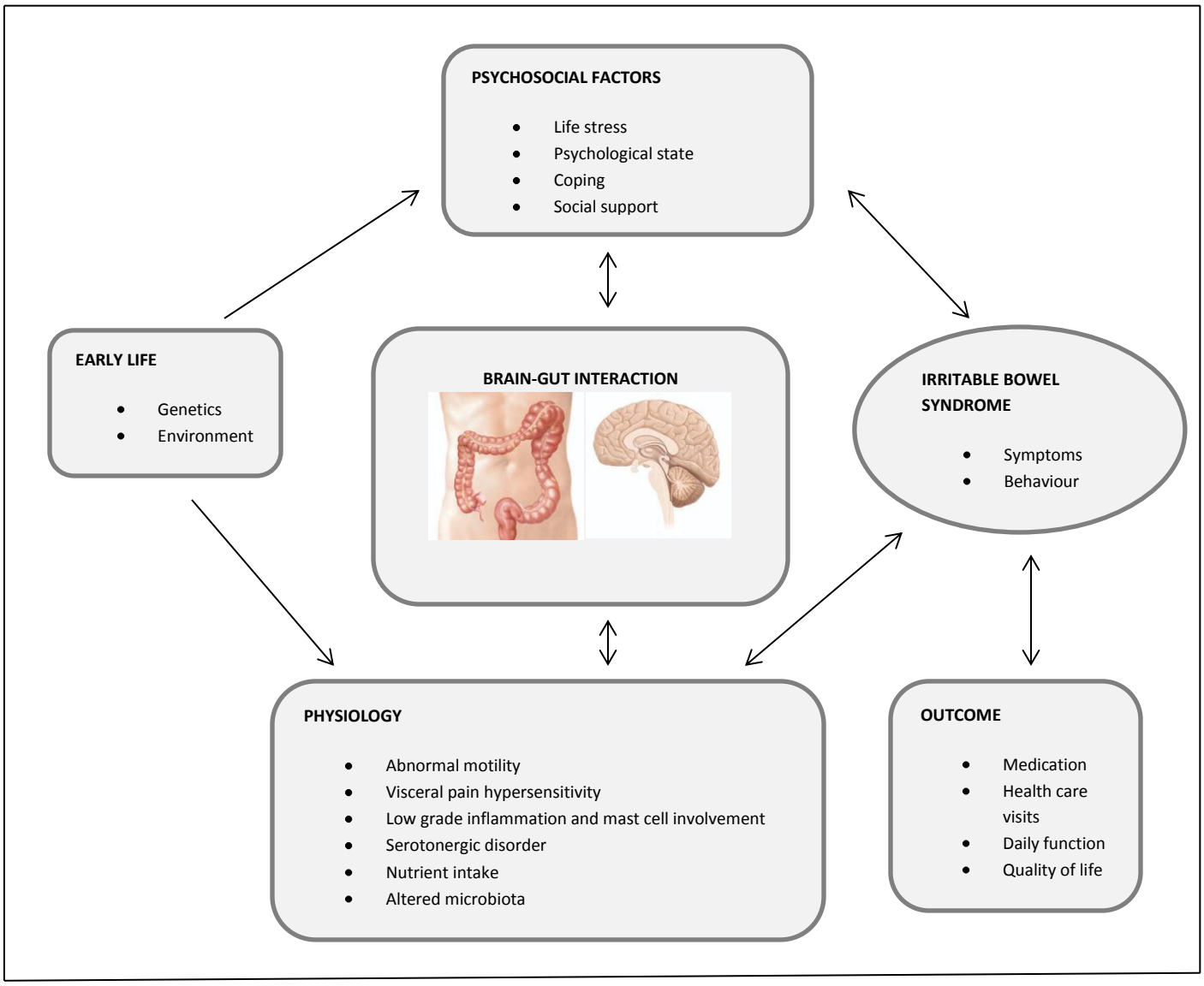

Figure 1. Biopsychosocial model of IBS depicting the relationship between pathophysiology, symptom expression and clinical outcome (modified from Drossman[1]).

Figure 1 illustrates the relationships between psychosocial and physiological factors and functional GI symptoms and clinical outcome. Early in life, genetics, in addition to environmental factors such as family influences on illness expression, abuse, major losses or exposure to infections, may affect one's psychosocial development in terms of one's susceptibility to life sress or psychological state and coping skills, as well as susceptibility to gut dysfunction – abnormal motility, altered mucosal immunity or visceral pain hypersensitivity.

Furthermore, these 'brain-gut' variables reciprocally influence their expression. Therefore, IBS is a clinical product of this interaction of psychosocial factors and altered GI physiology via the brain –gut axis. The clinical outcome will, in turn, affect the severity of the disorder (note the double-sideed arrow in **Figure 1**).[1]

1.4.1. Genetics

The possible role of genetics and putative susceptibility loci for IBS has been an area of growing investigation and interest. Multiple family studies have consistently reported that familial aggregation occurs in IBS.[24-27] Furthermore, the majority of twin studies have demonstrated a significant genetic liability in IBS.[27] Although it remains unclear whether these findings are secondary to common genetic versus early environmental factors, there has been an active interest in various candidate genes. Current IBS candidate genes consist of serotonin transporter (SLC6A4), norepinephrine transporter (NET), alpha-2A-adrenergic receptors (ADRA2A), interleukin-10 (IL-10), G protein β3 subunit (GNβ 3) and sodium channel (SCN5A).[28] Regarding genes controlling inflammation, a meta-analysis indicated that high producer IL-10 (-1082 G/G) polymorphism diminishes the IBS risk in the European IBS population, whereas tumor necrotic factor (TNF) (-308 G/G) polymorphism increases IBS susceptibility and TNF (-308 G/A) polymorphism decreases IBS susceptibility in the Asian IBS population.[29] Genetic variation in the genes controlling bile acid synthesis may contribute to abnormal bowel pattern and symptoms in IBS. Bile acid malabsorption stimulates colonic motility and secretion and has been associated with D-IBS.[30] Hepatic bile acid synthesis is partially controlled by feedback inhibition via the fibroblast growth factor 19 (FGF 19); FGF19 binds to the FGF receptor 4 and the co-receptor Klotho-beta (KLB), leading to the rate-limiting enzyme in bile acid synthesis.[31]

Wong *et al*. reported that a single nucleotide polymorphism in the KLB gene (rs17618244) is associated with accelerated colonic transit in D-IBS.[32] A genetic approach to IBS remains an exciting area of exploration. Future direction of investigation includes genome-wide approaches and further delineation of the role of epigenetic factors in IBS.[31]

1.4.2. Serotonergic disorder

Serotonin, or 5-hydroxytryptamine (5-HT), is a biogenic monoamine neurotransmitter that is largely contained within the GI tract in enterochromaffin cells.[34] Enterochromaffin cells release 5-HT in response to luminal stimuli, such as the passage of chyme, with 5-HT having a wide range of GI effects given that receptor subtypes are found on smooth muscle, enteric neurons and enterocytes.[35] Given the integral role of 5-HT in GI signalling and function, there has been growing interest in its potential role in IBS. It has been suggested that disruption of serotonergic equilibrium may have a role in IBS due to observations made in IBS patients, which include increased postprandial levels of circulating 5-HT in patients with D-IBS; elevated platelet-depleted plasma 5-HT levels in both fasting and fed states in patients with D-IBS; lack of elevation in plasma 5-HT after meal ingestion in patients with C-IBS and decreased mucosal 5-hydroxyindoleactic acid (5-HIAA)/5-HT ratio in those with C-IBS.[36-38] These findings suggest there may be relative 5-HT excess in D-IBS and 5-HT insufficiency in C-IBS. Furthermore, the role of serotonin transporter gene (SERT) function has been of interest given findings of reduced mucosal SERT activity in IBS.[39] However, the role of SERT function in IBS is unclear and differences in mucosal expression of SERT in patients with IBS has not been found.[40]

Perhaps some of the most compelling evidence that IBS is a serotonergic disorder stems from results from 5-HT receptor modulating agents as therapies in IBS; specifically, alosetron and tegaserod. Both alosteron and tegaserod were withdrawn from the United States of America (USA) market due to serious adverse events with constipation and ischemic colitis and post marketing reports of higher cerebrovascular and cardiac events, respectively. Nonetheless, both appeared to be effective therapeutic agents in IBS (and alosetron was re-released in the USA). As a 5-HT3 antagonist, alosetron was shown to be an effective agent in the treatment of D-IBS with improvements in global IBS symptoms, relief of abdominal pain, improvement of both frequency and consistency of bowel movements, inhibition of intestinal secretion, delay of colonic transit time, and central effects that may have resulted in beneficial effects on sensation in D-IBS.[41-46] Tegaserod, a selective partial agonist of the 5-HT4 receptor has been shown to improve global IBS symptoms and constipation in C-IBS patients.[42,45,47,48] Despite the significant adverse events associated with alosteron and those potentially reported for tegaserod, their consistently demonstrated effectiveness as targeted therapies for IBS is supported by the literature. They have served to further strengthen the hypothesis that serotonin is key in the pathophysiology of IBS, which has led to the development of other novel serotonergic agents.

1.4.3. Low grade inflammation and mast cell involvement

There is increasing evidence emerging on the role of low-grade inflammation in the pathogenesis of IBS, and in particular in the role of mast cells. Mast cells play a critical role in normal immune function and respond to antigen stimuli through degranulation resulting in the release of the inflammatory mediators histamine and tryptase.[49] Numerous studies have reported on the increased number of mast cells found throughout the GI tract in patients with IBS.[50-54]

Furthermore, both the degree of cellularity of mucosal mast cells and proximity to sensory nerves has been found to be correlated with abdominal pain in IBS.[52] Increased mast cell activity as measured by release of histamine and tryptase, has also been reported in patients with IBS.[50,52] The GI microbiota may contribute to the low-grade inflammation and intestinal immune activation described in IBS through effects of cytokine levels and toll-like receptor activity.[55-57] In a study of patients with IBS (n = 77) undergoing colonoscopy to rule out inflammatory bowel disease (IBD), patients were categorised based on biopsy as non-inflamed IBS, nonspecific microscopic colitis or lymphocytic colitis. Increases in lymphocytic populations were observed in all patient subgroups, even those without overt signs of inflammation, suggesting a pathophysiologic role of immune activation in IBS. The authors speculated that bacterial antigens could be one of the factors triggering immune activation.[58]

1.4.4. Abnormal motility

A variety of motor abnormalities have been described throughout the GI tract in IBS. Several distinct patterns of motility that vary in their intensity, type and location normally occur within the human GI tract. Overall, patients and healthy controls differ in quantitative, rather than qualitative, aspects of these motility patterns. In comparison with controls, IBS patients appear to have a delayed gastric emptying,[59,60] though not all studies support this.[61] Small bowel motility is altered in IBS in several ways: typical findings include a shorter duration of postprandial motor activity combined with episodes of clustered, recurring contractions correlating with abdominal pain.[62] Furthermore, abnormal duodenal pressure waves that correlate with symptom severity have been observed in D-IBS.[63] Small bowel transit time is significantly shorter in D-IBS and longer in C-IBS, compared to controls.[64] Both in the small

and large bowel, IBS patients show an exaggerated response to a range of provocative stimuli. For instance, hypermotility of the small bowel in IBS patients is seen after infusions of cholecystokinin, a fatty meal, or ileal distension,[65] and colonic motor activity is exaggerated after a meal, an anger stressor or cholecystokinin.[66-68] During cholecystokinin administration, abdominal pain coincided with >90% of the large-bowel high-amplitude contractions, suggesting that abnormalities in these vigorous colonic contractions may be one of the causes of pain.[68] Basal non-stimulated large-bowel motility parameters, such as the myoelectric activity[69] and sigmoid-colonic motor activity,[68,70] also appear to be altered in IBS. Similarly to the small bowel transit times, the whole-gut and colonic transit times are shortened in D-IBS and prolonged in C-IBS.[64,68] Although abnormal GI motor patterns are frequently observed in IBS, the mechanism behind such dysmotility is largely unknown. It has been proposed that disordered functioning of the enteric nervous system and serotonin signalling may be involved. This is corroborated by abnormal serotonin levels and turnover in IBS[37,39] and by the observation that endogenous 5-HT concentrations clearly correlate with the colonic activity index in IBS.[38]

1.4.5. Visceral pain hypersensitivity

Visceral hypersensitivity, defined as an increased sensation in response to intestinal stimuli, is one of the most commonly found hallmarks of IBS and other functional gastrointestinal disorders.[71] Evidence of visceral hypersensitivity in humans is principally based on barostat tests that measure the pain sensation caused by GI balloon distension. Lower pain threshold to colonic distension was observed in most of patients with IBS than healthy subjects, furthermore there was no difference in pain threshold to colonic distension between D-IBS and C-IBS.[72] Some brain regions such as the anterior cingulate cortex (ACC) may play a major role for

generating pain and/or pain-related emotion in humans. IBS patients showed greater activation in the perigenual ACC during painful rectal distension compared with healthy controls.[72] The influence of GI microbiota on visceral hypersensitivity has been suggested in a variety of animal models. In one study, the transfer of faecal microbiota of patients with IBS to germ-free rats was accompanied by a transfer of visceral hypersensitivity (assessed by colorectal distension) when these IBS human microbiota-associated (HMA) rats were compared with healthy HMA rats.[73] An investigation of whether changes in GI flora and GI inflammatory cell activity impacted visceral hypersensitivity in mice demonstrated that, in the absence of sterile precautions (i.e. allowing for the fluctuation of GI bacterial content), the mice had a substantial increase in visceral hypersensitivity over time that was associated with a slightly increased activity of inflammatory cells.[74] When an anti-inflammatory agent (i.e. dexamethasone) was administered, both inflammatory activity and visceral hypersensitivity were reduced, lending further support to the interplay between inflammation and visceral hypersensitivity. Overall, these results support the hypothesis that perturbations of the GI microbiota are associated with small changes in inflammatory activity in the GI tract that can change visceral perception, this is a possible rationale for the administration of certain probiotics for IBS.[56] In rats with induced post-inflammatory chronic hypersensitivity to colorectal distension, the administration of a probiotic resulted in the normalization of visceral hypersensitivity.[75] Although these animal data are interesting, the association between GI microbiota and visceral hypersensitivity remain speculative and require clinical investigations in patients with IBS.

1.4.6. Brain-gut interaction

Perturbations in the brain-gut axis are increasingly recognised as underlying pathophysiological factors in functional GI disorders.[76] Environmental, cognitive and emotional states can affect intestinal sensory perception. The brain-gut axis is considered a model describing the complex bidirectional neural pathways connecting the brain with the gut neuroendocrine centres, the enteric nervous system and the immune system.[77] Disturbed brain-gut communication is not an independent pathophysiological factor in IBS, as the brain-gut axis is the key regulator of e.g. gut motor activity and visceral perception, both known to be changed in IBS. Altered communication between the central nervous system (CNS) and the gut is thus tightly interlinked with other established pathophysiological phenomena in IBS. Overall, brain-gut interactions play an important role in the regulation of many vital functions both in health and in disease. Digestive functions, including motility, secretion, mucosal transport and blood flow are coordinated by the CNS in a top-down mann.[78] Conversely, signals from the gut play a role in reflex regulation and pain perception in a bottom-up manner.[79] The CNS functions as a "filter" with regard to the perception of peripheral afferent signals and the brain-gut communication is for the most part not consciously perceived: only very few of the signals reaching the brainstem and thalamus are consciously perceived in the cortex.[80] The brain-gut axis is stimulated by various stressors, as shown by the fact that acute intestinal inflammation is associated with central sensitisation,[81] whereas psychological events alter gut function.[82,83] Symptoms of IBS are thought to be produced by primary alterations in the CNS, by primary alterations in the periphery, or by a combination of both. Evidence for central alterations in IBS comes from studies using functional brain imaging techniques where different brain areas involved in pain processing are activated in IBS patients vs. controls following painful rectal stimuli.[84,85]

Moreover, IBS patients appear to have an altered processing of anticipated pain, since sham distension resulted in similarly low pain scores in IBS patients and healthy subjects, but a differential brain activation pattern.[85] Central processing may also distinguish between different bowel habits, as demonstrated by lower parasympathetic tone and higher autonomic nervous system balance in C-IBS vs. D-IBS patients.[86] The role of the central and the autonomic nervous system in IBS pathophysiology is supported by findings of sleep disturbances, and especially an enhancement of rapid eye movement sleep in IBS.[87] Studies presenting elevated levels of corticotrophin-releasing and adrenocorticotropic hormones as well as alterations of the visceral perception in IBS patients following mental stress also point towards disturbed brain-gut interaction.[88] Numerous neurotransmitters are involved in the regulation of the brain-gut axis. Amongst those, serotonin is of particular interest since its effects on gut motility, secretion and sensation as well as on cognition and mood make it of paramount relevance in IBS pathophysiology.[89] Acute lowering of serotonin synthesis reduces the threshold for 29 painful stimuli and induces a depression-like memory bias both in IBS patients and in control subjects, illustrating the essential role of serotonergic modulation in the brain-gut axis.[90] In contrast, increased 5-HT activity induced by citalopram is associated with enhanced affective memory performance biased towards positive words.[90] Another important group of molecules affecting the brain-gut axis are prostaglandins, which appear to exert their effects via peripheral mechanisms in the GI tract rather than via central mechanisms.[91]

1.4.7. Psychosocial factors

Psychosocial factors are not believed to cause IBS, but they exert a strong influence on some patients. Psychological stress and emotions produce GI symptoms in almost all individuals, but

IBS patients appear to be particularly susceptible to an exacerbation of symptoms by stress.[92] Psychological symptoms and comorbidity frequently exist in IBS, especially in those seeking health care. Commonly encountered conditions in these patients include depression, somatisation, anxiety disorder, panic disorder and phobic anxiety disorder.[93,94] Both physical and sexual abuses are common and underestimated among IBS patients.[95] In addition, these victims often manifest severe pain perception, psychological distress, and poorer health outcome.[96] Early life trauma is able to increase future visceral pain perception. Accordingly, maternally separated neonatal rodents were used to create a model to study the relationship between early life stress, visceral sensation and depression related disorders including IBS. It was indicated that water avoidance stress increased pain perception and activated somatosensory cortex, periaqueductal gray and hippocampus in the maternally separated rats.[97] In addition, maternally separated rats had significantly increased 5-HT content after colorectal distension.[98] This model also pointed out that the colon of maternally separated rats had elevated circulating levels of interleukin-6 in addition to gut dysfunction.[99] Considered together, neonatal maternal separation appears a stress in rats with exacerbated neurochemical, inflammatory responses, and visceral hyperalgesia in the colon and CNS comparable to IBS subjects. A study to explore the childhood events among IBS adults confirmed that loss and separation during childhood, in the current family and conflicted or dependent maternal relationships were common among some IBS patients.[100] Further psychosocial factors, for example coping strategies and social support are not further discussed here as they fall beyond the scope of the central theme being addressed.

1.5. Current IBS treatment

Treatment recommendations for IBS were presented in the Rome III consensus document for functional bowel disorders in 2006.[2] Since then no further up-to date treatment recommendations have been published by this working group. A number of reviews provide current treatment options, especially dealing with pharmacologic treatments.[101] There is no single curative treatment for IBS, and therapy is aimed at reducing the symptoms, often with very little success.[19] A caring and therapeutic physician-patient relationship is one of the cornerstones of managing IBS. A confident diagnosis, assurance of the benignity of the condition, an explanation as to why symptoms occur and suggestions on how to cope with them are key elements. The patient's need for reassurance and knowledge is reflected in health care utilization, as those patients feeling insufficiently informed will have more health care visits.[102] Overall, patients have more confidence in the effectiveness of education and advice about lifestyle modification than in pharmacotherapy.[103] The traditional approach to therapy for IBS has been largely limited to an individual symptom-specific approach. Such symptom-based therapies have had limited efficacy in treating the entire syndrome complex and have had no impact on the natural history of the disorder.[104] Current mainstays of treatment include; diet and lifestyle modification, pharmacological treatment, psychological treatment and pro, pre and symbiotics. Diet and probiotic treatment are discussed in sections 3.2 and 2.5 respectively.

1.5.1. Pharmacological treatment

Pharmacotherapy is not necessary for all IBS patients, but when needed, it should be directed towards the predominant symptom **(Table 2)**. Because of the abnormalities in bowel states associated with each IBS subtype, it is not likely that one agent would successfully treat all three

subtypes. As a result, clinical trials have focused, for the most part, on one IBS subtype. Over the past two decades very few agents have achieved regulatory approval for the treatment of IBS.[101] Significant methodological inadequacies were recognised in early IBS trials and the classic publication by Klein[105]over two decades ago concluded that not a single study offered convincing evidence that any therapy is effective in treating IBS. In spite of improvement in the design of more recent trials, meta-analysis sums up that many of the routinely used therapies for IBS are of dubious efficacy.[45] The traditional approach to therapy for IBS has largely been limited to an individual symptom-specific approach. These therapies include the use of bulking agents, antidiarrhoeal and antispasmodics. With the developing understanding of the mechanisms and mediators involved in GI motility and secretory function, novel therapies are emerging that hold promise. These therapies include new generation 5-HT4 agonists, novel 5-HT3 antagonists, secretagogues, anti-inflammatory agents, peripheral visceral analgesics and a centrally acting benzodiazepine receptor modulator. Diarrhoea is treated primarily with loperamide, constipation with dietary fiber or commercial fiber analogues and pain with smooth-muscle relaxants and serotonergic agents.

Table 2. Pharmacological treatment for IBS

Type of drug	Drug	IBS subtype	Rationale for use
Bulking agent	Psyllium (soluble fiber) Ispaghula (soluble fiber) Calcium polycarbophil (soluble fiber)	C-IBS	Historically, evidence for bulking agents role in IBS was limited.[106] However, meta-analysis has consistently reported the effectiveness of bulking agents in improving global IBS symptoms and reducing symptom severity.[107,108] Despite these findings, bulking agents have not been demonstrated to improve quality of life in IBS.[107] Given their benign safety profile, bulking agents appear to be an appropriate initial therapy in those with mild symptoms.
Antidiarrhoeal	Loperamide	D-IBS	A traditional antidiarrhoeal that has been used in the treatment of D-IBS. Although it has consistently demonstrated effectiveness in improving stool characteristics and improving diarrhoea, it has not been shown to be effective in ameliorating IBS symptoms or abdominal pain.[42,109] Therefore, loperamide appears to have a limited role in the effectiveness of D-IBS.
Antispasmodics	Hyosine Peppermint oil	D-IBS C-IBS	A recent well performed meta-analysis reported on the relative risk (RR) of persistent symptoms after therapy with antispasmodics compared with placebo 0.68 (95% CI 0.57-0.81) with a number -needed-to –treat (NNT) to prevent symptoms of IBS of five.[108] Hyoscine and peppermint oil appear to be the most effective agents in this class.[42,108]
Serotonergic (5-HT4 agonist)	Prucalopride		A 5-HT4 agonist with high selectivity and affinity that has promising findings without significant adverse events in patients with chronic constipation, which include accelerating colonic transit, improving bowel function and the frequency of bowel movements, improving satisfaction with bowel movements, decreasing perceived severity of constipation and improving constipation-related QoL.[110-113] Should these findings extend similarly for patients with C-IBS, then prucalopride may become an important therapy in the near future.
	Tegaserod (Zelnorm)	C-IBS	Significant improvement in the Subject's Global Assessment (SGA) of relief, bloating, stool frequency, stool consistency, abdominal pain/discomfort, bowel habit and satisfaction with bowel habit and number of days with straining.[114]
Secretagogues (prostone-stimulates chloride ion secretion in gut)	Lubiprostone (Amitiza)	C-IBS	A bicyclic fatty-acid derivative of prostaglandin E1 and an activator of CIC-2 (chloride channels), is thought to increase secretion of intestinal fluid and thereby have a positive enterokinetic effect on the small intestine and colon.[115-117] It has shown to improve abdominal discomfort, bloating, constipation severity, straining and stool consistency[118] and overall responder status in treatment compared to placebo group.[119]

Table 2. continued

Type of drug	Drug	IBS subtype	Rationale for use
Guanylate cyclase-C agonist – secretion of chloride and bicarbonate into intestinal lumen)	Linaclotide	C-IBS	A first-in-class guanylate cyclase-C (GC-C) agonist that activates GC-C receptors located on the luminal membrane of enterocytes which results in activation of cystic fibrosis transport regulator (CFTR) and leads to increased intestinal chloride, bicarbonate and fluid secretion.[120] Linaclotide has been found to significantly accelerate ascending colon transit time, improve ease of stool passage, improve stool consistency and increase stool frequency in women with C-IBS.[120] Significantly greater spontaneous bowel movement in treatment compared to control group.[121] Furthermore, there is growing evidence of its benefits on stool frequency and even QoL in patients with chronic constipation.[122,123]
Anti-inflammatory	Prednisolone		Has been investigated in one randomized, double-blind, placebo-controlled trial in post infectious IBS and was ineffective in improving symptoms.[124]
	Mesalazine (5-aminosalicyclic acid)		Traditionally used in the treatment of inflammatory bowel disease, has had positive preliminary findings in IBS. A recent RCT in IBS patients found that a significant reduction of colonic immune cells, inhibited mast cells and increased general well-being without serious adverse events, however, it did not significantly alter abdominal pain, bloating or bowel habits.[125]
	Ketotifen		A recent preliminary report found that it increased the threshold for discomfort in patients with IBS and visceral hypersensitivity versus 'normosensitive' IBS patients, significantly decreased abdominal pain and other IBS symptoms including bloating and diarrhoea and improved QoL.[126]
Antibiotics	Rifaximin	D-IBS A-IBS	A gut-selective nonabsorbable antibiotic that has broad activity against gram-positive and gram-negative anaerobes, has demonstrated the most promise.[127] Rifaximin therapy has been shown to improve IBS symptom as well as bloating.[128,129] However, most notable is that symptom improvements have been shown to be sustained for up to 12 weeks following therapy.[129] This particular finding from a phase-II trial is especially noteworthy as this suggests that rifaximin may alter the natural history of IBS. Statistically significant improvement on adequate relief of global IBS symptoms has been shown in a recent Phase – III trial.[130]
Peripheral visceral modulation (κ-opioid receptor agonist)	Asimadoline	D-IBS C-IBS A-IBS	Potential role in the visceral modulation of IBS as they are involved in the inhibition of noxious stimuli from the gut and are without the adverse side effects (e.g. constipation, opioid dependence) seen in μ-receptor agonists.[131] Asimadolene, a novel selective κ-opioid receptor agonist, has been demonstrated to significantly improve pain/discomfort in IBS, global IBS symptoms, improve urgency and frequency of stools and reduce pain scores.[132]

Table 2. Continued

Type of drug	Drug	IBS subtype	Rationale for use
			However, on-demand dosing of asimadoline in IBS has not been demonstrated to be an effective method of treatment.[133]
Benzodiazepine receptor modulation	Dextofisopam	D-IBS	An R-enantiomer of tofiospam that binds to 2, 3-benzodiazepine receptors found within the central nervous system which are thought to have a role in the modulation of autonomic function.[134] The role of dextofisopam in the treatment of D-IBS has been suggested with one RCT reporting significant improvements in consistency and frequency of bowel movements with D-IBS or A-IBS.[134]
Alosetron (5-HT3 antagonist)	Lotronex	D-IBS	Alosetron was the first new agent approved for the treatment of IBS in decades. Following its approval, it was withdrawn from the market within one year due to safety reasons related to constipation and ischaemic colitis. Alosetron was subsequently reintroduced under a risk management program and has only been approved in the USA. A total of six published articles have reported the results from seven phase 2 or 3 trials since the beginning of 2000[135-140] With each of these studies, alosetron showed significant improvement in patients with D-IBS across multiple symptoms of relevance. Evaluation of alosetron's treatment effect by gender and by IBS symptom severity showed greater efficacy in females and in subjects with more severe symptoms.[135,138,141] Specifically, significant improvement was seen with females on the adequate relief of IBS pain and discomfort, control of urgency, stool consistency and stool frequency.[135-139,141] Patients taking alosteron also reported greater treatment satisfaction as compared to placebo.[141] Although alosteron provided effective treatment for many IBS symptoms, a dose-dependent increase in the adverse event of constipation (ranging from 4-39%) was seen across the studies. Constipation was often reported as the main reason for early study withdrawal.[135-142]
Antidepressants	Amitriptyline (tricyclic antidepressant)	D-IBS	A phase II study has demonstrated a significant reduction in the treatment (as compared to the control) group for number of loose stools, sensation of incomplete evacuation and a greater treatment response (defined as an absence of all symptoms).[143]

C-IBS – constipation predominant irritable bowel syndrome; D-IBS – diarrhoea predominant irritable bowel syndrome; A-IBS – alternating irritable bowel syndrome; RCT – randomized controlled trial; QoL – Quality of life; RR – relative risk; NNT – number needed to treat; GC-C – guanylate cyclase-C.

Other pharmacological trial results include those for LX 1031, a locally acting, small molecule inhibitor of tryptophan hydroxylase, which is presumed to decrease mucosal production of serotonin. In a phase II clinical trial with D-IBS and M-IBS patients, clinically and statistically significant benefit was seen on the primary endpoint of adequate relief as well as stool consistency. Diarrhoea was the most common adverse event noted, although caution must be exercised as LX 1031 is a serotonergic acting agent.[144] Furthermore, confirmatory studies with crofelemer.[145] and agents acting at the neurokinin receptor[146] are needed for use in D-IBS. Phloroglucinol/trimethylphloroglucinol, an antispasmodic agent, has shown significant reduction in pain intensity in D-IBS and C-IBS patients,[147] but further confirmatory studies are needed.

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1.5.2. Psychological treatment

Psychological treatments may be useful in patients with moderate to severe symptoms when medical treatments have failed or when there is proof that stress or psychological factors exacerbate symptoms.[148] Interpersonal psychotherapy, relaxation/stress management and cognitive behavioural therapy are the most common approaches that have been considered in IBS. Critical evaluation of the efficacy of psychological treatments is hampered by the fact that trials cannot be double-blind, even though certain studies have included some form of control or placebo group. Generally, psychological treatment is time-consuming and expensive, and in many circumstances it is unavailable, which further limits its employment in IBS management.[2] Hypnotherapy, one form of cognitive behavioural therapy, is one of the most widely studied psychological treatments in IBS. Hypnosis can improve GI symptoms and quality of life[149,150] as well as rectal hypersensitivity in IBS.[151,152] However, a Cochrane review[153] identified 25 studies on hypnotherapy in IBS, but included only four in the final review after excluding

methodologically inadequate trials. Though hypnosis was found to be superior to usual medical management in those patients who fail standard therapy, the low number of high-quality trials does not allow for any conclusions to be drawn.

Although a great deal of research has been carried out into the area of the pathophysiology of IBS, it is complex and by no means completely understood. Furthermore the different treatments have varying degrees of success, illustrating the complex nature of the syndrome. It is important to bear in mind that IBS requires a holistic approach in determining effective treatment and understanding the underlying mechanisms.

2. GI microbiota

2.1 Human GI microbiota

The human gut consists of about 10^{13} micro – organisms, collectively known as microbiota or microflora and more than 50 genera and over 400 species of bacteria have been identified in human faeces.[154,155] The microbiota consists of bacteria, fungi, protozoa and viruses and plays an important part in digestive and metabolic processes necessary for general health. The bacterial microbiota are the best described and studied.[156] Importantly, the description of the human microbiota diversity is an on- going process and complete coverage has not been achieved.

The GI microbiota in healthy adults is generally considered highly individual and stable over time.[157,158] Environmental factors e.g. antimicrobials, certain dietary modifications, certain diseases and psychological stress can alter an otherwise stable microbiota.[159] A limited number

of bacterial groups make up the dominant microbiota. Based on molecular methods, the two dominant groups, *Clostridium coccoides-Eubacterium rectale* (28%) and *Clostridium leptum* (25%), represent more than half of the total bacteria. The most abundant bacterial groups after *C.coccoides* and *C.leptum* are *Bacteroides* (9%), *Bifidobacterium* (4%) and *Atopodium* (3%). The *Lactobacillus-Enterococcus* group represents approximately 2% of the total bacteria.[160]

The GI microbiota has a considerable influence on host health and disease, both in the GI tract and systemically.[161] Variations in the GI microbiota balance have been linked with several human diseases. These include obesity,[162,163] Crohn's Disease, ulcerative colitis and celiac disease.[164-166] These conditions have been linked to less species variation and abnormal immune responses to GI bacteria. Disturbances in the sensitive balance between the host and their GI microbiota (dysbiosis) can lead to changes in the mucosal immune system that range from obvious inflammation as seen in Crohn's Disease to low grade inflammation evidenced in a subset of IBS patients.[167] Research verifies the significance of the colonizing microbiota in determining the equilibrium of pro-inflammatory to regulatory cells in the gut.[168,169]

2.2 GI microbiota in IBS

The GI microbiota is comprised of two distinct ecosystems: luminal bacteria that are associated with faeces or food particles and mucosa-associated bacteria that are bound to the mucus layer adjacent to the intestinal epithelium.[170] The GI microbiota has been proposed to play a critical role in GI homeostasis, and has been proposed to have host effects from immune-microbial

interactions.[170] The theorized importance of the GI microbiota has led to investigations which seek to detect either quantitative or qualitative alterations of the microbiota in IBS.

Small intestinal bacterial overgrowth (SIBO) has been proposed as a quantitative alteration of the GI microbiota that results in symptoms of IBS. The role of SIBO in IBS has been supported by evidence that SIBO based on breath testing is highly prevalent in patients with symptoms of IBS, and that treatment of presumed SIBO resulted in improvements in global symptoms of IBS, abdominal pain, bloating and diarrhoea.[171] However, these findings have not been supported by other studies.[172] Furthermore, there is considerable controversy regarding the most appropriate standard by which to diagnose SIBO, breath tests versus small bowel aspirates and culture.[173] Although a recent systematic review and meta-analysis reported the prevalence of SIBO in patients with IBS to be between 4% and 64%, the significant heterogeneity among studies, funnel plot asymmetry, as well as attenuation of significant findings based upon SIBO criteria used, limited any conclusions that could be drawn.[174] Despite the uncertainty, it certainly is notable that recent preliminary studies have found rifaximin; to be effective not only in improving D-IBS symptoms, but also may have sustained effects after cessation of therapy.[129] Regardless of the uncertainty surrounding SIBO in IBS, disturbances of the GI microbiota in IBS have been found.

Post-infectious IBS (PI-IBS) is a common disorder wherein symptoms of IBS begin after an episode of acute gastroenteritis. The occurrence of IBS following episodes of bacteriologically-confirmed gastroenteritis has now been documented in several studies.[175-178] PI-IBS may explain

only a minority of cases of IBS (1-6,7% in one study).[179] But it does represent a clear link between exposure to an environmental agent and IBS in predisposed individuals.

The faecal microbiota of IBS patients differ significantly from that of healthy subjects.[180] Balsari *et al.* studied stool samples of 20 IBS patients and noted a decrease in *Coliforms*, *Lactobacilli* and *Bifidobacteria* compared to healthy individuals.[181] Similar results have been found in other studies.[182] In contrast to Balsari *et al*. a recent study, using similar plating methods, found a significantly higher number of *Coliforms* in IBS.[183] Among culture–independent methods, fluorescent *in situ* hybridisation (FISH) analysis indicates a higher total bacterial population in IBS patients vs. healthy subjects.[184] A further study that divided IBS patients according to subtype showed that D-IBS patients had lower numbers of *Lactobacilli* spp. while C-IBS patients had increased amounts of *Veillonella* spp.[185] A microarray based analysis with the Human Intestinal Tract Chip (HITChip) revealed that the total microbiota of IBS patients is more heterogeneous than healthy controls.[186] The microbiota in IBS is also characterised particularly by lower levels of *Bacteroides* and increased levels of the bacilli order.[186] No single deviance has been identified in IBS microbiota, but various quantitative and qualitative alterations in the GI bacterial composition have, nonetheless, been indicated by a range of techniques. An increasing amount of evidence supports the hypothesis of microbiota involvement in IBS pathophysiology. **Table 3** summarises the culture and molecular studies of the colonic microbiota in IBS to date. Results to date are inconsistent and sometimes contradictory. This may reflect differences in molecular techniques employed, the use of single samples not linked to fluctuating symptoms and other factors such as diet and phenotypic

characterization of patients. In addition it should also be realised that faecal samples do not necessarily reflect other parts of the GI tract.

Table 3. Summary of culture and molecular studies of colonic microbiome in IBS

Study	Subject	Sample	Method	Patient group	Main finding	Country of study
Balsari *et al.*[181]	IBS (n = 20) Ctrls (n = 20)	Faeces	Culture	IBS	↓Coliform bacteria ↓Lactobacillus spp. ↓ Bifidobacterium spp.	Italy
Si *et al.*[187]	IBS (n = 25) Crtls (n = 25)	Faeces	Culture	IBS	↓ Bifidobacterium ↑ Enterobacteriaceae ↓ C perfringens	China
Malinen *et al.*[185]	IBS (n = 27) Crtls (n = 22)	Faeces	qPCR	IBS D-IBS C-IBS	↓B catenulatum ↓CI coccoides group ↓Lactobacillus spp. ↑ Veillonella spp. ↑ Lactobacillus spp.	Finland
Mättö *et al.*[183]	IBS (n = 26) Ctrls (n = 25)	Faeces	Culture PCR-DGGE	IBS	↑ Coliform bacteria ↑ Aerobe to anaerobe ratio ↓ Temporal stability	Finland
Maukonen *et al.*[188]	IBS (n= 24) Ctrls (n=16)	Faeces	PCR-DGGE Affinity capture	IBS C-IBS	↓ Temporal stability ↓ CI coccoides group	Finland
Kassinen *et al.*[180]	IBS (n = 24) Ctrls (n = 23)	Faeces	GC profiling + sequencing of 16S rRNA genes qPCR	IBS	↓ Collinsella aerofaciens ↓CI cocleatum ↓ Coprococcus eutactus Subgroup diff (D, C, M)	Finland
Rajilić-Stojanović[186]	IBS (n = 20) Ctrls (n = 20)	Faeces	Microarray	IBS	Proteobacteria and specific Firmicutes ↑ Other Firmicutes, Bacteroidetes and bifidobacteria ↓	Finland
Kerckhoffs *et al.*[189]	IBS (n = 41) Ctrls (n = 26)	Faeces Duodenal mucosa	FISH qPCR	IBS	↓ Bifidobacterium spp. ↓B catenulatum	The Netherlands
Krogius-Kurikka *et al.*[190]	IBS-D (n = 10) Crtls (n = 23)	Faeces	GC-profiling + sequencing of 16S rRNA genes	D-IBS	↑ Proteobacteria ↑Firmicutes ↓Actinobacteria ↓Bacteroidetes	Finland
Lyra *et al.*[191]	IBS (n = 20) Ctrls (n = 15)	Faeces	qPCR	D-IBS C-IBS A-IBS	↑ R torques 94% ↓ CI thermosuccinogenes 85% ↑ R bromii-like ↓ R torques 93% ↑ CI thermosuccinogenes 85%	Finland
Tana *et al.*[182]	IBS (n = 26) Ctrls (n = 26)	Faeces	Culture qPCR	IBS	↑Veillonella spp. ↑Lactobacillus spp.	Japan
Codling *et al.*[192]	IBS (n = 41) Ctrls (n = 33)	Faeces Colonic mucosa	PCR-DGGE	IBS	↑ Temporal stability No significant difference faecal/mucosal	Ireland

Table 3. continued

Study	Subject	Sample	Method	Patient group	Main finding	Country of study
Carroll *et al.*[193]	IBS –D (n = 10) Ctrls (n = 10)	Faeces Colonic biopsies	Culture qPCR	D-IBS	↓ Aerobic bacteria Lactobacillus spp.	USA
Noor *et al.*[194]	IBS (n = 11) Crtls (n = 22) UC (n = 13)	Faeces	PCR-DGGE + sequencing of 16S rRNA genes	IBS	↓ Bacterial species ↓Biodiversity ↑Biological variability of predominant bacteria	UK
Malinen *et al.*[195]	IBS (n = 44)	Faeces	qPCR		R torques 94% symptom severity Other phylotypes neg assoc	Finland
Ponnusamy *et al.*[196]	IBS (n = 11) Crtls (n = 8)	Faeces	DGGE + qPCR of 16sRNA genes		↑Diversity in Bacteroidetes & Lactobacilli ↑Alanine & pyroglutamic acid & phenolic compounds	Korea
Rinttila *et al.*[197]	IBS (n = 96) Crtls (n = 23)	Faeces	qPCR	IBS	S aureus (17%)	Finland
Saulnier *et al.*[198]	IBS (n = 22) Crtls (n = 22) (Children)	Faeces	16 Metagenomic sequencing and DNA microarray	IBS	↑ γ Proteobacteria Classified IBS subtypes using sets of discriminant bacterial species	USA
Rajilić-Stojanović *et al.*[199]	IBS (n = 62) Crtls (n = 42)	Faeces	Phylogenetic 16S rRNA microarray and qPCR	IBS	↑ Proteobacteria and specific Firmicutes ↓Other Firmicutes, Bacteroidetes and bifidobacteria	Finland
Carroll *et al.*[200]	IBS-D (n = 16) Ctrls (n = 21)	Faeces Colonic mucosa	T-RFLP fingerprinting of 16S rRNA – PCR	D-IBS	Diminished microbial biodiversity in faecal samples	USA
Parkes *et al.*[201]	IBS-D (n = 27), IBS – C (n = 26) Ctrls (n – 26)	Colonic mucosa	FISH Confocal microscopy	IBS	Expansion of mucosa-associated microbiota; mainly bacteroides and clostridia; association with IBS subgroups and symptoms	UK
Jeffrey *et al.*[202]	IBS (n = 37) Ctrls (n = 20)	Faeces	Pyrosequencing 16SrRNA		Clustering of IBS patients – normal-like versus abnormal microbiota composition (increased ratio of Firmicutes to Bacteroidetes); association with symptom profile	Sweden

Reprinted with permission from Simren *et al.*[203]

N – number of randomised subjects, B – Bifidobacterium, C – constipation, C-Clostridium, ctrls – controls, d- diarrhoea, DGGE – denaturing gradient gel electrophoresis, FISH – fluorescent in situ hybridisation, IBS – irritable bowel syndrome, L – Lactobacillus, qPCR – quantitative PCR, R – Ruminococcus, S – Staphylococcus, T-RFLP – terminal restriction fragment length polymorphism

2.3 Overview of methods for analysing microbiota

Until recent years, cultivation-based methods were the most widely applied means of studying the GI microbiota.[204] Although broadly available, relatively inexpensive and having the potential for quantifying bacterial populations, its applicability is restricted to cultivable organisms. It has been proposed that only 30 – 40% of the GI tract microbiota can be cultured by currently available methods.[205] The development of culture-independent, molecular methods built on microbial nucleic acid sequence information has contributed to a dramatic increase in the knowledge of GI microbiota diversity. Examples of these methods are quantitative polymerase chain reaction (qPCR), temperature/denaturing gradient gel electrophoresis, FISH, DNA microarrays and pyrosequencing. Refer to Chapter 6 for further reading.

2.4 Probiotics – definition and health effects

The Food and Agriculture Organization of the WHO provides the most widely accepted definition of a probiotic as "live human microbial organisms that are administered to enhance the well-being of the host".[206] The most commonly used probiotics are lactobacilli, bifidobacteria and non-pathogenic yeasts such as *Saccharomyces boulardii.* Products that are labelled 'probiotic' are widely available; however few fulfil the above definition. From a microbiological perspective, some may not contain sufficient live organisms following commercial or domestic storage or have been adequately tested to ensure they will survive transit through the GI tract. From an application perspective, some may not confer a claimed health benefit, either because

they have not undergone efficacy testing in humans or because what evidence is available is inadequate or negative.[207] In the future, the definition of a probiotic may require modification, as there is experimental evidence that dead bacteria, bacterial components and substances secreted by bacteria (e.g. bacteriocins, conjugated linoleic acid) have physiologically relevant effects that contribute towards the well-being of an individual.[208] The more inclusive term 'pharmabiotic' has also been proposed to encompass entities that exert these potentially important effects.[209] There is growing interest in probiotics as a safe manner in which to change GI bacterial flora. Probiotic preparations can be found in the form of powders, tablets, capsules, pastes, sprays or fermented foods such as yoghurts, buttermilk, sour poi (a starchy paste made from the corm of taro plants) and miso (fermented soybean paste).

There are several mechanisms by which probiotics exert their favourable effects. Different strains of organisms have very different and specialised metabolic activity. Main mechanisms include i) mucosal adherence and inhibition of pathogenic bacteria adherence, ii) enhanced barrier function of epithelium, iii) secretion of bacteriocins, iv) acidification of the colon by nutrient fermentation, v) immuno-modulatory actins, vi) alteration in mucosal response to stress and vii) inhibition of visceral hypersensitivity.[210]

There is strong evidence for clinical effectiveness of probiotics in clinical conditions like antibiotic-associated diarrhoea (*S. boulardii*[211,212] and *Lactobacillus GG*[213,214]), pouchitis (VSL #3, a combination of eight different strains[215,216]) and in the treatment of childhood diarrhoea (*Bifodobacteria*[217]). There is suggestive evidence for the prevention of adult and childhood

diarrhoea (*L. GG,*[218,219] *L. casei,*[220,221] *L. acidophilus*[222,223] and *S. boulardii*[224]) and to improve immune response (*L. GG,*[225] *L.acidophilus,*[226] *B. lactis*[227] and *L. Johnsonii*[228]). There is evidence for the use of *B. infantis,*[229] VSL #3,[230] and *L. plantarum* 299v[231,232] in the treatment of IBS although not enough studies to make a strong recommendation.[233]

2.5 Probiotic clinical trials in IBS

A number of randomised, placebo-controlled trials on the efficacy of probiotics or combinations of probiotics in IBS have been published (Articles 1 and 2 in Chapters 2 and 3 respectively). Furthermore, a recent Rome Foundation group report identified 28 randomised controlled trials (RCTs) of probiotics in adults with IBS and four RCTs in children[203] and at least six systematic reviews (five of which included meta-analysis) that have been published on probiotics and IBS.[235] Most of the meta-analysis indicated a beneficial impact of probiotics on global symptoms, abdominal pain and flatulence, whereas the impact on bloating was equivocal. However, aggregating the effects of different probiotics into a meta-analysis should be undertaken with caution. Different probiotics have different microbiological characteristics that will inevitably impact on their efficacy. Therefore, converting the findings from a meta-analysis into clinical guidelines (e.g. probiotics improve global symptoms of IBS) implies that all probiotics will result in a similar benefit, which may not be the case.[235]

A systematic review by Brenner *et al*. assessed the effectiveness, safety and tolerability of probiotics in the treatment of IBS. An assessment of the RCT's methodology and statistical designs indicated that only *B. infantis* 35624 showed repeated efficacy. The remaining study

designs were limited by poor study design suggesting that there is inadequate data to determine whether probiotics are an effective treatment for IBS.[236] However the following studies do provide sufficient evidence to warrant further evaluation, particularly for single moieties *L. plantarum* 299v,[231,232,237] *L. salivarius* UCC4331[229] and *L. acidophilus* LB strain[238] and combination probiotics; VSL #3[230] and *L.rhamnosus GG*, *L.rhamnosus* LC705, *B. Breve* Bb99/*B. anamalis* spp. Lactis Bb12 and *P. freudenreichii* spp.shermanii JS.[239-241] The Rome methodology scores as assessed by Brenner *et al.*[236] for RCTs using *L.plantarum* 299v are given in **Table 4**. This data illustrates the poor study design used in these three RCTs.

Table 4. Methodology scores for RCTs assessing *L.plantarum* 299v in IBS

Study	***L. plantarum* 299V**[237]	***L. plantarum* 299V**[232]	***L. plantarum* 299V**[231]
ROME criteria used to define IBS	*		*
Randomization		*	*
Parallel study design		*	*
Double-blinding		*	*
Complete follow-up (intention-to-treat)	*	*	
No placebo run-in	*	*	*
Baseline observation period before trial initiation			*
Treatment duration of 8 – 12 weeks or longer	*		
Follow-up after treatment to assess symptoms			*
Treatment compliance measured			
Sample size calculation is provided/ adequate sample enrolled			
Primary outcome = improvement in global IBS symptoms	*		
Primary outcome based on patient Assessment	*	*	*
Validated scale used to measure improvement of IBS symptoms			
Rome methodology score	6/14	6/14	8/14

Adapted from Brenner *et al*.[236]

L. plantarum 299v has been the most studied strain from *L. plantarum* family. It has shown in rats that it can reduce mucosal inflammation by adhering to the mucosal membrane and reducing gram-negative bacteria, which contain endotoxins.[242,243] *In vitro L. plantarum* 299v increased IL-10 synthesis and secretion and thereby demonstrated beneficial immunomodulatory activity.[244] In a Swedish study of 60 patients with IBS given *L. plantarum* 299v or a placebo for 4 weeks, symptoms of flatulence and abdominal pain were significantly reduced in the intervention compared to control group.[231] Johansson *et al.* showed a significant increase in stool volume and

a decrease in flatulence and slightly softer stools in an intervention group receiving *L. plantarum* 299v as compared to controls.[245] *L. plantarum* 299v has the following properties (demonstrated in animal models and/or humans); being of human origin, non-pathogenic, resistant to intestinal acid and bile, demonstrating the ability to adhere to human epithelial cells, demonstrating an ability to temporarily colonise and be metabolically active within the human GI tract, survive transit through the GI tract and be free of side effects.[242,246-250]

The role of probiotics in GI disease, in particular IBS, has clearly not been determined adequately. Although questions exist on dosage and viability of probiotic strains, lack of industry standardization and potential safety issues (with specific regard to immuno-compromised or seriously ill patients),[251] there is substantial clinical evidence for the advantageous use of probiotics over a wide range of clinical conditions. Probiotics may be a safe and effective solution urgently needed in the treatment and management of IBS.

3. Nutrient intake and IBS

Postprandial worsening of symptoms, as well as adverse reactions to one or more foods are common in patients with IBS,[252] and self-reported food intolerance in IBS is associated with a high symptom burden and reduced quality of life.[253] In line with this, approximately two thirds of IBS patients exclude food items from their diet to improve symptoms.[254] Foods rich in fat[252] and carbohydrates[255-257] especially cause problems. Female gender and anxiety seem to predict a higher degree of food-related symptoms in IBS patients.[252] Food intake can be a trigger of GI

symptoms in IBS[252,258,259] and patients often consider food intolerance to be relevant for many of their symptoms.[260] Several studies report that people with IBS often avoid different food items as a way of coping with the disease, which possible could lead to a lower intake of essential nutrients.[252,254,261] However, patients with IBS seem to have a body mass index (BMI) comparable to the general population.[252] Despite the fact that IBS patients consider food and diet to be central issues,[262] few studies have thoroughly explored the dietary intake of patients with IBS and problems with existing studies include the use of retrospective food frequency questionnaires, with the risk of recall bias,[263-265] and the influence of IBS subtype is even less well studied.[253]

3.1. Reproducibility and validity of dietary data in IBS

To the writer's knowledge there is no published data evaluating the validity and reproducibility of dietary intake in IBS patients. The reproducibility of a dietary assessment method reflects the ability of the method to obtain identical results when administered at a later stage under similar circumstances, assuming nothing has changed in the interim.[266] While the validity reflects the ability of a dietary method to accurately measure what the participants have actually eaten.[267] Researchers commonly use these two characteristics to evaluate the measurement properties of dietary assessment methods.[268]

3.1.1. Reproducibility

Reproducibility sometimes called reliability is the minimum quality required for an assessment method.[268] In reproducibility studies the instrument is compared with an earlier administration of the same instrument. It should be recognised at the outset that a reproducibility study does not tell anything about whether the instrument is producing the correct answer, only whether it is producing the same answer. There are a number of reasons for doing reproducibility studies. Firstly, they are relatively easy to do and provide a partial answer to the question of validity; an instrument surely cannot give the correct answer every time if it cannot give more or less the same answer each time.[269] Reproducibility studies can also uncover problems in instrument design, respondent instructions, or quality control that can aid the investigator in improving the questionnaire. Furthermore, once the reproducibility of the questionnaire is known, investigators can use that information to estimate the gain that would accrue from the use of two administrations of the questionnaire, instead of one, in the study design, or to judge the degree of dietary change that could be detected between two administrations.[269]

Several factors can affect the reproducibility of estimates from a questionnaire, some of which are often overlooked. Respondents may be simply unable to estimate their diets reliability; however, it is likely that this explanation of poor reproducibility is less important than other, more methodological explanations. The most obvious of these is that in the elapsed time between the two administrations, real dietary change has taken place. The longer the elapsed time, the more likely this is to have occurred. For this reason, it might be preferable to administer the second questionnaire within a fairly short time, e.g. 4-8 weeks, long enough so

that the respondent is not simply remembering what he or she said before, and short enough to minimise real dietary changes in the interim. An important factor affecting the reproducibility of a questionnaire is the variability which it permits. An instrument which does not include variable portion sizes for each food permits less variability, and is likely to have a higher reproducibility score, than one which does not (e.g. food records). Similarly, a questionnaire which limits responses about the frequency of consumption to a few categories is likely to be more reproducible than one in which the respondent has an unlimited choice of responses. Because of this, a high level of reproducibility, though welcome, is not sufficient; a high level of validity is also necessary.[269] The error-proneness of the questionnaire design, or inadequate instructions to the respondent, can also affect reproducibility. Finally, poor reproducibility can result from poor quality control, again something under the control of the investigator. Inadequate attention to recoding and double keying can produce simple coding or typing errors which lead to apparent poor reproducibility.[269]

3.1.2. Validity

Several terms are used to refer to investigations that collect dietary data from a subset of study subjects by using two different assessment methods and which compare the data of the one method to the other, with the aim to determine the level of comparability or the relationship between the two methods. These terms include 'validation', 'calibration', and 'standardisation'.[270] Dietary methods designed to characterise usual intakes of individuals are the most difficult to validate, since the 'truth' is never known with absolute certainty.[272] Relative validity, in which a new method (i.e. test method) is compared to with an existing method known

to be valid (i.e. reference method) is the most practical validation method to use. Absolute validity implies that the reference method reflects the true dietary intake, while relative validity recognises that the reference method itself is subject to error.[267] Therefore, the extent of agreement between the test and reference methods is used to indicate the relative validity of the test method and the extent to which the reference method is believed to yield the truth.

All calibration studies may not have the same primary aim but the goal that has been most frequently described is to use information from the calibration study to adjust the relative risks estimated from the main epidemiologic study for the measurement error associated with the use of the questionnaire. It is well known that the measurement error biases the estimated relative risks, thereby creating the need for such an adjustment.[271] Another aim of calibration studies is to estimate the sample size required in the main study. This estimate is important because the sample size required for an investigation depends heavily on the degree of measured error associated with the questionnaire. A third aim is to estimate the correlation between intake from the food record or food frequency questionnaire and true usual intake. This evaluation may be critically important if a food frequency questionnaire has been modified extensively from earlier versions or is to be used in a population from which little information has been previously obtained. If the correlations are low, the investigators may postpone the main study until improvements are made in the design of the food frequency questionnaire or in the way in which the questionnaire is presented to the participants. Finally, many calibration studies are conducted to estimate the slope of regression of intake from the food record on usual intake, a variable that is important in assessing the patterns of bias that may result from the use of the questionnaire.[271]

Food records (e.g. 3 day estimated food record as used in this study) are often used as a reference method for the determination of the relative validity of other dietary assessment methods (e.g. food frequency questionnaires), few studies report on the validity and reliability of food records themselves. Numerous factors may affect the validity, or apparent validity, of a diet questionnaire. These factors include 1) respondent characteristics, 2) questionnaire design and quantification, 3) adequacy of the reference data and 4) quality control of data management. Of all of these, the latter three are likely to be more important than the first.[272]

3.1.3. Biochemical markers

Biomarkers are being increasingly used in nutritional epidemiology to assist in dietary measurement and to deal with the problems associated with self-reported dietary intake. The use of biomarkers, such as plasma fatty acids (FA), is a more objective approach to assess the validity of dietary intake data.[273] These methods may however, be expensive and impractical (e.g. the collection of 24-hour urine samples). The fundamental advantage of using a biomarker is that measurement errors are unrelated with errors in any dietary assessments, e.g. do not rely on memory, self-reported information or interviewer bias. There are no biomarkers that reflect absolute fat intake, however, measurement of FAs in various biological samples reflects to some extent, proportional intake of FAs.[274] The intake of FAs may be reflected in various serum (or plasma) lipids, platelet and erythrocytes phospholipids. The FA composition of plasma lipids reflects the type of dietary fat and may be an objective estimate of the type of fats proportionally consumed by an individual. The FA composition of plasma reflects medium-term (weeks to months) dietary intake.[275] Essential polyunsaturated FAs (linoleic acid and α-linolenic acid)

cannot be synthesized *de novo* by humans and play an important role in health. Øverby *et al*. performed a systematic review on 14 papers and found that blood lipid n-3 fatty acids correlated well with different dietary assessment methods. The blood lipid correlations were similar to those observed for subcutaneous fat, which literature describes as the best reference method.[276] As the source of these biologically active FAs is exogenous they may be particularly good biomarkers markers to use.[274]

3.2. Dietary management of IBS

The British Dietetic Association recently published a set of evidence-based guidelines for the dietary management of IBS in adults.[277] However, evidence-based guidelines for dietetic practice are difficult to give, as there are very few randomized, controlled trials on dietary treatment of IBS patients and much of the evidence is of poor quality and limited by the lack of suitable papers. First-line dietary advice consists of assessing dietary choices, eating habits and lifestyle factors. Before a first appointment, it may be useful to ask individuals to keep a food and symptom diary. Consider the frequency and timing of symptoms (e.g. meal-related, daily, nocturnal, weekdays, weekends, holidays, exercise induced and, for women, whether symptoms are related to their menstrual cycle). With specific food avoidances: explore how the individual thinks foods affect their IBS symptoms. Assess the eating pattern and usual dietary intake of dietary fiber, fatty foods, fluid, caffeine, alcohol and milk and/or lactose. Encourage healthy eating patterns with a good variety of foods to achieve nutritional adequacy. Use general healthy eating guidelines with special attention to regular eating, good eating behaviour (taking time over meals, sitting down to eat, chewing food thoroughly and not eating late at night) and drinking plenty of caffeine free, alcohol free, non-fizzy fluids throughout the day.[277] The clinical practice

recommendations as agreed by the IBS – dietetic guideline development group (IBS-DGDG)[277] are shown in **Table 5** below. Standard levels of evidence and grading recommendations as set out by the Scottish Intercollegiate Guidelines Network[278] were used, an A grade evidence ranking as the highest and D the lowest.

Table 5. Dietary clinical practice recommendations for IBS

	Grade of recommendation
1.0 Removing milk and dairy products to improve IBS symptoms	
In individuals where sensitivity to milk is suspected and a lactose hydrogen breath test is not available or appropriate, a trial period of a low lactose diet is recommended. This is particularly useful in individuals with an ethnic background with a high prevalence of primary lactase deficiency. A detailed dietary assessment of milk and/or lactose intake should be used not only to assess specific nutrient intake (e.g. calcium) but also to assess lactose intolerance.	D
Use a low lactose diet to treat individuals with a positive lactose hydrogen breath test. Gradual lactose reintroduction may be useful to determine an individual's lactose tolerance threshold	D
In individuals where milk is suspected as a problem food and symptoms do not improve on a low lactose diet, assess other components of milk (e.g. cow's milk protein) as a contributing factor. Recommend a milk free diet or, in some cases, an alternative mammalian milk (goat's or sheep). If cow's milk protein is not tolerated, the initial recommendation should be a non-mammalian alternative milk (e.g. soya, rice, oat, quinoa, nut, coconut or pea), preferably calcium fortified.	D
2.0 Non starch polysaccharides	
Avoid using dietary supplementation of wheat bran to treat IBS. Individuals should not be advised to increase their intake of wheat bran above their usual dietary intake	C
For individuals with C-IBS, dietary supplementation of ground linseeds (initial dose to start at one teaspoon to one tablespoon per day and build to a maximum of four tablespoons per day with a drink, can be added to food, e.g. yoghurt, breakfast cereal, soup and salads) can be recommended for a 3-month trial. Improvements in constipation, abdominal pain and bloating from linseed supplementation may be gradual	D
3.0 Fermentable carbohydrates	
For individuals with IBS and suspected or diagnosed fructose malabsorption, assess dietary intake of all short-chain fermentable carbohydrates (fructose, fructans, galacto-oligosaccharides and polyols). There is likely to be a benefit in reducing intake	B

Table 5. continued

3.0 Fermentable carbohydrates	Grade of recommendation
For individuals with IBS and abdominal bloating, abdominal pain and/or flatulence, assess dietary intake of fermentable carbohydrates because there may be a benefit in reducing intake	D
There may be individual tolerance levels to fermentable carbohydrates. A planned and systematic challenge of foods high in fermentable carbohydrates will identify which foods can be reintroduced to the diet and what individual tolerance levels are	D
4.0 Empirical and elimination diets	
Where food is considered to be a trigger for IBS symptoms, particularly D-IBS, an elimination or empirical diet can be considered	D
The initial phase of an elimination or empirical diet should be followed for 2–4 weeks, to complete they usually take 3-4 months, including the re-introduction phase	D
If there is no symptom improvement within 2–4 weeks of the initial phase of an elimination or empirical diet and foods consumed within the diet were not suspected symptom triggers, specific foods are an unlikely cause of IBS symptoms	D

Adapted from McKenzie *et al*.[277] IBS, irritable bowel syndrome; C-IBS, constipation predominant irritable bowel syndrome; D-IBS, diarrhoea predominant irritable bowel syndrome; IBS-M, irritable bowel syndrome with mixed bowel pattern.

3.2.1. Milk and dairy products

Many individuals with IBS have tried milk or dairy avoidance and often have low calcium intakes.[279] To avoid unnecessary exclusion and potential deficiencies, it is important to review the evidence for removing milk and dairy products, which includes lactose avoidance, and its effectiveness in improving IBS symptoms. Lactose is a disaccharide uniquely found in mammalian milk that is hydrolysed by the enzyme lactase. A genetically programmed decline in lactase activity after weaning resulting in lactase non persistence occurs in 70% of individuals, depending on ethnicity.[280] Lactose malabsorption is defined as incomplete hydrolysis of lactase resulting in unabsorbed lactose in the colonic lumen,[281] and leads to GI symptoms similar to those of IBS.[282] Because IBS and lactose intolerance have similar symptom profiles, a lactose hydrogen breath test can be useful to distinguish between the two and may assist with dietary management. However, hydrogen breath test facilities are not always available and the results may be inconclusive. In such circumstances, exclusion and challenge with lactose containing foods can be useful for assessing tolerance. Five non-randomized controlled trials assessed either a low lactose diet compared to no dietary restriction or no dietary intervention and were used to draw up the evidence based statements and recommendations[283-287] given in **Table 5**.

3.2.2. Non-starch polysaccharides (NSP)

NSP (dietary fiber) are composed of 'non-α-glucan polysaccharides that are mainly found in plant cell walls. This includes cellulose, hemicellulose, pectin, arabinoxylans, plant gums, ß-glucans.[288] Soluble fiber (e.g. pectin, ß-glucan from oats and barley, and gums in psyllium) generally undergoes significant fermentation, whereas insoluble fiber (e.g. celluloses, some hemicellulose and lignin) tends to undergo slow and incomplete fermentation and has a greater

effect on bowel habit by increasing faecal weight.[288] Alterations in NSP intake have traditionally been the mainstay of dietary management of IBS. There is conflicting evidence for increasing or decreasing intakes. The ten RCTs used to draw up the guidelines presented in **Table 5**,[289-298] assessed the research specifically relating to NSP that is provided within the diet, including food supplementation (i.e. using any wheat bran, ground linseeds and psyllium husk) and does not assess medicines or herbal preparations. Nor did any intervention use oats, other bran types or whole linseeds.[277]

3.2.3. Fermentable carbohydrates

Fermentable carbohydrates are poorly absorbed, osmotically active and undergo bacterial fermentation in the human GI tract, leading to loose stools and gas production.[299-300] They include fructo-oligosscarides (e.g. fructans in wheat and onion), galacto-oligosaccharides (GOS) (e.g. in beans and pulses), disaccharides [e.g. lactose in milk and dairy products, monosaccharides (in particular fructose in excess of glucose, e.g. mango, honey or a high fructose load, e.g. fruit juice, fructose ingredients in processed foods and drinks)], and polyols (e.g. sorbitol in various fruit and vegetables, polyol sweetened sugar-free manufactured foods and medicines) – acronym FODMAPS and resistant starches (e.g. in green banana, cold or reheated potato). Four RCTs met the inclusion criteria and were used to compile the evidence statements developed in **Table 5**. The studies assessed the intake of fructose, fructans, namely FOS, and sorbitol in relation to symptom provocation or *trans*-GOS in relation to symptom reduction. No studies assessed resistant starch.[255,301-303]

The hypothesis that reducing intake of FODMAPs can improve GI symptoms stems from clinical observation that a proportion of patients with IBS tolerate intake of certain short –chain carbohydrates poorly.[304] Moreover, these carbohydrates can be incompletely absorbed in the small intestine due to hydrolyzation (e.g. lactose maldigestion and non-digestible oligosaccharides), dependence on simultaneous intake of glucose for adequate absorption (fructose) or passive diffusion (certain monosaccharides and polyols).[305] Therefore, the absorption of short-chain carbohydrates in the small intestine varies depending on different factors such as presence/absence of enzymes to digest disaccharides (e.g. lactase), small intestinal transit time, dose of the carbohydrate, meal composition, and also on the presence of mucosal disease.[305]

The scientific evidence supporting a clinically relevant positive effect of reducing FODMAPS in IBS has so far been relatively limited, but gradually accumulating over the last few years and has, besides, observational reports, mainly been based on a randomized single-blinded FODMAP challenge study,[255] a nonrandomized, comparative study,[306] and a randomized, controlled trial comparing a low FODMAP diet with the habitual diet in IBS.[306] Moreover, a recent study also demonstrated that a general reduction of FODMAPs in the diet was effective in patients with suspected nonceliac gluten sensitivity, and no gluten-specific effect beyond that of the effect of the general FODMAP reduction could be demonstrated.[307] Because few clinical trials in this area exist, a very recent study by Halmos *et al*.[308] is very important because it provides high-quality evidence supporting the effectiveness of a low-FODMAP diet in IBS. By using a randomized, cross-over design (n = 30 IBS and n = 8 healthy controls), the authors were able to demonstrate a convincing reduction of the reported severity of all the key symptoms of IBS – abdominal pain,

bloating and bowel habit dissatisfaction – when the patients were on a low-FODMAP diet (22.8; 95% confidence interval (CI), 16.7-28.8mm) compared to when they received a standard Australian diet (44.9; 95% CI, 36.6-53.1mm; p <0.001). It is important to bear in mind that so far, no study has demonstrated that a low FODMAP diet is superior to the dietetic practise that has been used for patients with IBS before a low-FODMAP diet was suggested as a treatment alternative for IBS, that is, to encourage a regular meal pattern and a "healthy eating," to avoid large meals, reduce the intake of fat, discourage excessive fiber intake (especially soluble fiber), reduce caffeine, and avoid gas-producing foods, such as beans, cabbage and onions.[309]

3.2.4. Elimination and empirical diets

Empirical and elimination diets have traditionally been used to identify food intolerances in individuals with IBS. There is no standard diet describing which foods or ingredients should be excluded. An exclusion diet excludes one or two foods suspected to be responsible for symptoms. An elimination or few foods diet includes a selection of low allergen foods, usually one type of meat, one cereal, two fruit and vegetables, a milk substitute and a fat source. An empirical diet excludes common food allergens associated with a specific condition when a dietary source is suspected but cannot be identified.[310] Six studies met the inclusion criteria and were evaluated to draw up the evidence statements in **Table 5**. Two were RCTs comparing an elimination diet to sodium chromoglicate and the remaining four were intervention studies using an empirical or elimination diet followed by food challenge.[311-316]

4. The GI microbiota and diet

Diet is a factor that undoubtedly influences the composition of the GI microbiota. Diet provides nutrients for both the host and bacteria in the GI tract. Most of the enzymes needed to break down the structural polysaccharides in plant material are not encoded by mammalian genomes.[317] However, the GI microbiota produces a large collection of degradable enzymes that exhibit a broad range of metabolic activities.[318]

It is estimated that 20 – 60g of dietary carbohydrate reaches the colon on a daily basis, including resistant starch, NSP, plant cell wall polysaccharides and non-digestible oligosaccharides.[318] Some dietary proteins (e.g. collagen and elastin) as well as secondary plant metabolites (e.g. polyphenolic substances) can also reach the large intestine and undergo bacterial transformation.[319,320] Changes to the composition of the GI microbiota in response to dietary intake occur because different bacterial species are better equipped (genetically) to utilize different substrates.[321] Generally, bacteria favour carbohydrates as primary energy sources if they are available.[322]

Early studies comparing dietary patterns (e.g. Japanese vs. Western) or examining the impact of changing the proportions of food categories on the GI microbiota have found only moderate effects on the GI microbiota involving a few genera.[323-325] These studies relied on culture-based techniques and were, therefore, limited in their ability to detect changes in the finer detail of the composition of the GI microbiota. More recent studies have employed culture-independent

approaches and have further elucidated the role of diet in the determination of the composition of the GI microbiota in humans **(Table 6)**.

Table 6. Associations of the human intestinal microbiota with habitual dietary patterns or interventions

Authors	Methods	Study design	Subjects	Diets/nutrients	Microbial response
Claesson *et al.*[326]	16s rDNA sequencing	Cross-sectional	178 elderly subjects (age 64-102 years) – community, day hospital, rehabilitation and long-stay subjects	"community" diet – diverse with low-moderate fat/high fiber	↑ Diversity, ↑Firmicutes, ↑ Coprococcus, Roseburia
				"long-stay" diet –reduced diversity with moderate – high fat/low-moderate fiber	↓Diversity, ↑Bactericides, ↑Parabacteroides, Eubacterium, Anaerotruncus, Lactonifactor and Coprobacillus
De Filippo *et al.*[327]	16s rDNA sequencing and biochemical analysis	Cross-sectional	Twenty-nine children (1-6 years) – African children from Burkina Faso (n = 14) and European children from Florence, Italy (n = 15)	"Western" diet – high fat/ protein/sugar and low fiber	↑ Firmicutes, ↑Enterobacteriaceae
				"Rural diet – low fat/protein and high fiber	↑Bacteroidetes, Exclusively present: Prevotella, Xylanibacter, Butyrivibrio and Treponema, ↑SCFA
De Palma *et al.*[328]	FISH and qPCR	Feeding (1 month)	Ten healthy subjects (mean age 30.3 years)	Gluten-free diet (reduced polysaccharide)	↓ Bifidobacterium, Lactobacillus, Clostridium lituseburense and Faecalibacterium prausnitzii
Kabeerdoss *et al.*[329]	qPCR	Cross-sectional	Fifty-six healthy female subjects (age 18-27 years): n = 32 vegetarians and n = 24 omnivores	Vegetarian diet	↑ Enterobacteriaceae and Echerichia coli ↓Clostridium cluster XIVa, ↓Roseburia – Eubacterium rectale, ↓butyryl-CoA CoA-transferase gene
Liszt *et al.*[330]	qPCR and PCR-DGGE	Cross-sectional	Twenty-nine healthy subjects (age 19-34 years) – n = 15 vegetarians and n = 14 omnivores	Vegetarian diet	↑Bacterial DNA tendency for ↓ Clostridium cluster IV and ↑ Bacteroides (but not significant)
Muegge *et al.*[331]	16s RNA sequencing and shotgun metagenomics	Cross-sectional	Eighteen lean subjects (mean age 59.6 years) – members of a Calorie Restriction Society	Proteins	Associated with KEGG orthology groups
				Insoluble fiber	Associated with bacterial OTU content
Walker *et al.*[332]	16s rDNA sequencing and qPCR	Randomized cross-over (3-week intervention)	Fourteen overweight male subjects (age 27-73 years)	Diet high in resistant starch (type III)	↔Phylum level, ↑Ruminococcus bromii and E. rectale, ↑ Ruminococcaceae, ↑Oscillibacter valericigenes, ↑ Firmicutes bacteria related to Roseburia and E. rectale, ↔ Phylum level
				Reduced carbohydrate diet (weight-loss diet)	↓Collinsella aerofaciens, ↑O. valericigenes, ↓Firmicutes bacteria related to Roseburia and E. rectale

Table 6. continued

Authors	Methods	Study design	Subjects	Diets/nutrients	Microbial response
Wu *et al.*[333]	16s rDNA sequencing and shotgun metagenomics	Cross-sectional	Ninety-eight healthy subjects (age 18-40 years)	Fat	↑Bacteroidetes, Actinobacteria, ↓ Firmicutes, Proteobacteria
				Fiber	↓ Bacteroidetes, Actinobacteria, ↑ Firmicutes, Proteobacteria
				Animal fat and protein	Positively associated with Bacteroides enterotype
				Carbohydrates	Positively associated with Prevotelia enterotype
Wu *et al.*[333]	16s rDNA sequencing and shotgun metagenomics	Controlled feeding (10 day intervention)	Ten subjects having Bacteroides enterotype (high fat/protein)	Low-fat/high fiber diet or high-fat/low fiber diet	Changes in the composition of microbiome detectable within 24 hours of consuming diet; no stable switch in enterotype after 10 days
Zimmer *et al.*[334]	Culture-based methods	Cross-sectional	295 healthy subjects – 144 vegetarians, 105 vegans, 46 controls	Vegetarian diet	↓ stool pH
				Vegan diet	↓stool pH, ↓Bacteroides spp., Bifidobacterium spp., E.coli and Enterobacteriaceae spp.

Adapted from Power *et al.*[317] ↑ - increased, ↓- decreased, ↔ - no change, FISH – fluorescent in situ hybridization, qPCR – quantitative real time PCR, DGGE – denaturing gradient gel electrophoresis, KEGG – Kyoto Encyclopedia of Genes and Genomes, OTU – operational taxonomic unit.

Based on the available data, differences in the GI microbiota are demonstrable between groups of people living on different diets. These diet-associated changes in composition can lead to changes in the metabolic activity of the GI microbiota, which, in turn, may provoke changes in inflammatory and immune responses. Although attempts to change the composition of the GI microbiota by varying the diet have been successful in mice,[335,336] there is a relative paucity of human dietary intervention studies. A complex tripartite relationship exists between diet, microbes and the gut epithelium. Dietary patterns have a strong influence on the composition of the GI microbiota, as demonstrated by data in **Table 6**. Not surprisingly, diet and the microbiota are two factors in the pathogenesis of IBS. Although diet is a tempting intervention for GI dysbiosis, the understanding of how to manipulate diet to promote healthy microbiota is still in its early days. Bacteriotherapy provides a novel approach for restoring healthy homeostasis through GI microbiota. This can be achieved through the use of various interventions, including the removal of pathogenic bacteria with antibiotics, supplementation with probiotics and most recently, introduction of a new healthy microbial ecosystem by transplanting faecal bacteria from a healthy donor.

5. Rationale for the study

There is no single curative treatment for IBS. This leaves these patients very dependent on the support of their health care team. Often health care professionals are expected to advise or treat patients with anecdotal treatments that might be detrimental to their health. The findings of this thesis are relevant to health care workers, notably doctors, nurses and dietitians, and are important because patients as well as the general public are becoming increasingly aware of the

effects of diet and probiotics in IBS. The latter as a result of considerable media interest and intensive advertising campaigns. Consequently there is a need for health care professionals to provide advice on whether probiotics and dietary manipulation might be helpful for patients with IBS and, if so, what are these recommendations. Continual probiotic research necessitates that healthcare professionals keep a breast and informed of what is happening in this dynamic field, review articles provide a summary of the current understanding on this topic. This gap in knowledge is further illustrated in **Figure 2**, a conceptual framework.

To date, most RCTs on the utility of probiotics in IBS have not used appropriate study design and fall short on methodology. Areas of poor design include Rome criteria not used to define IBS, no randomisation, parallel study design, double blinding, placebo run-in, baseline observation period before trial initiation, short treatment duration (< 8 weeks), follow-up, validated scale to measure outcomes, incomplete follow–up (intention-to-treat), treatment compliance not measured and sample size inadequate.[236,337] The low quality design of the trials inevitably leads to the likes of concluding statements such as "further studies are needed to determine whether the probiotic under study may offer clinical benefits for IBS." Furthermore, effective treatment of IBS is often masked by its various groupings (C-IBS, D-IBS or PI-IBS) and their response to a particular treatment. Much of the published data do not differentiate between the groupings or subgroups, making interpretation of reported results difficult. Effective treatment outcomes are further compounded by variations in indigenous microbiota, as observed in stool microbiota, and possible varying aetiology among patients. Specific probiotic strains may work better in patients with either C-IBS or D-IBS. Most clinical trials base probiotic intervention response on a subjective assessment of symptom change. In this study

stool microbiota will additionally be used for a more objective assessment. These gaps in knowledge are further illustrated in **Figure 2** and sub-aims to conduct a well- designed RCT of a known probiotic as part of an intervention and to determine whether the probiotic can favourable influence GI microbiota and patient symptom outcomes addresses these gaps.

To our knowledge there is no published literature on the nutrient intakes of South African IBS patients, this is important information to know when up to two thirds of IBS patients exclude food items from their diet to improve symptoms.[254] Because of this, these patients may be a nutritionally at-risk group. Furthermore, the validity and reliability of any dietary data from IBS patients is unknown. These gaps in knowledge are further illustrated in **Figure 2**, a conceptual framework.

There is enough evidence to believe that dietary changes alter or modify the substrate for GI microbiota which influences normal gut symbiosis between GI microbiota and the intestinal mucosa (epithelial) layer as well as aiding in competitive relationships between GI bacteria. Although there is evidence that diets high in fiber provide substrate for certain microbiota, there are no clinical studies reflecting which microbiota are associated with certain nutrients in IBS patients. Part of this study will examine the relationship between nutrient intake (exposures), GI microbiota, and IBS (outcomes), and addresses this gap in knowledge as illustrated in **Figure 2**.

Specific gaps in knowledge relating to this research project are provided in **Figure 2** as part of a conceptual framework.

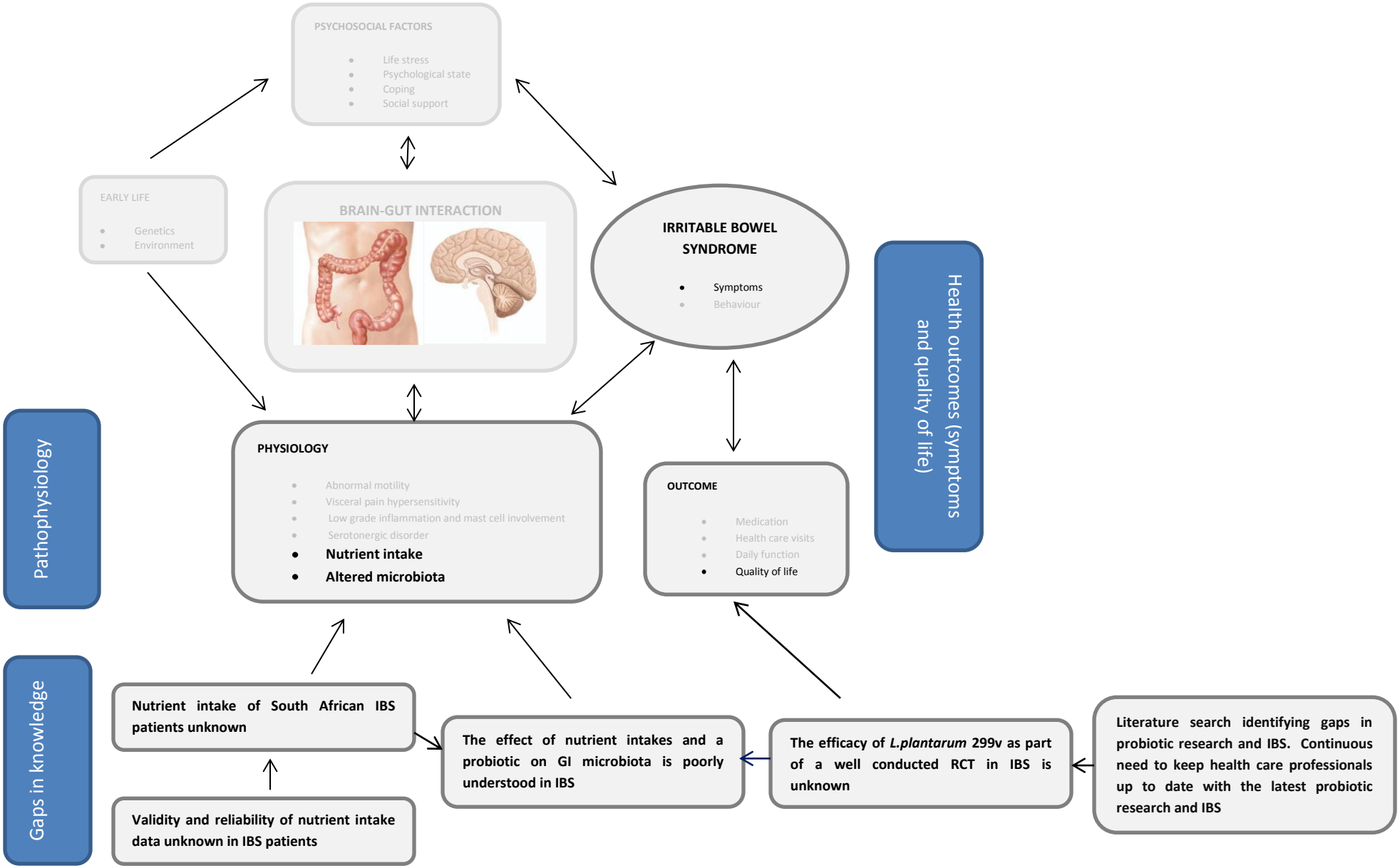

Figure 2. Conceptual framework depicting the possible influences of nutrient intake, the GI microbiota and probiotics on the pathogenesis of irritable bowel syndrome and health outcomes

5.1.Aim of the study

The overarching aim of this study was to investigate the nutrient intakes, GI microbiota and the impact of a probiotic, *L.plantarum* 299v in IBS patients.

5.2. Sub-aims The gaps in knowledge as identified in **Figure 2** will be addressed by the following sub-aims:

- *Literature review:* To update healthcare professionals on current probiotic information and provide an overview of probiotic treatment approaches, with special emphasis on IBS (Article I, Chapter 2).
- *Probiotic supplementation:* To conduct a well-designed randomised, double blind, placebo-controlled clinical trial with *L. plantarum* 299v as part of an intervention and establish whether a course of probiotics may alleviate undesirable symptoms of IBS and improve quality of life (Article 2, Chapter 3).
- *Nutrient intake:* To assess nutrient intake in patients with IBS compared to dietary recommendations. This is with the hypotheses that in a condition in which subjects insist that diet or trigger foods play a part in symptom generation, may lead to risk of nutrient inadequacy (Article 3, Chapter 4).
- *Validation and reproducibility of dietary data:* To validate and assess the reproducibility of food records in IBS patients (Article 4, Chapter 5).
- *Characterisation of probiotic action and the influence of nutrient intake on GI microbiota:* Identify possible nutrient risk components for establishing GI microbiota involved in IBS and as part of an intervention determine whether a course of probiotics may alter stool microbiota (Article 5, Chapter 6).

By answering the stated research aims, this study endeavours to make a contribution to knowledge in the area of the IBS, particularly in South Africa.

5.3. Study design

This study consisted of two phases. The first phase a descriptive, cross sectional, observational study was used for the collection of the dietary data (Article 3, Chapter 4). During the second phase, a RCT with an eight week course of probiotic was conducted (Article 2, Chapter 3). Data gathered during the RCT was used for Articles 4 and 5, Chapters 5 and 6 respectively.

5.4. Study population and participants

A multi-disciplinary team including a gastroenterologist, nursing sister, microbiologist and dietitian were involved in this study. The study was conducted at a private gastroenterology clinic, in Port Elizabeth, South Africa. The study population was men and women recruited at the gastroenterology clinic. They were screened by the gastroenterologist and recruited according to the study inclusion criteria and their willingness to participate. The subtypes C-IBS and D-IBS were included in the study. Eighty-one participants were recruited and formed a part of the RCT [information gathered and used in Articles 2, 4 and 5, (Chapters 3, 4 and 6 respectively)]. One hundred and twenty-two participants were recruited and their dietary data used for Article 3, Chapter 4.

5.5.The following null hypotheses were stated

- An intervention of the probiotic, *L.plantarum* 299v at a dose of two daily 5 X 10^9 colony forming units (cfu), will not alleviate i) the undesirable symptoms of IBS nor ii) improve quality of life (Article 2, Chapter 3) in C-IBS and D-IBS subjects.
- The nutrient intakes of IBS (C-IBS and D-IBS) subjects place them at risk of inadequacy compared to Dietary Reference Intake (DRI) recommendations (Article 3, Chapter 4).
- Three day estimated food records demonstrate poor i) reproducibility and ii) validity in IBS (C-IBS and D-IBS) subjects (Article 4, Chapter 5).
- Nutrient intakes do not have an influence on the faecal microbiota (i.e. *Bifidobacterium bifidum, Lactobacillus plantarum* and *Bacteroides* spp.) of IBS (C-IBS and D-IBS) subjects (Article 5, Chapter 6).
- *L.plantarum* 299v does not have an influence on the faecal microbiota (i.e. *Bifidobacterium bifidum, Lactobacillus plantarum* and *Bacteroides* spp.) (Article 5, Chapter 6).

5.6. Chapter overview, contribution of candidate and scope of work

- Chapter 2

  This involved a thorough literature research and the compilation of a review article on probiotics, with special emphasis on their role in the management of irritable bowel syndrome.

  Article: **Review: Probiotics, with special emphasis on their role in the management of irritable bowel syndrome.**

  Cheryl Stevenson and Renée Blaauw

  South African Journal of Clinical Nutrition 2011; 24 (2): 63-73.

Candidate contribution:

The first author performed the literature search and was responsible for writing the manuscript.

- Chapter 3

This involved a RCT with 81 participants. Participants were randomised to receive either probiotic or placebo for eight weeks.

Article: **Randomized clinical trial: Effect of *Lactobacillus plantarum 299v* on symptoms of irritable bowel syndrome**

Cheryl Stevenson, Renée Blaauw, Ernst Fredericks, Janicke Visser and Saartjie Roux

Nutrition 2014; 30: 1151-1157.

Candidate contribution:

The first author took part in the study design, performed the data collection, data capturing, data analysis, liaised with statistician, evaluated the results and was responsible for writing the manuscript.

- Chapter 4

This study looked at the nutrient intakes of patients with irritable bowel syndrome and compared to healthy controls and international recommendations. A total of 122 subjects participated.

Article: **Food avoidance in irritable bowel syndrome leads to a nutrition deficient diet.**

Cheryl Stevenson, Renée Blaauw, Ernst Fredericks, Janicke Visser and Saartjie Roux

South African Journal of Clinical Nutrition 2014; 27 (1):25-30.

Candidate contribution:

The first author took part in the study design, performed the data collection, data capturing, data analysis, liaised with statistician, evaluated the results and was responsible for writing the manuscript.

- Chapter 5

  Eleven irritable bowel syndrome participants were involved in the test-retest reliability and validity testing of three day estimated food records.

  Article: **Validation and test-retest reliability of estimated food records in irritable bowel syndrome patients.**

  Cheryl Stevenson, Renée Blaauw, Diana Kimono, Janicke Visser and Saartjie Roux

  Manuscript.

  Candidate contribution:

  The first author took part in the study design, performed the dietary data collection, data capturing, dietary data analysis, liaised with statistician, evaluated the results and was responsible for writing the manuscript.

- Chapter 6

  Faecal microbiota analysis of stool samples was performed on a subset of irritable bowel syndrome patients enrolled in the RCT. The effects of nutrient intake and the probiotic on GI microbiota were analysed.

Article: **The effect of nutrient intakes and a probiotic on gastrointestinal microbiota in irritable bowel syndrome**

Cheryl Stevenson, Renée Blaauw, Ernst Fredericks, Janicke Visser and Saartjie Roux

Manuscript

Candidate contribution:

The first author took part in the study design, performed the data collection, dietary data capturing, dietary data analysis and calculations, actively participated in the laboratory microbial analysis, liaised with statistician, evaluated the results and was responsible for writing the manuscript.

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Chapter 2

Review: Probiotics, with special emphasis on their role in the management of irritable bowel syndrome

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Probiotics, with special emphasis on their role in the management of irritable bowel syndrome

Stevenson C, Blaauw R
Division of Human Nutrition, Stellenbosch University and Tygerberg Academic Complex, South Africa.
Correspondence to: Cheryl Stevenson, e-mail: cheryl@retaildc.co.za
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Abstract

Probiotics are live microorganisms, and when administered in adequate amounts, bestow beneficial effects on the host. The therapeutic and preventative application of probiotics in several disorders is receiving increasing attention, and this is especially true when gastrointestinal microbiota is thought to be involved in their pathogenesis, as in irritable bowel syndrome (IBS). Given the increasingly widespread use of probiotics, a thorough understanding of their risks and benefits is important. The purpose of this review is to update healthcare professionals on current probiotic information, and provide an overview of probiotic treatment approaches, with special emphasis on IBS.

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Introduction

The scientific literature on probiotics commences with the Russian Nobel laureate, Elie Metchnikoff, who suggested that ingestion of lactic acid-producing bacteria, such as that found in yoghurt, was a protective factor, enhancing longevity and potentially beneficial in treating digestive diseases.[1] Probiotic research is a relatively young, but rapidly expanding field. By mid-2010, there were 7 180 publications in the PubMed database, of which 26% (1 863) were reviews. By comparison, the search term "antibiotics" yielded 509 728 publications, of which 8.5% (43 515) were reviews. Therefore, it can be concluded that the field of probiotics does not suffer from too few reviews, but rather from insufficient original research.[2]

The term "probiotic" is derived from the Latin "pro" (meaning for) and the Greek "bios" (meaning life). The World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations have defined probiotics as "live microorganisms, that when administered in adequate amounts, have beneficial effects for the host."[3] Some definitions have also changed the word "administered" to "consumed."[4] Although this is the acknowledged scientific definition, there is no legal definition for the term "probiotic". Unfortunately, the definition is often used by industry, even when the minimum scientific criteria for probiotics are not met.[5]

Colonisation and diversity of gut microbiota

The gut microbiota comprises a complex ecological system, consisting of at least 500 different bacterial species, yeasts, protozoa, viruses and fungi, and this microbiota plays an integral part in the digestive and metabolic processes that are essential for general well-being.[6] The bacterial microbiota is the most reported and researched.[7] Inherent colonisation of microbiota occurs at birth with organisms that inhabit the skin, oral cavity, vagina and gastrointestinal tract.[8] This colonisation is influenced by the route of delivery (vaginal vs. Caesarean section), gestational age (prematurity vs. full term), and use of antibiotics in the perinatal period, especially in the neonatal intensive care unit setting. For example, vaginal births are associated with a greater intestinal colonisation by bifidobacteria, but not lactobacilli, compared to Caesarean section deliveries.[9] In comparison, Caesarean section births are associated with increased colonisation by *Klebsiella, Enterobacter* and *Clostridium*. These organisms are common in hospital settings.[10] Early feeding practices also influence microbial colonisation. Breastfed infants have less intestinal permeability, compared to formula-fed infants.[11] Formula feeding is associated with an increased presence of both *Clostridium* and *Bacteroides* in the intestinal tract.[10] Poor microbial variety in infancy seems to be related to a greater risk of atopic disease later in childhood.[12] The initial acquisition of intestinal microbiota plays a key role in the development of immune processes and protection against pathogens. A reduction in the variety of the gut microbiome often occurs in a number of conditions that are potentially related to dysbiosis, including inflammatory bowel disease and chronic diarrhoea.[13]

Dysbiosis

Disturbances in the sensitive balance between the host and the intestinal microbiota (dysbiosis) can lead to changes in the mucosal immune system that range from obvious inflammation, as seen in Crohn's disease, to low-grade inflammation, evidenced in a subset

of irritable bowel syndrome (IBS) patients.[14] Research verifies the significance of the colonising microbiota in determining the equilibrium of proinflammatory to regulatory cells in the gut.[15,16] Variations in the intestinal microbiota balance have been associated with obesity,[17,18] Crohn's disease, ulcerative colitis and coeliac disease.[19-21] These conditions have been linked to less species variation and abnormal immune responses to intestinal bacteria.

The faecal microbiota of IBS patients differs significantly from that of healthy subjects.[22] Balsari studied stool samples of 20 IBS patients and noted a decrease in coliforms, lactobacilli and bifidobacteria, compared to that in healthy individuals.[23] Similar results have been found in other studies.[24,25] A further study that divided IBS patients according to subtype, showed that diarrhoea-predominant IBS (D-IBS) patients had lower numbers of lactobacilli, while constipation-predominant IBS (C-IBS) patients had increased numbers of *Veillonella* spp.[26] Despite the fact that dysbiosis has progressively become better documented in various intestinal diseases,[27,28] it remains to be seen whether this is, in fact, a cause-and-effect relationship.

Probiotic organisms

In the stomach, small numbers of probiotic organisms [0-10^3 colony-forming units (CFU) per gram] are found, consisting mainly of lactobacilli, streptococci, staphylococci, enterobacteriacae and yeasts. These small numbers are primarily because of the low intragastric pH. Subsequently, there is an increase from 0-10^5 CFU per g in the duodenum, to 10^8 CFU per gram in the ileum, and 10^{10}-10^{12} CFU per gram in the colon because of the neutral intestinal pH, a slower transit time and the availability of nutrients. In the colon, > 99% of the microorganisms are strictly anaerobic, such as bifidobacteria, *Bacteroides* spp., *Clostridium* spp., *Eubacterium* spp., *Fusobacterium* spp. and peptostreptococci.[6,29] As part of the normal microflora, *Lactobacillus* and *Bifidobacterium* genera vary from 10^6-10^{10} in different individuals. For probiotic bacteria to be effective, they need to transit through the gastrointestinal tract that contains gastric juices, bile and pancreatic juice. Adhesion to the intestinal mucosa is considered to be a prerequisite for interaction with the immune system.[30]

Nomenclature of probiotics

Probiotics need to be classified according to their genus (e.g. *Lactobacillus*), species (e.g. *rhamnosus*) and strain (e.g. GG). This level of specificity in describing a probiotic is important, as effects can be and are, strain-specific. For example, *L. plantarum* 299v may have a different mechanism of action and effect to *L. plantarum* MF1298. It is important to look for probiotics that are supported by strain-specific research.[4,5]

Clinical application of probiotics

Probiotic usage is likely to attract the interest of two groups of people: healthy people who are interested in probiotics to keep them that way, and people with specific health concerns, about which evidence of probiotic efficacy is available. The second group is motivated and has a measurable outcome: symptom improvement.[5] Several functions of the gut microbiota may be influenced by probiotics beneficially. Probiotics have been studied in a number of diseases, especially when intestinal bacteria are thought to be involved in their pathogenesis.[2] The main study areas and application of probiotics are a direct result of their mechanisms of action. The clinical benefits of probiotic usage include those related to improvement of the gut epithelial or mucosal layer, strengthening of the immune response, and prevention of diseases later in life, e.g. eczema, atopic eczema, allergic rhinitis and cancer. The consensus recommendations for the correct clinical use of probiotics in various scenarios, as well as examples of probiotic strains and their associated published benefits, are tabulated in Table I.

Table I: Examples of probiotic strains and their associated published benefits[5,31]

Indication	Genus, species, strain
Infant diarrhoea	*Lactobacillus rhamnosus* GG *L. casei* DN-114001 *L. reuteri*
Inflammatory bowel conditions (primary evidence in pouchitis)	Multistrain probiotic containing three *Bifidobacterium* strains, four *lactobacillus* strains, and *Streptococcus thermophilus* (VSL#3) *Escherichia coli* Nissle
Antibiotic-associated diarrhoea, (*Clostridium difficile)*	*Saccharomyces boulardii* *L. rhamnosus* GG *L. casei* DN-114011 *L. acidophilus* CL1285 plus *L. casei* *L. bulgaricus*
Gut transit time	*Bifidobacterium animalis* DN-173 010
Keeping healthy	*L. reuteri* ATCC 55730 *L. casei* DN-114001
Atopic dermatitis	*L. rhamnosus* GG *B. lactis*
Lactose intolerance	Most strains *L. bulgaricus* and/or *S. thermophilus*
Colic in infants	*L. reuteri* ATCC 55730
Immune support	*B. lactis* HN019 *B. lactis* Bb12 *L. casei* DN-114001 *L. rhamnosus* GG *L. plantarum* *L. acidophilus* *B. lactis* *L. johnsonii*
Vaginal applications	*L. rhamnosus* GR1 plus *L. reuteri* RC14 *L. acidophilus*
Irritable bowel syndrome	*L. plantarum* 299v *B. infantis* 35264

Mechanism of action

The microbiota performs many significant functions for the host. These include the production of vitamins, degradation of bile acids, conversion of (pro)carcinogenic substances and digestion of nutrients.[30] Anaerobic bacteria are of benefit to the host by performing metabolic functions, such as fermentation, providing

short-chain fatty acids (SCFAs), producing vitamins, adding to the trophic action of the epithelium, and aiding in the development of the immune system.[32] Saccharolytic fermentation of unabsorbed and indigestible carbohydrates by intestinal bacteria occurs mainly in the colon. This is essential, as SCFAs (i.e. acetate, propionate and butyrate) are produced.[33] Butyrate, a major energy source for intestinal epithelial cells, affects cell proliferation and differentiation, increases mucus secretion and decreases inflammation.[34] Proteolytic bacterial fermentation usually takes place in the more distal colon, where carbohydrates are no longer available, and results in the production of toxic compounds like ammonia, phenols, cresols and paracresols.[35]

The exact mechanism by which probiotics exert their favourable effect has not been fully elucidated. Different strains of organisms have very diverse and specialised metabolic activity. Proposed mechanisms include those responsible for the manipulation and regulation of the intestinal microbial balance, those that protect the mucosa against pathogenic invasion (adhesion and translocation), and those that modulate an appropriate immune response.[36,37]

In the gastrointestinal tract, probiotics can aid with the following:

- The secretion of antibacterial substances, e.g. bacteriocins and acids, which result in a reduction in the luminal pH, with decreased growth ability of the pathogens.
- The production of intestinal mucin, which influences bacterial colonisation, and human β-defensins (peptides with antibacterial properties), which affect mucosal adherence, and inhibit pathogenic bacteria adherence.
- The expression of receptors (toll-like receptors 2 and 4), that sense bacterial components and trigger an appropriate immune response through the release of protective cytokines (IL-6).
- An increased release of secretary IgA that can protect the microflora against bacterial attachment.
- The regulation of epithelial cell apoptosis.
- The acidification of the colon by nutrient fermentation.[36-39]

The immune response is modulated by controlling levels of circulating inflammatory cytokines (NF-κB and TGF-β), restoring the imbalance between Th1 and Th2 responses, and increasing the expression of heat-shock proteins which are essential for the maintenance of the epithelial barrier function. Through an appropriate pro- and anti-inflammatory response, the immune function is regulated suitably for each condition in a strain-specific manner.[36,37,39,40] Certain probiotic strains also exhibit anticarcinogenic effects by increasing faecal mutagen excretion, and inhibiting the conversion of precarcinogens to carcinogens by reducing the enzyme β-glucuronidase.[39] Indirectly, this anticarcinogenic effect is seen by an increased immune response (as discussed previously).

Dose

Dose levels of probiotics should be based on levels that are found to be efficacious in human studies.[41] The necessary amount and duration of use depends on the specific strain and the health condition being studied. Studies demonstrating beneficial results at levels < 100 million (10^8) CFU/day are uncommon in published literature. For example, the efficacy of *Bifidobacterium infantis* 35264 has been documented at 10^8 CFU/day,[42] whereas the recommended dose of VSL#3 is 1.8 x 10^{12}CFU/day (a four-log-cycle difference).[43] This disparity underlines the inaccuracy in making general dose recommendations.[5]

Single- vs. multiple-strain vs. multi-species products

The value of using a single-strain probiotic over a combination of probiotic strains or species is a topic of ongoing debate. Microorganisms may behave differently when administered in combinations, compared to in isolation. The use of combinations or cocktails concerns some investigators because attempts to classify the mechanism of action are then difficult to define.[13] Within a product containing eight to 20 strains or even more, it may be fair to say that a few dominant strains will exert a greater effect, or discount the effects of others. Uncertainty exists as to whether the correct strain will be effective at the right time, and in the correct location. Each strain within a probiotic cocktail has been selected for a specific characteristic, such as the induction of a certain immune parameter. However, the same strain may have another immune modulating parameter that is not desired in the concerned application. These disadvantages do not mean that cocktails are undesirable, the so-called multistrain (containing more strains of the same genera, e.g. several *Lactobacillus* spp.) and multi-species (containing strains of different genera, e.g. lactobacilli, bifidobacteria, streptococci) may be more advantageous over mono-strain probiotics, particularly in people who are interested in probiotics to keep them healthy, and not aid management of a specific health concern.

Probiotic preparations can be found in the form of powders, tablets, capsules, pastes, sprays or fermented foods, such as yoghurts, buttermilk, sour poi (a starchy paste made from the corm of taro plants) and miso (fermented soybean paste). The method of delivery, e.g. yoghurt vs. milk, may have an impact on the viability of the bacterial colonies. The probiotic product needs to have a good taste and smell, and an acceptable shelf-life.[30]

Storage

Environmental conditions, such as moisture, oxygen, acid and heat, affect susceptible probiotic strains in different ways. Micro-encapsulation or coating technologies (e.g. enteric coating) have been developed by manufacturers to ensure that a live probiotic, in the correct quantities, is delivered on ingestion. However, once a probiotic package is opened, these barriers are compromised. Generally microbes survive better at lower temperatures, but properly stabilised non-refrigerated products can retain potency at room temperature. Refrigerated products are also not necessarily of a better quality than non-refrigerated probiotics. Products need to be chosen from reputable companies that are labelled to reflect viability "through the end of shelf-life" and not "at time of manufacture". Some products contain dried probiotics, and if these bacteria have been dried and stabilised properly, they remain alive,

although dormant, and start to grow again after they reach the moist environment inside the body.[5] By definition, the term "probiotic" can never be used to describe products comprising dead bacteria primarily, even though in some cases, dead bacteria or bacterial cell products have been shown to have physiological effects.[44] For example, the administration of heat-killed *Enterococcus faecalis* to healthy dogs increased neutrophil phagocytes. These dead cells exert an anti-inflammatory response in the gastrointestinal tract. The variable amounts of dead cells found in probiotic products might contribute to the variation in response that is often seen with probiotic cultures.[44]

Adverse effects and safety issues

Although probiotics are generally considered to be safe, some research has revealed that probiotics may be inappropriate in specific populations. Probiotics have the potential to result in bacterial translocation across the gastrointestinal mucosa, and to transfer antibiotic resistance to other microorganisms. For these and other reasons, some adverse events have been linked to the use of probiotics in certain clinical settings.

Patients receiving nutritional support have been studied extensively with regard to the use of probiotics in various scenarios, e.g. antibiotic-associated diarrhoea and *Clostridium difficile*-associated diarrhoea. Conditions where gastric pH is increased through medications, or where the stomach is bypassed, i.e. jejunal feeding, result in the increased survival of probiotics in the small bowel. Patients with a central venous catheter (CVC) are also a known risk category.

In one study, the efficacy of a multi-species probiotic was tested in patients with severe pancreatitis in an ICU setting. A significantly increased risk of death was reported in the group receiving the probiotic. The patients who died had evidence of necrotising jejunitis. In this study, a multi-species probiotic of various strains, previously not tested, was administered nasojejunally.[45] This finding raised the possibility of an impaired splanchnic circulation that was further compromised by direct delivery of a high concentration of microorganisms into the proximal intestine. To date, this is the only study to associate probiotic use with increased risk of death in a clinical setting. However, Oláh et al did show that early nasojejunal feeding with a symbiotic preparation may prevent organ dysfunction in the late phase of severe acute pancreatitis, highlighting once again how much research still needs to be carried out in this area.[46]

A recent systematic review evaluated the safety of probiotic administration to patients receiving nutritional support (either enteral or parenteral nutrition).[47] Bacteraemia (n = 5), fungaemia (n = 27) and endocarditis (n = 2) were reported, and the causative strains were *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii*. The risk factors identified for these adverse events were patients receiving antibiotics with a CVC in situ, those at increased risk of bacterial translocation (i.e. with colitis or sepsis), and those with immune suppression [sepsis, human immunodeficiency virus (HIV) and necrotising enterocolitis]. It is argued that the two strains identified are the most commonly used in an ICU setting, and thus the reason for being cited. Similarly, the identified risk factors are general factors that are prevalent in most ICU patients, i.e. patients with a CVC and those receiving antibiotics. This systematic review evaluated studies published between 1996-2009, and only identified 32 cases of adverse events out of a total of 4 131 patients receiving probiotics.

As a result of the reported side-effects, the recommendations for the use of probiotics in various clinical settings are unclear. According to some, caution must be exercised when prescribing probiotics in newborns, immunocompromised patients, patients with pancreatitis, those with short-bowel syndrome, with a CVC in situ, and those with severe underlying illness.[4] Others propose classifying the risk factors responsible for probiotic sepsis into major and minor categories. A major risk factor includes immunocompromised patients. Minor risk factors include a CVC, jejunal administration of probiotics, impaired intestinal epithelial barrier function, cardiac vascular disease *(Lactobacillus* only), and administration of a broad-spectrum antibiotic to which a probiotic is resistant.[48] Finally, some advocate that it is not contraindicated to prescribe probiotics to patients receiving various forms of nutrition support, or those that are immunocompromised, provided that it is done under proper medical supervision and with good monitoring systems in place.[31,47]

Irritable bowel syndrome

The definition of IBS, according to the Rome III criteria, is that of a chronic disorder characterised by abdominal pain or discomfort associated with disordered defecation, either C-IBS, D-IBS, or mixed and alternating symptoms of constipation and diarrhoea.[49] The patient group is heterogeneous. It is estimated that IBS affects 3-25% of the general population.[50] The prevalence of IBS in South Africa is unknown. However, the progressive Westernisation of diets and lifestyles of less privileged populations is likely to be associated with an increased incidence of bowel disease and IBS. IBS patients can account for up to 30-50% of gastroenterology clinic visits.[50] Various factors have been linked to the pathophysiology of IBS. These include altered bowel motility, enhanced visceral sensitivity, neurotransmitter imbalances, low-grade inflammation of the gastrointestinal mucosa, altered microflora and increased proinflammatory cytokine secretion.[40,51-53] Elevated levels of cytokines IL-6, IL-6R, IL-1β and TNF-α,[54,55] and a lower IL-10/IL-12 ratio,[56] have been reported in IBS patients vs. controls.

There is no single curative treatment, and therapy is aimed at reducing the symptoms, often with very little success.[50] Pharmacological treatment comprises the use of bulking agents, antispasmodics, dopamine antagonists and antidepressants. The handful of therapeutic agents that were previously useful in the management of global IBS symptoms have either been removed, or limited, due to adverse side-effects.[57] Current treatment aims at strengthening or improving gastrointestinal epithelial function, and

improving the host's immune ability. This has led to numerous clinical trials investigating the therapeutic benefit of probiotics in IBS.

Clinical trials involving probiotics and irritable bowel syndrome

Many of the clinical trials on probiotics and IBS have important weaknesses in trial design, study execution and data analysis. These weaknesses include not using the intention-to-treat group for analysis, involving only a specific group (e.g. C-IBS), while others have included both C-IBS and D-IBS, not stipulating whether C-IBS or D-IBS patients are being used, and using a crossover design where the treatment may "wash over" into the non-treatment period. There is a wide variety in dosing regimens, species used, and clinical end-points in probiotic or IBS clinical trials. Guidelines have been developed for clinical trials involving functional gastrointestinal disorders (including IBS).[58] Recently, there have been two systematic reviews[40,59] and four meta-analyses, one with particular emphasis on *S. boulardii*.[50,60-62]

Twenty-eight double-blind, placebo-controlled, randomised trials were identified for the purposes of this review. Only those trials where the strain of the probiotic was clearly identified were used, regardless of type, dose and duration of treatment. The probiotics varied from one to multiple strains, and no symbiotic preparations were included.[63,64] In the clinical trials, there had to be clear primary end-points. Single-blinded studies,[65,66] those not using a control,[63] and non-randomised trials[67] were excluded. To date, there have been two clinical trials that have focused on children aged six to 20 years[68] and six to 16 years.[69] However, in this review, only those

Table II: Clinical trials with probiotic use in IBS

Author	n	Probiotic preparation	Treatment duration	Results
O'Sullivan and O'Morain[70] (2000)	24	*Lactobacillus casei* GG	20 weeks	No significant differences between the two groups.
Nobaek and Johansson[71] (2000)	60	*L. plantarum* 299v = DSM 9843	4 weeks	Reduction in flatulence and pain.
Niedzielin and Kordecki[72] (2001)	40	*L. plantarum* 299v	4 weeks	Pain resolution and improvement in [a]GSS.
Sen and Mullan[73] (2002)	12	*L. plantarum* 299v	4 weeks	No significant difference between the two groups.
Kim et al[74] (2003)	25	VSL#3 (*Bifidobacterium longum, B. infantis, B. breve, L. acidophilus, L. plantarum, L. casei, L. bulgaricus, Streptococcus salivarius* spp. *thermophilus*)	8 weeks	Improvement in abdominal bloating, but no significant difference between the two groups.
Kim et al[75] (2005)	48	VSL#3	4-8 weeks	Significant reduction in flatulence.
Saggioro[76] (2004)	70	*L. plantarum* LP01 and *B. breve* BR03 or *L. plantarum* LP01 and *L. acidophilus* LA02 or placebo	4 weeks	Significant decrease in GSS and abdominal pain with probiotic combinations, compared to placebo.
Kajander et al[77] (2005)	103	*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99, *Propionibacterium freudenreichii* spp. *shermanii* JS	6 months	Significant reduction in GSS (abdominal pain, distension, flatulence, and borborygmi).
Lyra et al[78] (2010)	42	*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99, *Propionibacterium freudenreichii* spp. *shermanii* JS	6 months	Significant decrease in stool *Bifidobacteria* spp. in probiotic group.
Kajander and Kroglus-Kurikka[79] (2008)	86	*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. animalis* spp. *lactis* Bb12, *P. freudenreichii* spp. *shermanii* JS	5 months	Significant reduction in GSS.
Niv et al[80] (2005)	54	*L. reuteri* ATCC 55730	6 months	No significant differences between groups. Trend towards improvement in constipation and flatulence in treatment group.
O'Mahony et al[56] (2005)	80	*L. salivarius* UCC4331 or *B. infantis* 35624	8 weeks	*B. infantis* reduced GSS, while *L. salivarius* reduced abdominal pain and discomfort, bloating and straining.
Whorwell et al[42] (2006)	362	*B. infantis* 35624 in three different doses	4 weeks	With 10^8 CFU, significant improvement in abdominal pain, bloating, bowel movement satisfaction, straining, passage of gas and evacuation.

Author	n	Probiotic preparation	Treatment duration	Results
Guyonnet et al[81] (2007)	274	*B. animalis* DN-173 010, *S. thermophilus*, *L. bulgaricus*	6 weeks	Significant improvement in quality of life, bloating and stool frequency in constipated participants.
Drouault-Holowacz et al[82] (2008)	116	*B. longum* LA101, *L. acid* LA102, *L. lactis* LA103, *S. thermophilus* LA104	4 weeks	No significant difference in GSS.
Agrawal et al[83] (2008)	41	*B. lactis* DN-173 010	4 weeks	Significant improvements in objectively measured abdominal girth, gastrointestinal transit time. Reduced symptoms.
Sinn et al[84] (2008)	40	*L. acidophilus* SDC 2012, 2013	4 weeks	Significant improvement in treatment group with abdominal pain, pain while straining to pass a stool, bowel habit satisfaction and sense of incomplete evacuation.
Enck et al[85] (2008)	297	*Escherichia coli* DSM 17252, *Enterococcus faecalis* DSM 16440	8 weeks	Significant reduction in GSS and pain.
Enck et al[86] (2009)	298	*E. coli* DSM 17252	8 weeks	Significant reduction in GSS and pain.
Dolin[87] (2009)	61	*Bacillus coagulans* GBI-30, 6086	8 weeks	Significant reduction in number of bowel movements in D-IBS participants.
Hun[88] (2009)	44	*Bacillus coagulans* GBI-30, 6086	8 weeks	Significant improvement from baseline abdominal pain and bloating to end-point scores.
Williams et al[89] (2009)	52	*L. acidophilus* CUL60, CUL21, *B. lactis* CUL34, *B. bifidum* CUL20	8 weeks	Significant improvement in GSS, quality of life, days with pain and bowel habit satisfaction.
Hong et al[90] (2009)	70	*B. bifidum* BGN4, *B. lactis* AD011, *L. acidophilus* AD031, *L. casei* IBS041	8 weeks	Significant reduction in abdominal pain.
Ligaarden et al[91] (2010)	16	*L. plantarum* MF 1298	2 x 3 weeks	Significantly higher symptomatic relief satisfaction while on placebo.
Simrén et al[92] (2010)	74	*L. paracasei* ssp. *paracasei* F19, *L. acidophilus* La5, *B. lactis* Bb12	8 weeks	Significant improvement in GSS in both groups.
Søndergaard et al[93] (2011)	64	*L. paracasei* ssp. *paracasei* F19, *L. acidophilus* La5, *B. lactis* Bb12	8 weeks	No significant improvement in abdominal pain.
Guglielmetti et al[94] (2011)	122	*B. bifidum* MIMBb75	4 weeks	Significant improvement in GSS and quality of life in probiotic group.
Choi et al[95] (2011)	67	*S. boulardii*	4 weeks	Significant improvement in quality of life, but not symptoms.

a = global symptom score

studies involving adults are presented. This review covers the important clinical studies over the past ten years. An overview of studies carried out on the use of probiotics in IBS is given in Table II, in which the main benefits (if found) are given in the results column.

In the following discussion, the various strains are not discussed individually. Rather, focus is placed on the strains that have provided positive results when treating IBS.

L. plantarum 299v

There are three small studies in which a liquid form of *L. plantarum* 299v was used in the treatment of IBS. Two studies showed some benefit over placebo. One showed improved flatulence,[71] and the other a reduction in abdominal pain.[72] The third trial showed no significant benefit, but it was underpowered.[73] There were differences in enrolled populations, study designs, outcomes and statistical analyses in these three trials. Tolerability and adverse events were poorly recorded. These smaller trials, although showing promising results, have never been followed up with larger, multi-centre clinical studies.

L. reuteri ATCC 55730

A single trial of 54 IBS participants using *L. reuteri* ATCC 55730 over a six-month period, showed an improved global symptom score (GSS) from baseline until the end of the trial in both groups. There was a large placebo effect, and therefore failure in showing benefit over the controls.[80] The study group was small, and was further reduced due to non-compliance during the trial. Compliance and adverse events were well reported.

L. salivarius UCC4331

A single study, carried out by O'Mahony et al, used *L. salivarius* UCC4331 as part of a three-arm study evaluating the efficacy of *L. salivarius* UCC4331 and *B. infantis* 35624 compared to placebo for the treatment of IBS.[56] After a four-week run-in period, 50 participants received either *L. salivarius* or placebo for eight

weeks, followed by a four week washout period. At the end of the trial, a significant reduction in abdominal pain and discomfort was seen at weeks two and seven with *L. salivarius* UCC4331, but this effect was not sustained. This was a well-designed study, and limited only by a lack of statistical power.

***B. infantis* 35624**

In the previously mentioned trial of O'Mahony et al,[56] 77 patients with IBS were randomly assigned *B. infantis, L. salivarius* or placebo. The *B. infantis* (not *L. salivarius*) was shown to reduce pain, bloating and bowel satisfaction scores. The benefit of *B. infantis* has been replicated in a large multicentred research trial in 362 female patients with IBS. Participants were randomised to receive either 10^6, 10^8 or 10^{10} CFU/day, or placebo.[42] The group taking 10^8 CFU/day scored significantly better than the placebo in all symptom groups, including global assessment of IBS relief as the primary end-point. The bacteria in the group taking 10^{10} CFU/day were found to be nonviable later, perhaps explaining the lack of efficacy.

B. animalis* subsp. *lactis

(Sometimes commercially known as *B. lactis* DN-173 010*)*

Several well-designed, large multicentred trials of the use of *B. animalis* subsp. *lactis* in IBS have failed to demonstrate benefit, again often in part as a result of a high placebo response.[63,81] A French multicentre trial of *B. animalis* subsp. *lactis* in 274 patients with C-IBS in primary care, demonstrated symptomatic relief compared with baseline in its primary end-point, but not over placebo.[81] However, subgroup analysis of patients with fewer than three bowel motions a week (n = 19) at baseline showed a significant increase in stool frequency compared with controls (p-value < 0.001). In a single trial carried out by Agrawal et al,[83] 34 IBS patients were randomised to either receive fermented milk containing *B. animalis* subsp. *lactis* or placebo for a four-week period. Compared with the control product, the test product resulted in a significant change in maximal distension [median difference -39%, 95% CI (-78, –5; p-value = 0.02]. An accelerated orocaecal [-1.2 hours (-2.3, 0); p-value = 0.049], as well as colonic [-12.2 h (9-22.8, -1.6); p-value = 0.026] transit, was observed, and overall symptom severity [-0.5 (-1.0, -0.05): p-value = 0.032] also improved. The probiotic resulted in improvements in objectively measured abdominal girth (distension) and gastrointestinal transit, as well as reduced symptomatology.[83]

***Escherichia coli* DSM 17252**

A primary-care-based, placebo-controlled trial[86] was conducted in 298 patients with IBS, diagnosed by a primary care standard (not Rome criteria[96]) and was defined as "clinical remission" with complete resolution of IBS symptoms.[97] In comparison with the placebo, the treatment arm was reported to have achieved complete remission in 18.4% vs. 4.6% (p-value < 0.0004) of the patients studied. In addition, a 50% decrease in abdominal pain scores was recorded (18.9% vs. 6.7% in the treatment and placebo groups, respectively (p-value = 0.001)). This trial was based on a much earlier trial of *E. coli* DSM 17252 in combination with *Enterococcus faecalis* (DSM 16440), originally published in 1993,[98] and more recently reanalysed[85] by redefining the clinical end-points to give a GSS in accordance with modern guidelines. This reanalysis showed a significantly better response rate, defined by a decrease of 50%, in the treatment arm than the placebo (68.5% vs. 37.8%; p-value < 0.001). Although both these arms failed to use Rome II[96] or the definitions of Manning et al[99] as their inclusion criteria, they were otherwise large and well-designed trials. Data from primary care, rather than secondary care patients, are particularly useful, given that the majority of IBS patients are treated by primary care physicians.

The role of probiotics in gastrointestinal disease, and in particular IBS, has clearly not been determined adequately. Although questions exist on the dosage and viability of probiotic strains, lack of industry standardisation and potential safety issues (with specific regard to immunocompromised or seriously ill patients),[100] substantial clinical evidence of the advantageous use of probiotics over a wide range of clinical conditions exists. As there is currently no curative treatment for IBS, the relief that probiotic usage may provide, no matter how small, may motivate patients and caregivers to utilise them. Continuing research will recognise and characterise existing strains, identify specific outcomes, determine optimal doses needed for certain results, and assess their stability through processing and digestion.[56] The heterogeneity of IBS and very high placebo response (up to 50%) are problems that are associated with clinical trials. Inevitably, the low-quality design of the trials on IBS and probiotics has led to concluding statements such as: "Further studies are needed to determine whether the probiotic under study may offer clinical benefits for IBS".

Future studies should use Rome III guidelines for the appropriate design of functional gastrointestinal trials.[101] These guidelines also include sample size calculation, which should be based on the expected behaviour of the primary outcome measure. A study must have sufficient power to detect the minimal clinically important difference.[102] With these data, clinicians will be better able to guide patients to efficacious and safe probiotics. Probiotics may be a safe and effective solution, and are urgently needed in the treatment and management of IBS.

Regulatory aspects of probiotics

Testing for the probiotic potential of various microorganisms commences at the preclinical level, and includes animal studies and evaluations of antibiotic resistance, safety and potential efficacy.[103] Many studies, both in animal and human clinical trials, report success in reducing the severity of diseases by the use of a certain probiotic strain, but not by the use of others for the same condition. The need for research to determine the underlying mechanisms of action of specific probiotics will help in determining which specific organism is most likely to benefit a specific disease condition.[13] The specific bacterium should be defined by its genus and species, as well as its

strain level. This is not always adhered to in scientific publications.[104]

It is often incorrectly stated that probiotic products are unregulated. The US Food and Drug Administration has regulatory authority over probiotic products and regulates manufacturers' responsibilities, including the labelling and safety of these products, whether in food, supplement or drug form.[41] In South Africa, permissible statements regarding the health benefits of probiotics are included in the regulations governing labelling and advertising in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972; www.doh.gov.za). The South African guidelines and regulations need to be revised regularly to accommodate the results of ongoing scientific research in the field of probiotics.[105]

Conclusion

Effective treatment of IBS is often masked by its various groupings (C-IBS, D-IBS or post-infectious IBS) and their response to a particular treatment. Much of the published data do not differentiate between the groupings or subgroups, making interpretation of reported results difficult. Effective treatment outcomes are further compounded by variations in indigenous microbiota, as observed in stool microbiota, and possible varying aetiology among patients. Specific probiotic strains may work better in patients with either C-IBS or D-IBS. The strains that have shown good results to date include bifidobacteria, lactobacilli, *E. coli* and mixtures of different bacterial strains. Both *B. infantis* 35624 and *L. plantarum* 299v have demonstrated promising initial results in IBS clinical trials as single composites, but as yet, they have not been studied in combination, and a combination or "cocktail" probiotic of these two strains does not exist. It would be beneficial to assess the effects of these two probiotic strains in the treatment of IBS symptoms.

It is important that probiotic clinical findings are not extrapolated to other clinical settings. Knowledge of the various properties of probiotics will prove to be fundamental in improving patient management. To date, it has been difficult to demonstrate a specific mechanism of action via the intestinal immune system, the enteric nervous system, or otherwise.[106] This knowledge would help to answer the question of whether we have the relevant probiotics to manage IBS.

Probiotics and their benefits are an area of intensive research in various domains. Functional foods, with complex modes of action, may provide an alternative to the pharmacological approach in patients who require lifetime probiotic treatment, and/or who suffer from serious side-effects or drug resistance development. It is important to balance the potential benefits against the harms. Probiotics need to be carefully selected in a strain-specific manner. Thoroughly assessed, probiotic strains will possibly present as alternatives to individuals for whom traditional medical therapies have been unsuccessful, and perhaps, in the future, even serve as a first choice of therapy for some patients.

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Chapter 3

Randomized clinical trial: Effect of *Lactobacillus plantarum* 299v on symptoms of irritable bowel syndrome

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Applied nutritional investigation

Randomized clinical trial: Effect of *Lactobacillus plantarum* 299 v on symptoms of irritable bowel syndrome

Cheryl Stevenson M.Sc.[a,*], Renée Blaauw Ph.D.[a], Ernst Fredericks M.B.Ch.B., M.Med., B.Sc.(Hons)[b], Janicke Visser M.Nutrition[a], Saartjie Roux D.Med.Science[b]

[a] Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
[b] Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa

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ABSTRACT

Objectives: Irritable bowel syndrome (IBS) is a common diagnosis in gastroenterology. Its etiology is unknown and therapeutic options limited. Trials suggest probiotics may be beneficial. The aim of this study was to assess the symptomatic efficacy of *Lactobacillus plantarum* 299 v (*L. plantarum* 299 v) for the relief of abdominal pain in patients with IBS fulfilling Rome II criteria.
Methods: This study was conducted in a referral hospital. Trial participants were randomized to receive either two capsules of *L. plantarum* 299 v at a dosage of 5×10^9 cfu per capsule or placebo daily for 8 wk. Severity of abdominal pain was assessed using a visual analog scale at each visit and a quality-of-life IBS (QoL-IBS) questionnaire was also completed.
Results: There was no significant difference in abdominal pain relief between the study and placebo groups ($P = 0.800$). There was also no difference in QoL- IBS scores between the groups ($P = 0.687$). Both groups had a significant improvement in abdominal pain scores over the study period, from an average of 251.55 to 197.90 ($P < 0.0001$) indicating a large placebo effect.
Conclusion: An 8-wk treatment with *L. plantarum* 299 v did not provide symptomatic relief, particularly of abdominal pain and bloating, in patients fulfilling the Rome II criteria.

Background

Multiple clinical trials within the past decade have aimed to study the safety and efficacy of various probiotic strains in treating patients with irritable bowel syndrome (IBS). These trials provide guidance to health care professionals as they strive to treat patients suffering with IBS. Both positive and negative trial results provide a way forward and assist in the decision-making process. Health care workers are assailed with advertisements, representative details, patient expectations, and questions. Trials that contribute toward completing the picture on the use of specific strains are urgently needed.

Often, probiotic clinical trials have suffered from inherent design weaknesses. Areas of poor design include Rome criteria not used to define IBS, no randomization, no parallel study design, no double-blinding, no baseline observation period before trial initiation, short treatment duration (<8 wk), no follow-up, non-validated scales to measure outcomes, incomplete follow-up (intention to treat), treatment compliance not measured, and inadequate sample size [1]. The low quality design of the trials inevitably leads to the likelihood of concluding statements such as "further studies are needed to determine whether the probiotic under study may offer clinical benefits for IBS."

The incomplete picture provided by previous trials makes it difficult to determine whether truly efficacious probiotic therapies exist [1]. Comparisons among clinical trials are much easier when variables throughout the trial's design and execution are limited [2]. In 1999, a working committee was charged with developing guidelines for the design and conducting an analysis of treatment trials in the functional gastrointestinal disorders [1].

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* Corresponding author. Tel.: +27 21 938 9259; fax: +27 21 933 2991.
E-mail address: Cheryl@retaildc.co.za (C. Stevenson).

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These guidelines have helped improve the standard of the clinical trials conducted.

Most probiotic trials have suggested that the *Bifidobacteria* and *Lactobacillus* genuses appear to be the most effective in the treatment of IBS [3,4]. A systematic review assessed the effectiveness, safety, and tolerability of probiotics in the treatment of IBS. A thorough literature search of multiple databases was performed and 16 randomized controlled trials (RCTs) met the stringent inclusion criteria. An assessment of the methodology and statistical designs of the RCTs indicated that only *B. infantis* 35624 showed repeated efficacy [3,5]. Of the remaining studies, sufficient evidence to warrant further evaluation, particularly for single moieties, included *L. plantarum* 299 v [6–8], *L. salivarius* UCC4331 [9], and *L. acidophilus* LB strain [10] and certain combination probiotics [11–14].

The lactobacilli species exhibit the following desirable properties: They usually produce antimicrobial substances like bacteriocin, which have a broad spectrum of antagonist effects against closely related gram-positive and gram-negative pathogens. They often produce polymeric substances such as exopolysaccharides, which increase the colonization of probiotic bacteria by cell–cell interactions in the gastrointestinal (GI) tract [15]. Lactobacilli probiotics also have demonstrated the ability to increase fecal concentration of carboxylic, acetic, and propionic acids [16]. Generally, short-chain fatty acids lower colonic pH, which assists in controlling the growth of undesired pathogens [17].

L. plantarum 299 v has been the most studied strain from *L plantarum* family. It has been shown to reduce mucosal inflammation by adhering to the mucosal membrane and reducing gram-negative bacteria, which contain endotoxin [18,19]. In vitro *L plantarum* 299 v has increased interleukin-10 synthesis and secretion, thereby demonstrated beneficial immunomodulatory activity [20]. A previous study demonstrated a significant increase in stool volume and a decrease in flatulence and slightly softer stools in an intervention group receiving *L plantarum* 299 v for 3 wk compared with controls [16]. *L plantarum* 299 v has the following properties: It is of human origin, non-pathogenic, and resistant to intestinal acid and bile. It demonstrates the ability to adhere to human epithelial cells and to temporarily colonize and be metabolically active within the human GI tract. It can survive transit through the GI tract, and it can be free of side effects [18,21–25].

The role of probiotics in GI disease, in particular IBS, clearly has not been determined adequately. There is substantial clinical evidence for the advantageous use of probiotics over a wide range of clinical conditions. Probiotics may be a safe and effective solution urgently needed in the treatment and management of IBS. The need arose to conduct a well-designed study because of promising data coming from previous trials but still no clear conclusion regarding to the efficacy of *L. plantarum* 299 v in the management of IBS.

Methods

Participants

The present study was performed as a double-blind, placebo-controlled trial. Patients were recruited at a private gastroenterology clinic in Port Elizabeth, South Africa between January 2011 and April 2013. They were screened and recruited according to the study inclusion criteria and their willingness to participate. Constipation-predominant IBS (C-IBS) and diarrhea-predominant IBS (D-IBS) were included; the mixed type of IBS was excluded. Only C-IBS, D-IBS, and a placebo group were included in the study to make a clear distinction between the groups and to determine whether the probiotics may have an advantage in only one group (i.e., C-IBS or D-IBS) or both. All patients provided informed, written consent and the study was approved by the Health Research Ethics Committee of Stellenbosch University (reference no. N10/08/270) and followed the guidelines of the World Medical Association's Declaration of Helsinki. The trial is registered on the ClinicalTrials.gov registry (reference no. NCT01886781).

The inclusion criteria were as follows:

1. Fulfilment of the Rome II criteria for IBS.
2. Aavailability of at least one colonoscopy report within the previous 3 years.
3. A positive diagnosis of IBS by a gastroenterologist.
4. Age ≥18 at the time of screening.
5. Provision of written informed consent.
6. Commitment of availability throughout a 12-wk study period.

Exclusion criteria were:

1. Major abdominal surgery in the past other than appendectomy, cesarean delivery, tubal laparoscopic cholecystectomy, abdominal wall hernia repair, or hysterectomy.
2. Current use of antibiotics
3. History of organic intestinal disease.
4. Pregnant or breast-feeding mothers.
5. Chronic infectious disease such as HIV or tuberculosis.
6. Unable to understand English or Afrikaans.

Study design

The overall study length was 12 wk. Treatment was preceded by a 1 to 2 wk run-in period, depending on the scheduling of a colonoscopy, if necessary. Patients scoring <75 on the Francis Severity IBS score (see later) at the end of the run-in period were considered to be in remission and were excluded from the study. The rest of the patients were considered as an intention-to-treat (ITT) group. Each patient was identified by a serial number and the entire cohort underwent double-blind (investigator and participant) randomization into three equal groups (D-IBS, C-IBS, and placebo). Six visits were scheduled: visit 1 (−2 wk) took place at the beginning of the run-in period, visit 2 (baseline) involved randomization and commencement of treatment, and visits 3 to 6 were scheduled 2, 4, 8, and 10 wk into the trial, respectively. The treatment phase was 8 wk, followed by a 2-wk washout phase.

Study products

The study product contained 5×10^9 cfu of *L. plantarum 299 v* and was tested against placebo capsules, filled with micro-crystalline cellulose powder, of identical taste, texture, and appearance by the manufacturer (Ferlot Manufacturing and Packaging (PTY) Ltd, South Africa). The expiry date of the test product was not exceeded. The capsules were put in identical bottles and with no way to distinguish between the two products. The dose was two capsules taken orally every morning.

Assessments and end points

The primary end point was a decrease in abdominal pain at week 8 of the trial. A total quality-of life IBS (QoL-IBS) score [26] and the scores of the individual components at the end of the study compared with baseline scores was a secondary outcome measure. The clinical severity of the IBS symptoms (pain and distension) was evaluated by the Francis Severity Score questionnaire [27] at each visit and by the QoL-IBS questionnaire at visits 2, 5, and 6. Both questionnaires are validated tools for use in IBS [26,27]. The severity score contained five questions, each given a value from 0 (*no symptoms*) to 100 (*most severe*) for measuring the severity and frequency of abdominal pain. The sum of scores of these questions was considered the severity score, with a maximum possible score of 500. The QoL-IBS questionnaire consisted of 34 questions, each rated from 1 (*not at all*) to 5 (*extremely*), and the sum of them yielded the total QoL-IBS score. The questions of this questionnaire were further subdivided into eight categories: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual, and relationships. The participants were instructed not to change their eating habits throughout the entire study period. Of the 81 patients, 5 were on probiotics and stopped at least 2 wk before entrance into the study. The patients were asked not to change their regular medications. Adverse events and medication compliance were monitored throughout the study period.

Sample size and randomization

To determine an effect size of 0.64 with 80% power, a sample size of 27 for each group (D-IBS, C-IBS, and controls, N = 81), with a type 1 error of 5% using a

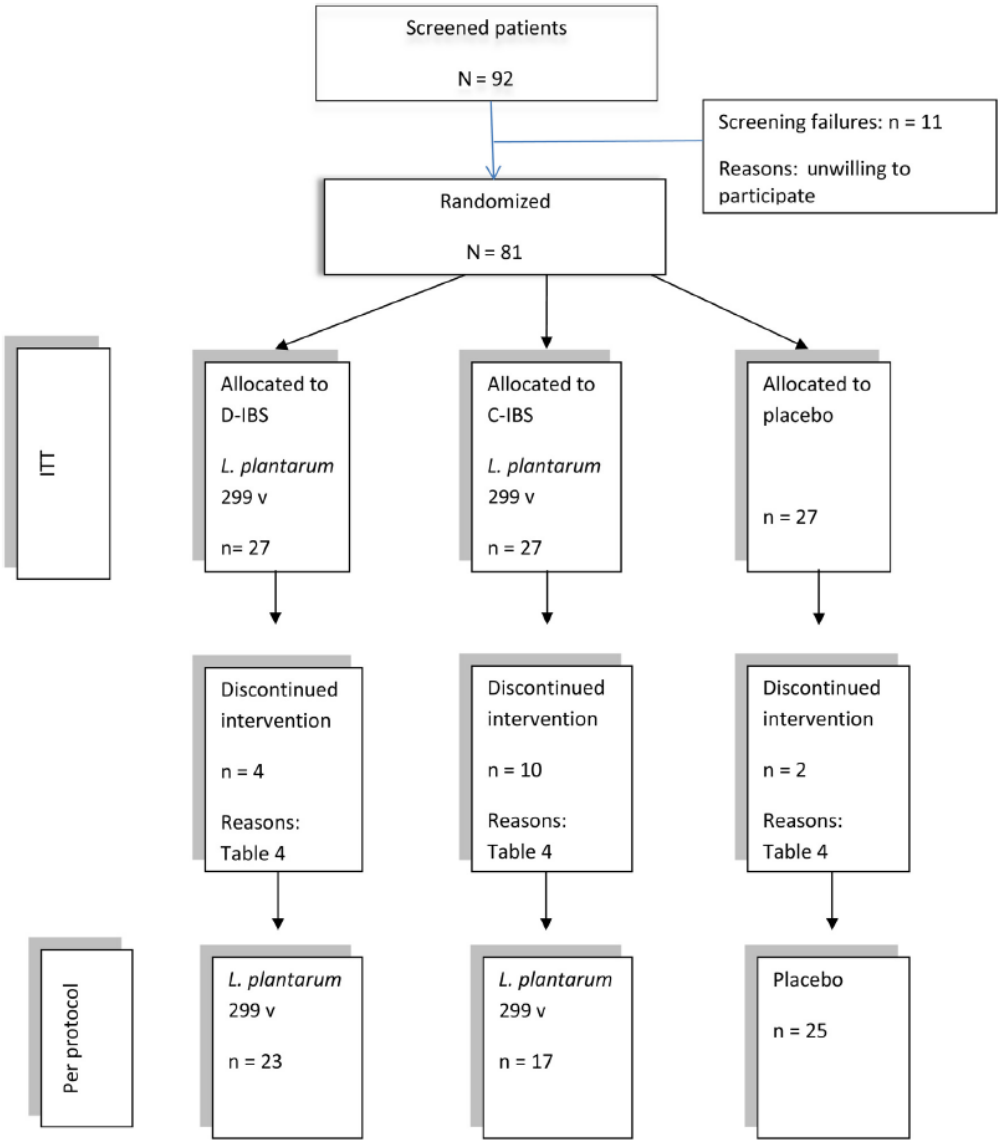

Fig. 1. Flow chart of study. C-IBS, constipation-predominant irritable bowel syndrome; D-IBS, diarrhea-predominant irritable bowel syndrome; ITT, intention-to-treat; *L. plantarum*, *Lactobacillus plantarum*.

two-sided test would be sufficient. The sample size calculation is based on the expected behavior of the primary outcome measure (i.e., abdominal pain relief at the end of week 8 on the visual analog scale [0–100%]) pain severity score. The minimally clinically important difference based on the primary outcome measure with this instrument was 50 points [27]. Computer-generated randomization charts were generated for packaging and labeling. The randomization code was kept in a secure place during the study. Investigators as well as participants were blinded to the intervention.

Statistical analysis

The intention-to–treat (ITT) analysis was performed on all patients who underwent randomization (N = 81). An additional analysis included only those patients who completed the study, per protocol analysis (n = 64). The results revealed no statistically significant differences between these two groups, and those of the ITT are presented here.

Changes in severity and QoL-IBS scores during follow-up were examined using the mixed model for analysis of variance to account for missing data (dropouts). This model allows the evaluations of the effect of each factor on the outcome as well as the interactions between factors. The mixed model used group (treatment versus control) and time as factors. If residuals proved not to be normally distributed the Bonferroni multiple comparisons with Bootstrap multiple comparisons to accommodate for the non-normality of residuals was done. A P-value < 0.05 was considered significant and multiple comparisons were done with Fisher's least significant difference test and 95% confidence intervals.

Results

The flow chart of the study is given in Figure 1. Eighty-one patients met the inclusion criteria, passed the run-in period, and were randomized. Fifty-four (66%) patients were

Table 1
Demographic data of the study participants*

	Study group n = 54 (%)	Placebo group n = 27 (%)	*P*-value
Female/Male	52/2 (96/4)	27/0 (100/0)	0.221
Mean age (y)	48.15 ± 13.48	47.27 ± 12.15	0.779
BMI (kg/m^2)	28.83 ± 7.12	28.88 ± 7.74	0.978
Mean duration of IBS (y)	9.58 ± 10.32	10.05 ± 9.36	0.340

BMI, body mass index; IBS, irritable bowel syndrome
* Mean ± SD.

randomized to receive *L. plantarum* 299 v and 27 (33%) the placebo; ratio of 2:1. Of the 54 patients randomized to receive the *L. plantarum* 299 v (50%, n = 27, were C-IBS and the other half, n = 27, D-IBS), the placebo was also equally distributed among D-IBS and C-IBS participants. The results are presented as the study C-IBS and D-IBS groups combined versus placebo group. There were no significant differences when the C-IBS versus D-IBS versus placebo were analyzed as three separate groups.

The baseline demographic characteristics are given in Table 1. Although mainly women, there were no statistical differences in sex distribution between the two groups. The mean age of the study group was 48.15 ± 13.48 y (range 25–75 y) and that of the placebo group 47.27 ± 12.15 y (range, 31–72 y), with no significant differences between the groups. Both groups had mean body mass indexes that fell in the overweight range. IBS was longstanding for most the participants, with a mean duration of 9.58 ± 10.32 y for the study group and 10.05 ± 9.36 y in the placebo group.

Table 2 shows the clinical severity of IBS in both groups before treatment (visit 2, week 0), there was no statistical difference between the study and placebo groups, (259.66 ± 106.90 versus 256.04 ± 104.27; $P = 0.884$). The total QoL-IBS scores at baseline (visit 2, week 0) also did not differ significantly between the groups (49.95 ± 21.43 versus 44.72 ± 22.22; $P = 0.725$). The analysis of the different components of the QoL-IBS score also did not show any significant differences between the groups at baseline. The changes in the severity score and the different components of the total QoL-IBS score were analyzed for the study and placebo groups over the entire trial and are shown in Table 2. The groups were also further divided into C-IBS versus placebo and D-IBS versus placebo, no significant differences between the C-IBS versus placebo and D-IBS versus placebo groups were found and only the data for the study group versus placebo are presented. The entire study population (N = 81) improved significantly over time in all study parameters ($P < 0.0001$) and there were no significant differences between those receiving the *L. plantarum* 299 v and those receiving the placebo for severity scores (259.66 ± 106.90 to 199.13 ± 119.70 versus 256.04 ± 104.27 to 201.98 ± 97.44; $P = 0.800$) and QoL scores (49.95 ± 21.43 to 44.42 ± 23.96 versus 44.72 ± 22.22 to 32.29 ± 23.22; $P = 0.687$) (Figs. 2 and 3).

Further characteristics of the study population are presented in Table 3. More than half of the patients identified either diet, stress, or a combination of the two as a trigger for their IBS symptoms. Fourteen (25.9%) and eight (29.6%) participants were current smokers in the study and placebo groups, respectively. There was similar rates of family histories of IBS and metabolic syndrome in both groups and the study group had a slightly higher percentage use of anti-IBS treatment than the placebo groups, although not significant (57.4% versus 48.1%, $P = 0.779$).

Table 2
Comparison of severity score and of QoL scores between the groups*

	Visit 1 (week −2)		Visit 2 (week 0)		Visit 3 (week 2)		Visit 4 (week 4)		Visit 5 (week 8)		Visit 6 (week 10)		*P* value
	Study group	Placebo group	Study group	Placebo group	Study group	Placebo group	Study group	Placebo group	Study group	Placebo group	Study group	Placebo group	
Total Francis Severity Score	272.89 ± 93.76	277.56 ± 88.12	259.66 ± 106.90	256.04 ±104.27	188.77 ± 116.23	201.04 ± 135.00	203.10 ± 107.83	198.52 ± 108.34	199.13 ± 119.70	201.98 ± 97.44	209.08 ± 110.63	185.30 ± 96.52	0.800
Total QoL			49.95 ± 21.43	44. 72 ± 22.22					44.42 ± 23.96	32.29 ± 23.22	41.81 ± 27.28	33.22 ± 26.47	0.687
Dysphoria			50.89 ± 25.84	44.32 ± 24.77					39.66 ± 29.90	30.54 ± 22.88	37.27 ± 30.79	28.98 ± 27.74	0.884
Interference with activity			52.18 ± 23.87	44.81 ± 26.73					43.38 ± 24.22	31.66 ± 24.95	44.69 ± 27.69	31.98 ± 26.26	0.538
Body image			61.76 ± 24.03	57.67 ± 23.45					45.39 ± 29.72	41.19 ± 30.05	46.13 ± 32.05	42.61 ± 33.63	0.992
Health worry			51.19 ± 25.56	53.03 ± 25.54					39.09 ± 27.95	38.26 ± 31.57	40.67 ± 27.93	39.02 ± 32.83	0.756
Food avoidance			64.88 ± 28.00	74.01 ± 27.59					59.13 ± 29.12	51.89 ± 30.85	74.01 ± 91.18	51.89 ± 28.68	0.643
Social reaction			45.42 ± 26.52	41.76 ± 26.79					36.28 ± 26.90	26.70 ± 28.16	36.59 ±9.26	28.13 ± 29.42	0.420
Sexual			30.49 ± 36.65	27.27 ± 31.57					22.87 ± 35.55	15.34 ± 26.14	25.91 ± 36.26	25.57 ± 28.82	0.504
Relationships			35.71 ± 25.37	26.89 ± 26.23					30.36 ± 26.15	19.70 ± 23.65	30.75 ± 28.90	22.35 ± 25.71	0.888

QoL, quality of life
* Mean ± SD.

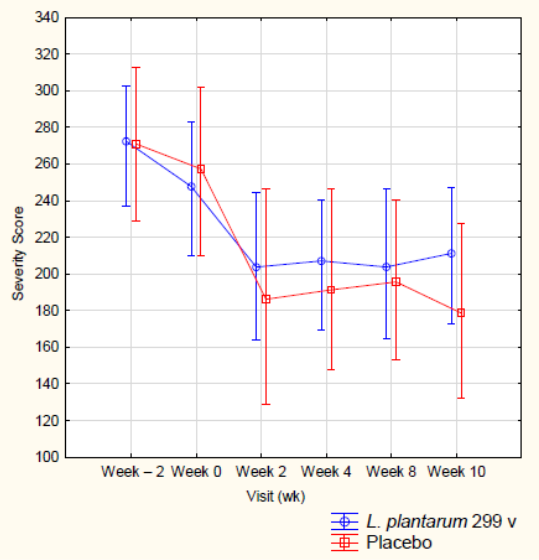

Fig. 2. Changes in severity score with each visit, $P = 0.800$, *Lactobacillus plantarum* 299 v group versus placebo group.

Figure 1 and Table 4 present the data on compliance and adverse events (AEs). Eighty percent of participants completed the trial period; however, 16 (19.8%) were lost to follow-up, the reasons for non-completion of the trial are given in Table 4. There was a significant difference between the groups in terms of completing the 12 wk period of the study ($P = 0.048$), the drop-out rate was higher in the study group than in the placebo group. Furthermore, the C-IBS study group had 10 dropouts versus 4 in the D-IBS group. Most of the dropouts where either due to non-compliance (n = 5) and those needing an antibiotic (n = 6) (one patient developed hepatitis B, one had a gynecologic condition, and three developed chest infections) and two left the study due to worsening of their IBS symptoms. The severe rash indicated as an AE in Table 4 was initially thought to be related to the probiotic but continuous follow-up with the patient and doctor showed that the rash was related to a gynecologic condition. There was no difference in the groups in terms of completing 50% of the visits and the rate of AEs, was very low. The tolerability of the test product was good.

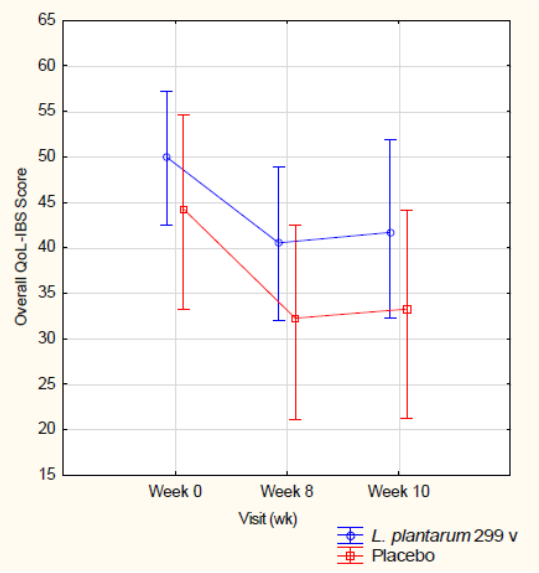

Fig. 3. Changes in overall QoL-IBS score with each visit, $P = 0.687$, *Lactobacillus plantarum* 299 v group versus placebo group. QoL-IBS, quality of life-irritable bowel syndrome.

Table 3
Further characteristics of the study participants*

	Study group n = 54 (%)	Placebo group n = 27 (%)	*P*-value
Trigger of IBS symptoms			
Diet	20 (37)	10 (37)	0.713
Stress	12 (22)	5 (18.5)	0.884
Other	2 (3.7)	0 (0)	0.221
Frequent antibiotic usage during childhood	8 (14.8)	4 (14.8)	0.753
Current smoker	14 (25.9)	8 (29.6)	0.517
Family history of:			
Colorectal cancer	9 (16.7)	5 (18.5)	0.669
Inflammatory bowel syndrome	0	0	
Metabolic syndrome	17 (31.5)	9 (33.3)	0.617
Irritable bowel syndrome	16 (29.6)	8 (29.6)	0.756
Lactose intolerant	5 (9.2)	4 (14.8)	0.362
Current use of anti-IBS treatment	31 (57.4)	13 (48.1)	0.779

IBS, irritable bowel syndrome
* Mean ± SD.

Discussion

The aim of this study was to conduct an appropriately well-designed randomized, double-blind, placebo-controlled clinical trial with *L. plantarum* 299 v and to determine its efficacy in IBS. No significant beneficial effects by the probiotic were seen on the severity of symptoms (abdominal pain) and quality of life.

To our knowledge this is the longest RCT reporting the effects of *L. plantarum* 299 v. Earlier interventions with *L. plantarum* 299 v had a 4-wk treatment phase [6–8,28]. Given the chronicity of functional GI disorders, a minimum treatment duration of 8 to 12 wk has been recommended for IBS trials [1,29].

The participants of this study responded very well to the probiotic therapy, however, they also responded very well to

Table 4
Compliance and adverse events

	Study group n = 54 (%)	Placebo group n = 27 (%)	Total n = 81 (%)	*P*-value
Number of patients completing all 6 visits	40 (74.1)	25 (92.6)	65 (80.2)	0.048
Number of patients with at least 3 visits (50%)	50 (92.6)	25 (88.89)	74 (91.4)	0.999
Adverse events				
Severe rash	1 (1.9)	0	1 (1.%)	0.477
Reason for dropping out				
IBS worsening	1 (1.9)	1 (3.75)	2 (2.5)	0.613
Adverse event	1 (1.9)	0	1 (1.2)	0.477
Antibiotic needed	5 (9.3)	0	5 (6.2)	0.103
Poor compliance	5 (9.3)	0	5 (6.2)	0.103
Patient requested to exit due to personal reasons, no worsening of IBS symptoms	2 (3.7)	1 (3.75)	3 (3.7)	0.999

IBS, irritable bowel syndrome

placebo. The rate of such a high placebo response in this study could result from patients with IBS having very burdensome symptoms and being anxious to be treated. They may respond to any alteration in therapy, even if that alteration is placebo. There is a high degree of subjectivity in many outcome measures in IBS clinical trials. The allocation to either treatment or placebo in this study was done on a blind basis and was not manipulated in any way, which could have led to the group being skewed. However, both the experimental and control groups were well balanced and equally represented, which means this group of IBS patients was fairly heterogonous.

The use of placebos in RTCs is the gold standard for evaluating the effectiveness of new therapies [30]. In clinical trials of conventional therapies for the treatment of IBS, between 16% and 71% (mean of 40%) of patients respond to placebo [31].

In a recent meta-analysis of 73 RCTs, the pooled placebo response rate was 37.5% [32]. Rates were higher in European RCTs; RCTs that used physician-reported outcomes; and RCTs that used shorter duration of therapy. The trials involved antispasmodics, peppermint oil, antidepressants, 5-HT_3 antagonists, 5-HT_4 agonists, and mixed 5-HT_3 antagonists/5-HT_4 agonists, but not probiotics [32]. Results from one RCT demonstrated that even placebos administered openly were an effective treatment for patients with IBS [33]. The magnitude of the placebo response also may be influenced by the wording of the question used to define treatment response or by the use of a compound question. A meta-analysis suggested that placebo response rate is larger when a responder is defined by a global improvement in IBS symptoms compared with defining a responder by a reduction in abdominal pain [34].

Informal feedback from the participants in this study also indicated that many of them felt that regular visits to a health care practitioner who was willing to take time and listen to their problems and complaints translated into an improved sense of well-being. This could have compounded the placebo effect in this group of patients. This informal feedback aptly describes "The Hawthorne effect," a form of reactivity whereby patients improve or modify an aspect of their behavior being experimentally measured simply in response to the fact that they know they are being studied, not in response to any particular experimental manipulation [35]. Further external factors that could have contributed toward making it difficult to detect a treatment effect include a natural variation in symptoms, regression toward the mean, and unidentified or unintended cointerventions [29].

A study limitation is the drop-out rate in the treatment group, particularly the C-IBS group. The number and timing of the drop outs was reported and no specific reasons for this were noted. The drop-out rate fell just within the recommendations of 10% to 20 % for functional GI disorders [29]. To account for the drop-out rate, the last observation for the patient while in the study was used, in order not to lose all the information gathered from the individual. However, the significant loss of patients from the treatment group could have compounded the large placebo effect. This trial was performed according to Rome III guidelines on design of trials for functional GI disorders [29] to demonstrate statistical superiority to placebo with a double-blind, placebo-controlled, parallel design and outcome measures including both the effect of the treatment on the primary outcome, abdominal pain, and a global assessment of the treatment to obtain adequate symptom relief. Since 1999, most published pharmaceutical trials for IBS have used "adequate relief of abdominal pain and discomfort" as their primary outcome measure [29].

Four studies using *L. plantarum* 299 v were published before this trial [6–8,28]. There are three small single-centre studies using a liquid form (rose hip drink/oatmeal soup: Proviva) of *L. plantarum* 299 v, at different dosages in IBS. Two studies showed some benefit over placebo, one improving flatulence [7] and the other a reduction in abdominal pain [6]. The third trial showed no significant benefit although it was underpowered [8]. Among these three trials there are significant differences in enrolled populations, study designs, outcome variables, and statistical analyses, making it impossible to make comparisons across the studies. There was poor recording of tolerability and AEs in all three trials. These three trials all used a rose hip/oatmeal soup drink and the oats in addition to the probiotic might have improved clinical outcomes as they may have acted as a prebiotic for the *L. plantarum* 299 v, the advantage of these trials might have been the added prebiotic [6–8]. Until recently, these smaller trials with promising results were not followed up with larger, 12-wk, double-blind studies.

The fourth RCT was a large multicenter trial enrolling 214 participants. Significant superiority of *L. plantarum* 299 v was demonstrated over placebo with regard to the primary outcome measure, improvement of the frequency of abdominal pain episodes. The patients were recruited via general practitioners and the dosage of probiotic was one capsule per day containing 10 billion cfu [28]. As the dosage was the same used in this study, we investigated whether there was a significant improvement in this study at week 4 and whether this possible mimicked the earlier study, however, this was not a finding. This highlights the importance of follow-up over longer periods of time and also might explain why the results of this study differ from previous studies. If the follow-up period had been only 3 wk in this study, the results would have been interpreted differently. It seems as if the effect of *L. plantarum* 299 v is best seen over the short term. The participants in our study were recruited at secondary-level care by a gastroenterologist, which might indicate that the type of patient we enrolled was experiencing a greater severity of symptoms or more resistant conditions. Significant differences in treatment response may exist between primary and referred patients [36,37].

Conclusion

This study has demonstrated that an 8-wk treatment with *L. plantarum* 299 v did not provide relief for patients with moderate IBS symptoms over placebo and a very large placebo effect was demonstrated.

It is of the utmost importance that researchers strive to assess the efficacy of probiotics over placebo in treating patients with IBS and this means using more objective assessment methods. Probiotics may also prove to be a better treatment option in patients whose symptoms are on the lower end of the severity scale and not those consulting at referral centers.

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Chapter 4

Food avoidance in irritable bowel syndrome leads to a nutrition deficient diet

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Food avoidance in irritable bowel syndrome leads to a nutrition-deficient diet

Stevenson C,[1] Master of Science, Postgraduate Student; **Blaauw R,**[1] PhD, Associate Professor and Acting Head
Fredericks E,[2] BSc(Hons), MBChB, FCP(SA), MMed, CertGastro(SA), Gastroenterologist, Private Practice
Visser J,[1] Master of Nutrition; Postgraduate Programme Coordinator and Senior Lecturer; **Roux S,**[2] DMedScience, Associate Professor
[1]Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University
[2]Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth
Correspondence to: Cheryl Stevenson, e-mail: cheryl@retaildc.co.za
Keywords: irritable bowel syndrome, dietary intake, fibre, fructose

Abstract

Objective: The objective was to assess the dietary intake of subjects with irritable bowel syndrome (IBS) and to compare it to that of international recommendations. The hypothesising assumption of this study was that a situation in which subjects insist that diet or trigger foods play a part in symptom generation may lead to an unbalanced dietary intake.

Design: This was a descriptive observational study, with an analytical component.

Setting: A private, secondary care-level clinic in South Africa.

Subjects: The study population comprised 122 participants. Each subject completed an estimated, three-day dietary record. The data were analysed using a computerised food analysis programme. The fructose intake was analysed semi-quantitatively. IBS subjects' protein and carbohydrate intake were significantly higher than the recommended dietary allowance for protein and carbohydrate (p-values < 0.000 and < 0.000, respectively).

Outcome measures: The identification of dietary risk factors that affect IBS.

Results: The IBS subjects' daily total dietary fibre (15.13 g $\pm$ 13.11) was significantly lower (p-value < 0.000) than the dietary reference intake (DRI) target intake of 24.76 g/day, and the intake of micronutrients,(calcium, iron and folate) was significantly less than the DRI. There was no significant difference in macronutrient intake between the diarrhoea-predominant IBS (D-IBS), constipation-predominant IBS (C-IBS) and the control groups. The total number of fructose serves per day was not statistically significant between the three groups (C-IBS 2.68 $\pm$ 1.68, D-IBS 2.15 $\pm$ 1.86, and controls 3.17 $\pm$ 2.39, p-value = 0.157).

Conclusion: The IBS subjects in this study consumed diets that were deficient in key micronutrients and total fibre when judged against the recommended DRIs. Dietary adjustments may have been tailored by subjects to minimise symptom development and this led to nutritionally deficient diets.

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Introduction

Irritable bowel syndrome (IBS) is a chronic disorder that is characterised by abdominal pain or discomfort, and associated with disordered defecation, either C-IBS, D-IBS, or mixed or alternating symptoms of constipation and diarrhoea.[1] It is estimated that IBS affects 3-25% of the general population. Patients with IBS can account for up to 30-50% of gastroenterology clinic visits.[2] Treatment and management of the condition is often unsatisfactory, and there is no single curative treatment. Despite numerous reviews on IBS and diet, it is very difficult to give general dietary advice to patients with IBS. This is partly because of the complexity of the condition, heterogeneity of the patient population and poor understanding of the aetiology of the disorder. Dietary experts may play a very positive role in managing such patients. However, more definitive guidelines that outline a constructive approach for the dietitian are needed to treat patients with IBS. Definitive guidelines would support the unique skills of dietitians with regard to habitual eating and therapeutic dietary manipulation assessment. There is a dire need for further prospective research on dietary factors and IBS. Published data suggest that IBS symptoms may be caused or exacerbated by one or more dietary components in at least 25% of patients.[3] Many patients restrict their dietary intake or eliminate certain provocative dietary agents in order to reduce the symptoms. The restriction of certain trigger foods could potentially distort macro- and micronutrient intake and place IBS individuals at risk of low nutrient intake.

Recent work has identified a collection of short-chain carbohydrates that are poorly absorbed in the small intestine, namely fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs).[4,5] These

include fructo-oligosaccharides, galacto-oligosaccharides and polyols.[6] Failure to completely absorb fructose in the small intestine leads to its delivery into the colonic lumen, together with water, because of its osmotic effect. If sufficient fructose reaches the colon, luminal distention may occur because of the osmotic load, as well as rapid gas production. Potentially, this leads to bloating, abdominal discomfort and motility changes. These symptoms are commonly experienced by patients with IBS. Malabsorption of dietary fructose may trigger symptoms in patients with IBS and the removal of fructose from the diet may improve them.[7]

Dietary fibre, classified as soluble or insoluble, is a collective term for different plant substances that are resistant to digestion by human gastrointestinal enzymes. In the USA and the European Union, adult dietary fibre intake falls well below the recommended range of 20-35g/day.[8] Currently, fibre supplementation is recommended to patients with IBS and constipation because greater insoluble fibre intake results in softer and bulkier stools, thus promoting colonic peristalsis and easing defecation.[9] Many patients with IBS self-prescribe fibre supplements before consulting a physician because of the belief that alimentary habits play a significant role in their symptom development.[10,11]

Actual dietary behaviour in patients with IBS may differ from that of healthy controls, and has not been extensively reported. To date, only a few studies have assessed the dietary intake of patients with IBS.[12-14] It is important that the latter is fully understood before any dietary recommendations or interventions are given. The aim of this study was to propectively assess the dietary intake of patients with IBS and to compare this with the international recommendations for healthy dietary intake, namely the dietary reference intakes (DRIs),[15-18] and secondly, to compare the intake of patients with IBS to that of healthy controls. The hypothesising assumption was that a situation in which subjects insist that diet or trigger foods play a part in symptom generation may lead to an unbalanced diet.

Method

Study subjects

This study was a descriptive observational study, with an analytical component, that identified dietary risk factors that affect IBS. The study population was men and women recruited at a private, secondary care-level clinic in South Africa. Data were collected between July 2008 and June 2012. Subjects were screened by a gastroenterologist and recruited according to the study inclusion criteria and their willingness to participate.

Exclusion criteria for both controls and cases included:

- Being unable to give informed consent.
- Being younger than 18 years of age.
- Being unable to understand English or Afrikaans.
- Having had a chronic infectious disease, e.g. human immuno-deficiency virus (HIV) or tuberculosis.
- Being unable to tolerate a colonoscopy procedure.
- Pregnant or breastfeeding mothers.
- Having had any previous abdominal surgery, besides an appendectomy, a Caesarean, tubal laproscopic cholecystectomy, abdominal wall hernia repair or a hysterectomy.
- Currently taking antibiotics or gastrointestinal motility medication.
- Having a history of organic intestinal disease.

In order to establish whether or not there was a difference in dietary intake among the studied IBS population, subjects were divided into subtypes: C-IBS and D-IBS. However, patients with alternating or mixed-type IBS were excluded for the purposes of the study. A diagnosis of IBS was made using Rome II criteria. Controls included any patients who visited the clinic for routine screening procedures, for example, cancer screening and to exclude IBS. They were also included based on their willingness to participate in the study. Cases and controls were neither age, nor sex matched.

Written informed consent to participate was obtained from each participant on enrolment. The study was approved by the Research Ethics Committee at Nelson Mandela Metropolitan University (Reference No HUM07.70.2), and was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki.

Medical information

Collected pertinent medical information included recent antibiotic usage, current medication, background medical history, previous bowel surgery, a family history of IBS, being a smoker or non-smoker, IBS duration, IBS symptom triggers and anti-IBS-specific medication.

Dietary assessment

A registered dietitian explained and orientated each study subject on the required procedure to complete a prospective, estimated three-day dietary record. Portion sizes were determined using household food measures; for example, spoons, cups, bowls and a ruler. The results were analysed using FoodFinder™ III, a computerised nutritional analysis programme for South African foods.[15] To examine the energy and nutrient content of the subjects' diets, the intakes were compared to the DRI values for adults aged 31-50 years and 51-70 years.[16-19] Micronutrient (calcium, iron, vitamin C, folate and vitamin A) intake was compared to the relevant DRIs. The percentage energy intake for protein, fat and carbohydrates was compared to that outlined in the UK National Diet and Nutrition Survey.[20,21] The subjects' diets were also analysed semi-quantitatively, using tables of known FODMAP content by a dietitian for fructose intake, based on their three-day food record. This method has been described elsewhere.[4,7]

Statistical analyses

Summary statistics were used to describe the variables. Mean values were used as the measures of central location for continuous responses, and standard deviations (SDs) as indicators of spread. The relationship between continuous response variables and nominal input variables was analysed using appropriate analysis of variance (ANOVA); for example, for homogenous variance among the groups. One-way ANOVA was utilised to assess variance. When heterogeneity of variances was detected with the Brown-Forsythe test, the data were analysed as per the Welch-Satterthwaite ANOVA. If the residuals were not normally distributed, a nonparametric ANOVA method, e.g. the Kruskal-Wallis test, was used. Multiple comparisons were assessed as per the Bonferroni test, provided that the ANOVA p-value was significant ($p < 0.05$). A p-value of less than 0.05 represented statistical significance. Statistica® version 9 was employed for all data analysis, and Statistical Analysis Software® version 9.1 for the Brown-Forsythe tests.

Table I: The demographics of the 122 subjects

	All subjects (n = 122)	Controls (n = 19)	C-IBS (n = 69)	D-IBS (n = 34)	p-value
Age in years (mean ± SD)	48.29 ± 13.56	56.9 ± 13.66	47.2 ± 12.45	45.7 ± 14.14	0.008
BMI in kg/m^2 (mean ± SD)	27.82 ± 6.88	26.80 ± 7.48	28.07 ± 6.81	27.89 ± 6.81	0.779
Gender (male/female)	20/102	9/10	4/65	7/27	0.000

BMI: body mass index, C-IBS: constipation-predominant irritable bowel syndrome, D-IBS: diarrhoea-predominant irritable bowel syndrome, SD: standard deviation

Results

Subjects' demographics

The subjects' demographics are shown in Table I. In total, 122 subjects participated in the study and completed the three-day food record. There was a significant difference in age between the subjects in the three groups at baseline. Fifty-seven per cent (n = 69) of the subjects were diagnosed with C-IBS and 28% (n = 34) with D-IBS, while 16% (n = 19) were controls (non-IBS). The majority (75%) of IBS subjects were female (n = 92). Nine per cent of the IBS subjects were male (n = 11). As expected, the majority of the subjects were women. There was an almost equal number of males (9) and females (10) in the control group. The mean body mass index (BMI) of the three groups fell within the overweight range.

Table II: The daily energy and nutrient intake of the irritable bowel syndrome group (combined constipation-predominant irritable bowel syndrome (C-IBS) and diarrhoea-predominant irritable bowel syndrome (D-IBS) (mean ± standard deviation) compared with dietary reference intake (recommended daily allowance or adequate intake where available)

Total daily intakes	IBS participants (n = 103)	RDA/AI	p-value
Energy intake (MJ)	8.63 ± 4.7	8.9[a]	0.560
Carbohydrates (g)	216.37 ± 101.86	130[b]	< 0.000
Protein (g)	88.01 ± 119.04	47.1[c]	< 0.000
Fat (g)		-	-
% energy from carbohydrates	44.68 ± 9.54	47[d]	0.015
% energy from protein	15.7 ± 6.51	15[d]	0.281
% energy from fat	36.54 ± 7.77	33[d]	< 0.000
Total dietary fibre (g)	15.13 ± 13.11	24.76[e]	< 0.000
Calcium (mg)	619.33 ± 308.04	1077.67[f]	< 0.000
Iron (mg)	11.26 ± 9.38	13.24[g]	0.035
Vitamin C (mg)	93.3 ± 89.68	76.6[h]	0.061
Folate (µg)	186.23 ± 103.01	400[i]	< 0.000
Vitamin A (µg)	712.86 ± 600.45	721.36[j]	0.886

AI: adequate intake, IBS: irritable bowel syndrome, RDA: recommended daily allowance
a: Estimated average requirement for energy intake, weighted for the study population[18]
b: Recommended dietary intake for carbohydrates[18]
c: Recommended dietary intake for protein, weighted for the study population. The recommended daily allowance for protein for men and women aged 31-70 years is 56 g/day and 46 g/day, respectively[18]
d: Population average, based on the UK survey[22]
e: Adequate intake for total dietary fibre, weighted for the study population. Adequate intake of total fibre for men aged 31-50 years and 51-70 years is 38 g/day and 30 g/day, respectively. Adequate intake of total fibre for women aged 31-50 years and 51-70 years, is 25 g/day and 21 g/day, respectively[18]
f: Adequate intake for calcium, weighted for the study population. Adequate intake of calcium for men and women aged 31-50 years is 1 000 mg/day, and for men and women aged 51-70 years, 1 200 mg/day[19]
g: Recommended daily allowance for iron, weighted for the study population. The recommended daily allowance of iron for men aged 31-70 years is 8 mg/day. The recommended daily allowance of iron for women aged 31-50 years and 51-70 years is 18 mg/day and 8 mg/day, respectively[19]
h: Recommended daily allowance for vitamin C, weighted for the study population. The recommended daily allowance for men aged 31-70 years is 90 mg/day and for women aged 31-70 years, 75 mg/day[20]
i: Recommended daily allowance for folate[19]
j: Recommended daily allowance for vitamin A, weighted for the study population. The recommended daily allowance for men aged 31-70 years is 900 µg/day, and for women aged 31-70 years, 700 µg/day[19]

The dietary intake of subjects with irritable bowel syndrome compared with that in the dietary recommendations

The mean (± SD) daily intakes for energy, macronutrients and selected micronutrients for the subjects are presented in Table II, and were compared to DRIs, recommended dietary allowances (RDAs) and adequate intakes, where available. Protein and carbohydrate intake was significantly higher than the RDAs for protein and carbohydrates (p-values < 0.000 and < 0.000, respectively). When this was expressed as the percentage energy intake from the diet, carbohydrate intake significantly differed to that of the population-based average in the UK study. However, protein intake did not.[20,21] There is no DRI for absolute intake of fat. However, the percentage of energy from fat was significantly higher than that of the population-based average in the UK study.[20,21] Daily total dietary fibre (15.13 g ± 13.11) was significantly lower (p-value < 0.000) than the DRI target intake of 24.76 g/day. The intake of micronutrients (calcium, iron and folate) was significantly less than the DRI (Table II). The subgroups, C-IBS and D-IBS, were also compared separately to the RDA. No significant differences were found when the two groups were combined, except for iron intake. The dietary intake for the constipation-predominant IBS group was not significantly different to RDA, but it was significantly different for the diarrhoea-predominant IBS group (9.925 µg ± 5.786) (p-value = 0.002).

The dietary intake of participants according to the different groups (constipation-predominant irritable bowel syndrome and diarrhoea-predominant irritable bowel syndrome and controls)

The data were analysed to establish whether or not there was any difference between the IBS subtypes and the control group. The mean (± SD) daily intake of energy, macronutrients and selected micronutrients are summarised in Table III. Total energy intake and macronutrient distribution did not differ significantly between the groups. The constipation-predominant IBS group had the highest intake of fat at 86.6 ± 62.78/day.

There was no significant difference in dietary intake between the three groups

Figure 1 shows that total dietary fibre intake did not reach the recommended intake for all three groups, but was

Table III: The daily energy and nutrient intake of the study population (mean ± standard deviation), constipation-predominant irritable bowel syndrome, diarrhoea-predominant irritable bowel syndrome and controls

Intake	D-IBS (n = 34)	C-IBS (n = 69)	Controls (n = 19)	p-value
Energy kJ (kcal)	8 036.44 ± 3 552.91 (1 913 ± 846)	8 928.9 ± 5 166.18 (2 126 ± 1 230)	7 808.65 ± 2 128.66 (1 859 ± 507)	0.476
Carbohydrates (g)	205.57 ± 89.61	221.7 ± 107.60	219.07 ± 58.16	0.710
Protein (g)	70.23 ± 38.15	96.77 ± 142.53	75.21 ± 26.43	0.466
Fat (g)	78.92 ± 42.39	86.8 ± 62.78	68.97 ± 27.52	0.411
Carbohydrates (% energy)	44.16 ± 8.49	44.93 ± 10.07	49.17 ± 8.82	0.157
Protein (% energy)	14.81 ± 3.42	16.13 ± 7.57	16.32 ± 3.17	0.541
Fat (% energy)	36.98 ± 8.37	36.31 ± 7.51	32.91 ± 7.78	0.167
Saturated fatty acids	25.75 ± 12.31	29.32 ± 26.97	22.31 ± 10.38	0.417
Polyunsaturated fatty acids	18.61 ± 10.51	20.77 ± 11.98	15.06 ± 8.23	0.132
Monounsaturated fatty acids	26.90 ± 16.5	28.47 ± 25.11	24.19 ± 10.93	0.733
Cholesterol (mg)	246.03 ± 139.5	333.36 ± 407.73	250.01 ± 132.18	0.345
Total dietary fibre (g)	15.14 ± 9.22	15.13 ± 14.72	17.50 ± 6.04	0.746
Insoluble dietary fibre (g)	4.36 ± 2.91	3.66 ± 2.17	5.16 ± 3.16	0.064
Soluble dietary fibre (g)	3.96 ± 3.51	2.83 ± 1.66	3.93 ± 2.26	0.055
Calcium (mg)	572.60 ± 358.34	642.35 ± 279.98	662.68 ± 269.45	0.464
Iron (mg)	9.92 ± 5.79	11.92 ± 10.70	11.47 ± 5.06	0.561
Vitamin C (mg)	81.76 ± 71.45	98.97 ± 97.4	123.47 ± 108.54	0.293
Folate (µg)	177.46 ± 76.13	190.54 ± 114.22	189.32 ± 54.14	0.808
Vitamin A (µg)	582.04 ± 419.65	777.32 ± 665.26	899.11 ± 694.20	0.154

C-IBS: constipation-predominant irritable bowel syndrome, D-IBS: diarrhoea-predominant irritable bowel syndrome

higher in the control group than it was in the IBS groups. The IBS groups had a poorer intake of fibre compared to that in the controls. Although it was not statistically significant, there was a trend for the constipation-predominant IBS group to have the poorest intake of soluble fibre, when compared to both the D-IBS and control groups (p-value = 0.055).

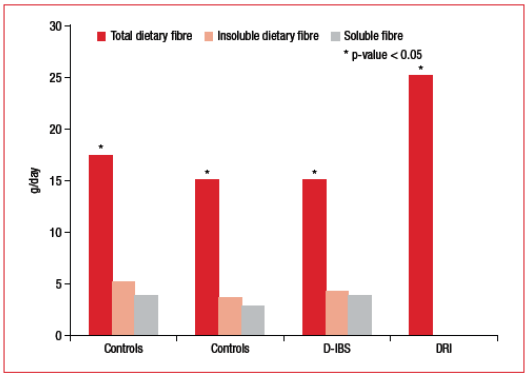
Fructose intake

Fructose intake was assessed in n = 115 subjects [consti-pation-predominant IBS (n = 64), D-IBS (n = 33) and controls (n = 18)]. The total number of fructose serves per day was not statistically significant among the three groups [constipation-predominant IBS (2.68 ± 1.68), D-IBS (2.15 ± 1.86) and controls (3.17 ± 2.39), p-value = 0.157]. The diarrhoea-predominant IBS participants had the lowest intake of fructose (in excess of glucose) per day. The high fructose intake in all three groups was mostly attributed to the intake of high corn starch-containing soda or fizzy drinks (the fructose to glucose ratio is 55:45), and secondly to fruits with a high fructose content.

Discussion

The purpose of this observational study was to assess the dietary intake of subjects with IBS and to establish whether or not any alteration in dietary intake impacted on nutrient intake, or whether or not any dietary intake could explain the presentation of IBS symptoms.

The results of this study revealed nutritional inadequacies in the IBS population. The subjects in this study reported on the consumption of a diet with a macronutrient composition that met recommended intakes, with the exception of total dietary fibre intake, which fell



C-IBS: constipation-predominant irritable bowel syndrome, D-IBS: diarrhoea-predominant irritable bowel syndrome, DRI: dietary reference intake
*:p-value < 0.05 was significantly different to dietary reference intake

Figure 1: The dietary fibre intake of constipation-predominant irritable bowel syndrome, diarrhoea-predominant irritable bowel syndrome and the controls, compared to the dietary reference intake

significantly below the recommendation. The latter finding is similar to that of Park et al.[8] In that study, it was observed that American and European diets contain inadequate fibre. A further study by Park et al also highlighted poor fibre intake in a group of women with IBS.[22] A randomised, multicentre, open clinical trial[23] compared the effect of a partially hydrolysed guar gum (PHGG) supplement (soluble fibre) with that of a wheat bran supplement (insoluble fibre) on a large number of patients with C-IBS and D-IBS. Significant improvements in bowel movement normalisation and abdominal pain relief were found in patients receiving the 5 g/day PHGG, compared to those receiving the 30 g/day of wheat bran. In a recent systematic review

and meta-analysis of 12 studies that compared fibre with placebo or no treatment in 591 patients, ispaghula husk (soluble fibre) and not wheat bran, was shown to be an effective treatment for IBS.[24] The latter finding highlights the beneficial effects of increasing soluble fibre, particularly PHGG, in patients with C-IBS and D-IBS.

Short-chain fatty acids (SCFAs) are linked to a decreased likelihood of IBS.[24,25] The rate and amount of SCFA production depends on the species and number of microflora present in the colon, the substrate source and gut transit time.[26] The poor fibre intake seen in the participants with IBS in this study would influence SCFA production. SCFAs aid in the relaxation of resistance vessels in the colonic vasculature, help support blood flow to the liver and colon,[27] and enhance colonic muscular contraction, thereby contributing to laxation and relief from constipation.[28]

Therefore, SCFAs may alleviate certain IBS symptoms, particularly if altered central nervous system sensory processing and disturbed autonomic nervous system regulation are aetiological factors for IBS.[29] In a study by Tana et al, patients with IBS showed significantly higher levels of acetic, propionic, and total organic, acid, than that in controls.[30] Higher organic acid levels were also associated with more severe symptoms and impaired quality of life.

Recent published data strongly suggest that dysbiosis in the microbiota of patients with IBS patients plays an important role in the presentation of IBS. The faecal microbiota of patients with IBS differs significantly to those of healthy subjects.[31] Tana et al showed that patients with IBS had significantly higher counts of *Veillonella* and *Lactobacillus,* but did not differentiate these findings between IBS subtypes.[30] Codling et al found that there was significantly more variation in the gut microbiota of healthy volunteers than in those of patients with IBS, and concluded that the gut microbiota within the colon are influenced by the disease in IBS.[32] Owing to the positive role that fibre plays in SCFA production and gut microbiota symbiosis, the recommendation to increase soluble fibre intake, in particular, in this group of patients with IBS may be beneficial.

The C-IBS group had the highest BMI, although it was not significantly different from that of the diarrhoea-predominant IBS and control groups. A potential relationship exists between overweight/obesity and constipation. A few studies have reported on an association between obesity and constipation.[33,34]

Inflammation may play a role in functional gastrointestinal disorders. It has been hypothesised that obesity may increase the risk of functional gastrointestinal disorders, owing to the release of proinflammatory cytokines. Secondly, the association could be attributed to excess abdominal fat exerting extra pressure on the colon, leading to slower mobility and possible metabolism. Or alternately, the longer that fermentable food remains in the colon, the longer the gut microbiota can ferment it and release extra energy, contributing to obesity. The higher fat content of the C-IBS subjects diets could have aggravated the development of symptoms in this subset of subjects. Dietary advice to decrease dietary fat content may be valuable.[35]

To date, there has been no evidence that patients with functional bowel symptoms consume more FODMAPs than those without symptoms.[36] In this study, it was found that subjects with IBS consumed a very comparable amount of fructose to that of healthy controls. The number of fructose serves per day eaten by IBS subjects was similar to that found by Croagh et al.[37] Fructose is a common part of the Western diet. FODMAPs are not the cause of functional bowel disorders, but may be a useful tool in the dietary treatment and management of IBS.[38] The efficacy of a dietitian-delivered, low-FODMAP diet has been demonstrated in published studies. Reducing the dietary intake of FODMAPS has been shown to lead to a marked improvement in bowel symptoms.[7,39,40] In view of the latter findings, it is probable that the IBS subjects in this study would benefit from dietary counselling aimed at a reduction in fructose intake, particularly as the majority of fructose consumed by the subjects was from high-fructose corn syrup in sweetened cold drinks.

The IBS subjects in this study had a poor micronutrient intake of calcium, folate and iron. However, the symptoms for a poor calcium, folate and iron intake are not synonymous with IBS symptoms. Hence, it would appear that subjects altered or restricted their diets in order to manage their IBS symptoms, which may have adversely affected their nutrient intake.

The D-IBS group showed an insufficient intake of vitamin A. This is similar to the findings of Aller et al.[41] Vitamin A and its derivatives have been shown to regulate the growth and differentiation of gastrointestinal epithelial cells. Amit-Romach et al showed that a vitamin A-deficient diet compromised the function of the gastrointestinal mucosal epithelial barrier.[42] This may be one of the underlying mechanisms in the aetiology of diarrhoea-predominant IBS.

There are inherent limitations in utilising an estimated three-day food record to assess nutritional intake. This prospective method of dietary assessment may be influenced by the presence of active IBS symptoms, which could have distorted usual dietary intake. Hence, it is important to concurrently assess symptom severity. Further research to assess the validity and reliability of the three-day food record or diary in patients with IBS would be useful.

Lastly, supplementation usage was recorded, but not reported, because less than 10% of subjects used supplements (varying brands).

Conclusion

The findings of this study suggest that IBS may have an impact on the dietary intake of patients with IBS. Therefore, patients with IBS should be assessed for inadequate intake of key nutrients, specifically fibre. Dietary advice should be given and supplements recommended, where appropriate. Patients with IBS may be at risk of low micronutrient intake.

All three groups demonstrated a poor fibre intake, which is a growing concern in the Westernised diet.[43] There were more similarities than differences in the IBS groups and controls with regard to the consumption of foods that have frequently been implicated to worsen IBS symptoms. The lack of differences in dietary intake may be the result of an absence of awareness or education that certain food may worsen symptoms, namely, fructose. This prospective study evaluated and compared the dietary content in C-IBS and D-IBS subjects, and found the diets to be deficient in micronutrients and fibre. The IBS subjects may have adjusted their diets to alleviate symptom development, and this may have resulted in nutritionally deficient diets.

Patients with C-IBS were found to have a higher fat intake, while those with D-IBS had an insufficient vitamin A intake, both of which could have contributed to their IBS symptoms. It is recommended that patients with C-IBS and D-IBS are managed differently as their clinical presentation may vary in pathogenesis. Further research on the cause-effect relationship between dietary composition and the resultant SCFAs or gut microbiota is needed. The role that the organic acids and gut microbiota composition play in the aetiology of IBS symptoms has yet to be strongly demonstrated. There is a dire need for research that demonstrates a strong relationship between dietary composition, microbiota and SCFAs found in patients with either C-IBS or D-IBS. The role that diet plays with regard to microbiota and their by-products, and to what extent this may contribute to the pathogenesis of C-IBS and D-IBS, also requires further exploration.

Declaration

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Conflict of interest

Prof Roux, Prof Blaauw, Dr Fredericks and Mrs Visser declare no potential conflict of interest.

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CHAPTER 4 ADDENDUM 1

Usual intake distributions were calculated and are presented in **Table 1**. The mean intake of the estimated three day food record, non-consecutive days, was calculated to represent the observed intake distributions for energy, macro and micronutrients. In this study adjustments to the observed intake distribution to obtain usual intake distribution estimates were made using the method described by Carriquiry.[1] This is because, in all probability, an individual's observed intake during a particular three-day period will differ from observed intake in a different three day period, and both three-day observed intakes will differ from true usual intake. An individual's observed mean intake over a few days may not be an accurate estimate of that individual's usual intake.[2] A number of statistical methods that have been developed to estimate usual intake distributions from observed intake distributions obtained using food records.[2]

Table 1. Prevalence of risk of inadequate nutrient intakes in the IBS (combined C-IBS and D-IBS groups) group using the EAR cut-point method[a]

Nutrients	EARs or EER or AI	IBS participants (n = 103) mean intake	Prevalence below EAR (%) or p value
Energy (MJ)	9.28[b]	7.45	p < 0.0000
Carbohydrates (g)	100[c]	189.11	4.85
Protein (g)	76.29[d]	58.60	85.44
Total dietary fiber (g)	25.04[e]	10.72	96.12
Calcium (mg)	873[f]	529	95.15
Iron (mg)	6.83[g]	6.33	61.17
Vitamin C (mg)	62.2[h]	64.5	67.96
Folate (µg)	320[i]	157.67	99.03
Vitamin A (µg)	518.4[j]	512.49	66.02

EAR – Estimated Average Requirement, EER – Estimated Energy Requirement, AI – Adequate Intake, IBS – Irritable Bowel Syndrome

[a]The Estimated Average Requirement (EAR) is the appropriate Dietary Reference Intake to use when assessing the adequacy of group intakes. The EAR is defined as the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group. Comparing the mean nutrient intake of a group either to the EAR or the Recommended Dietary Allowance (RDA) should not be used for assessment or to imply relative nutrient adequacy.[2] The EAR cut-point method, used in Table I above, examines the prevalence of nutrient inadequacy in groups by estimating the proportion of individuals in the group with usual intakes below the EAR (the median requirement) for a specific nutrient.[2]

[b]Weighted for the study population for males and females as per Estiamted Energy Requirment (EER) formula, assumption of physical activity factor of 1.12 (low active) as average BMI >25kg/m^2. Due to the high correlation between energy intakes and requirements, energy adequacy cannot be assessed using either the probability approach or the cut-point method[2] and a t - test was used to test for significance.

[c]EAR for carbohydrates for males and females ≥ 19 years is 100g/day.

[d]EAR for protein for males and females ≥ 19 years is 0,66g/kg/day.

[e] Adequate Intake (AI) for total dietary fiber, weighted for the study population, AI for total fiber for males 19-50 years is 38g/day and males' ≥ 51 years is 30g/day; for females 19-50 years is 25g/day and ≥ 51 years is 21g/day.

[f]EAR for calcium, weighted for the study population, EAR for males and females 19 – 50 years is 800mg/day; females 51-70 years is 1000mg/day; males 51-70 years is 800mg/day; males and females > 71 years is 1000mg/day.

[g] EAR for iron, weighted for the study population, EAR for all males ≥ 19 years is 6mg/day; females 19 – 50 years is 8,1g/day; females' ≥ 51 years is 5mg/day.

[h]EAR for vitamin C, weighted for the study population, EAR for males ≥ 19 years is 75mg/day and for females ≥ 19 years is 60mg/day.

[i]EAR for folate for males and females is 320mg/day.

[j]EAR for vitamin A, weighted for the study population, EAR for males ≥ 19 years is 625µg/day and for females ≥ 19 years is 500µg/day.

Table 2. Comparisons of percentage macronutrient intakes with the AMDRs[a] (adults > 18 years; using usual intake distributions) in the IBS group (D-IBS and C-IBS groups combined)

		Prevalence below AMDR	Prevalence within AMDR	Prevalence above AMDR
	AMDR	% IBS	% IBS	% IBS
Protein (%)	10-35%	8.74	91.26	0
Fat (%)	20-35%	2.91	62.14	34.95
Carbohydrate (%)	45-65%	60.19	39.81	0

AMDR – Acceptable Macronutrient Distribution Range; IBS – Irritable Bowel Syndrome

[a]The AMDRs are defined as range of intakes for a specific energy source that is associated with reduced risk of chronic diseases while contributing adequate intakes of essential nutrients. Although mainly directed at individuals, the AMDRs also allow for assessment of groups and populations.[3] By determining the proportion of the group that falls below, within and above the AMDRs, it is possible to determine the proportion that is outside the range and to examine adherence to recommendations. If considerable proportions of the group fall outside the range, concern for potential adverse consequences could be increased.[3]

ADDENDUM 1 OF CHAPTER 4

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Chapter 5

Validation and test-retest reliability of estimated food records in irritable bowel syndrome

Manuscript

Validation and test-retest reliability of estimated food records in irritable bowel syndrome patients

Authors: Cheryl Stevenson[1], cheryl@retaildc.co.za

Renee Blaauw[1], rb@sun.ac.za

Diana Kimono[2], dianakim35@yahoo.com

Janicke Visser[1], jconrad@sun.ac.za

Saartjie Roux[2], saartjie.roux@nmmu.ac.za

[1] Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

[2]Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa

Corresponding author: C Stevenson

Postal Address: Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg 7505, Cape Town, South Africa

Telephone: +27 21 938 9259

Email: cheryl@retaildc.co.za

Fax number: +27 21 933 2991

ABSTRACT

Background: The reliability and validity of dietary data gathered from irritable bowel syndrome (IBS) patients is unknown. This would be useful information to know, especially when patients insist that diet or trigger foods play a part in symptom generation.

Aim: To examine the reliability and validity of three day estimated food records in a group of IBS patients.

Design: Patients attending a private gastroenterology clinic in South Africa were recruited as part of a larger IBS study. A small subsample of the total study group (n = 81) were used to assess validity (n = 5, 6.2%) and reproducibility (n = 6, 7.2%). Reliability was assessed with a test-retest (eight week interval) of a three day estimated food record. Validity was assessed using dietary fatty acid (FA) intake from three day food records and comparing to plasma FA profiles. Validity was analysed using Pearson and Spearman correlation coefficients and paired T-tests were used to analyse reliability. Both absolute and percentage total FA intake were used for plasma and dietary FAs.

Results: Correlation coefficients for validity ranged from -0.03 to 0.69, $p > 0.05$. None of the macro and micronutrients differed significantly from each other in the reliability testing, except that of percentage energy intake from protein 12.33 ± 1.29 vs 17.48 ± 3.18g/day ($p = 0.015$).

Conclusions: A three day estimated food record in a group of IBS patients demonstrated good reliability, however using plasma FAs to validatie dietary intake data was poor, suggesting that further research and testing are needed in a larger grouping of IBS patients.

Keywords: irritable bowel syndrome, validity, reliability, dietary intake, biomarker

BACKGROUND

Irritable bowel syndrome (IBS) is a chronic disorder characterized by abdominal pain or discomfort associated with disordered defecation, either constipation predominant IBS (C-IBS), diarrhoea predominant IBS (D-IBS) or mixed/alternating symptoms of constipation and diarrhoea [1]. The natural history of IBS is one of relapsing and remitting symptoms [2]. The aetiology of IBS is multi-faceted and ill defined, however the majority of IBS patients believe that certain food items are important triggers of their gastrointestinal (GI) symptoms. This is especially true for foods containing carbohydrates and fat and also may be relevant for histamine-releasing food items and foods rich in biogenic amines [3]. Many IBS patients associate one or more foods with the onset of IBS symptoms and approximately two thirds of IBS patients exclude food items from their diet to improve symptoms [4, 5]. The validity and reliability of dietary data gathered from IBS is necessary as patients insist that diet or trigger foods play a part in symptom generation. This may lead to them avoiding certain foods and consuming nutritionally deficient diets. Generally, fairly large numbers are needed for the reliability and validity testing of dietary data and this is possibly a reason why this data is not always reported. However to give credibility to dietary data, validity and reliability testing is required. In this study we assess the validity and reliability of dietary data in a small subsample of IBS patients.

The reliability of a dietary assessment method reflects the ability of the method to obtain identical results when administered at a later stage under similar circumstances [6]. The reliability of dietary data is not as often reported on as the validity. Validity reflects the ability of a dietary method to accurately measure what the participants have actually eaten [7]. Dietary methods designed to characterize usual intakes of individuals are the most

difficult to validate, since the 'truth' is never known with absolute validity [8]. Relative validity, in which a new method (i.e. test method), is compared with an existing method known to be valid (i.e. reference method), is the most practical validation method to use. Absolute validity implies that the reference method reflects the true dietary intake, while relative validity recognizes that the reference method itself is subject to error [7]. Therefore, the extent of agreement between the test and reference methods is used to indicate the relative validity of the test method and the extent to which the reference method is believed to be the truth.

While food records are often used as a reference method for the determination of the relative validity of other dietary assessment methods, few studies report on the validity and reliability of food records themselves. Fat is one of the most difficult dietary components to measure for several reasons. Fat is sometimes very difficult for an individual to recognise and quantify. For instance, fat used in food preparation for frying and cooking or as sauces and dressings – is often added by someone other than the individual under study, making it nearly impossible to identify the source and brand of fat. Even if this were known, it would be particularly tedious to report in detail [9]. In addition, the accuracy of reporting fat is especially prone to bias. Underreporting of fat intake is greater among individuals whom are overweight because of social implications [10].

Biomarkers are being increasingly used in nutritional epidemiology to assist in dietary measurement and to deal with the problems associated with self-reported intakes. The use of biomarkers, such as plasma fatty acids (FA), is a more objective approach to assess the validity of dietary intake data [11]. The fundamental advantage of using a biomarker is that

measurement errors are unrelated with errors in any dietary assessments, e.g. do not rely on memory, self-reported information or interviewer bias.

No biomarkers reflect absolute fat intake, however, measurement of FAs in various biological samples reflects to some extent, proportional intake of FAs [9]. The intake of FAs may be reflected in various serum (or plasma) lipids, platelet and erythrocytes phospholipids. The FA composition of plasma lipids reflects the type of dietary fat and may be an objective estimate of the type of fats proportionally consumed by an individual. The FA composition of plasma reflects medium-term (weeks to months) dietary intake [12]. Essential polyunsaturated FAs (linoleic acid and α-linolenic acid) cannot be synthesized *de novo* by humans and play an important role in health. As the source of these biologically active FAs is exogenous they may be particularly good biomarkers markers to use [9].

In IBS patients, actual dietary intake, has not been extensively reported. To date there have only been a few prospective studies assessing dietary intakes of IBS patients [13-15]. The reliability and validity of IBS dietary data is unknown and to the author's knowledge there is no published data. This is pertinent information to know in a condition where diet is thought to play such a significant role. The aim of this methodological study was to assess the validity and reliability of dietary data gathered from estimated food records in a group of IBS patients. This sub-group of patients (n = 6, 7.4% for reliability and n = 5, 6.2% for validity testing) formed part of a larger IBS research study (n = 81). More specifically the null hypothesis of whether three day estimated food records demonstrate poor i)reproducibility and ii)validity in IBS patients will be tested.

METHODS

Study Participants

Participants for this study were a sub-group randomly selected from a larger probiotic clinical trial (Clinical Trials Registry number NCT01886781), where n = 81. The study was approved by the Research Ethics Committee at Stellenbosch University (reference number: N10-08-270) and was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki. Written informed consent to participate was obtained from each participant on enrolment. For the reliability (test-retest) analysis 7.4% (n=6) of the total study sample were selected and a further 6.2% (n=5) for the validity of the dietary information study.

Medical information

Participants were screened by a gastroenterologist and recruited according to the study inclusion criteria and their willingness to participate. The diagnosis of IBS was made using Rome II criteria. Pertinent medical information collected included recent antibiotic usage, current medication, background medical history, previous bowel surgery, family history of IBS, smoker/non-smoker, duration of IBS, triggers for IBS symptoms and 'anti-IBS specific' medication. Participants underwent physical measurements, including weight and height, which were used to calculate body mass index (BMI kg/m^2).

Dietary Assessment

A registered dietitian explained and trained each participant on the procedure for completing a prospective three day estimated dietary record (two weekdays and one weekend day included). Portion sizes were estimated using household food measures like spoons, cups and bowls and a ruler, food portion photographs were also utiliased (MRC kit). The importance of recording food(s) eaten immediately after it was eaten was emphasized. The results were

analysed by FoodFinder™ III – a computer-based data evaluation system for South African foods [16]. For the validity testing, the 3-day estimated food record was correlated against plasma FA profiles, and the reliability of the dietary data was done by a test-retest with an eight week time difference between the two three day estimated food records.

Blood sampling, processing and analysis

A five ml of blood was collected from each participant in a sterile vacutainer, with a clot-activated gel, using a 21G needle. Blood samples were collected following a 12 hour fast. Blood samples were taken within the same week as the three day estimated food records were completed. Samples were left to stand for 15 min then centrifuged for 20 min at 3500g. The serum was transferred into small sterile tubes and stored in freezer at -22°C for a maximum of a month before being transferred to a -80°C freezer until analyses. Samples were thawed at room temperature. Four hundred microlitres of each sample was spiked with 200µl/l of internal standard (C17.0) then extracted using a modified Bligh and Dyer method [17]. The samples were extracted in 2ml chloroform methanol (2:1) and one ml of 0.05% sulfuric acid for 60 minutes each. One ml of 0.9% NaCl solution saturated with extraction solvent was added, and further shaken. The samples were then centrifuged at 2000g for 30 minutes. The aqueous and protein layer discarded and the organic phase, containing the lipids, was filtered completely dried under a stream of nitrogen gas at 40°C and the mass of extracted lipid determined. The obtained lipid was then derivatised in 3% methanoic sulphuric and methyl esters (FAMEs) extracted in hexane. The FAMEs were diluted (X2) and analysed by gas chromatography (model 7890, Agilent) and mass spectroscopy (model 5975, Agilent) (GC-MS). The FAMEs were identified using an 11 component custom mix (Sigma) and a 37 component mix (Sigma), on the highly polar HP-88 column (J&W 112 88-A7; 100m X 0.25 ID X 0.2 micrometers; Agilent technologies). The GC and MS data obtained was analysed using Chemstation software (Agilent). The GC-MS analytical conditions used were as

follows: Injection volume 1 µl (split-less mode), carrier gas Helium (2 ml/min constant flow); inlet temperature 250^{o}C while the detector temperature was 280^{o}C. The oven temperature was at 120^{o}C for one minute then raised to 170^{o}C at 10^{o}C/min, 210^{o}C to 5^{o}C/min for five minutes. And finally increased to 230^{o}C at 5^{o}C/min and held for seven minutes.

Statistical analysis

In order to assess the validity the Pearson and Spearman correlation coefficient were calculated. This was done for the absolute FA values as well as percentage of total FAs. Correlations were evaluated as poor (<0.2), moderate (0.2-0.6) or good (>0.6). To evaluate the reproducibility, paired T-tests between the first and second food record (or test) were calculated. A p value < 0.05 was considered as significant.

RESULTS

Validity testing

Demographic data of the participants for the validity study are shown in **Table 1**. All five participants were women. The average BMI fell within the normal range and one (20%) participant was a smoker.

Table 1 Demographics of validity study population (n = 5)

Demographics	Mean ± SD
Age (years)	49.91 ± 11.23
Height (cm)	1.65 ± 0.11
Body weight (kg)	67.6 ± 21.67
BMI (kg/m^2)	24.58 ± 5.68
Smokers (percentage current)	20% (n=1)

BMI – Body mass index

Table 2 shows the dietary data of selected key nutrients. Dietary data indicate a macronutrient composition within the normal recommendations with regards to percentage total energy contributions.

Table 2 Dietary data of validity study population (n = 5)

Dietary intake	Mean ± SD
Energy intake (MJ/day)	7.9 ± 2.3
Total fat intake (g/day) (percentage of total energy)	63.72 ± 26.68 30.65 ± 7.50
Protein intake (g/day) (percentage of total energy)	80.34 ± 4.71 19.16 ± 7.88
Carbohydrate intake (g/day) (percentage of total energy)	208.07 ± 95.38 42.43 ± 12.41
Alcohol intake (g/day) (percentage of total carbohydrate)	9.02 ± 8.87 4.15 ± 4.39
SFA (g/day) (4:0) (6:0) (8:0)	 0.43 ± 0.26 0.24 ± 0.14 0.17 ± 0.10

(10:0)	0.37 ± 0.19
(12:0)	0.7 ± 0.35
(14:0)	2.41 ± 0.94
(16:0)	11.0 ± 3.62
(18:0)	5.11 ± 1.68
(20:0)	0.12 ± 0.21
(24:0)	0.07 ± 0.08
MUFA (g/day)	
(14:1)	0.11 ± 0.06
(16:1)	1.08 ± 0.71
Oleic (18:1)	18.3 ± 8.56
(20:1)	0.13 ± 0.09
(22:1)	0.13 ± 0.15
n-6 PUFA (g/day)	
Linoleic (18:2n-6)	11.65 ± 7.78
(percentage of total energy)	5.6 ± 3.74
Arachidonic (20:4n-6)	0.07 ± 0.04
n-3 PUFA (g/day)	
Linolenic acid (18:3n-3)	0.40 ± 0.16
(18:4n-3)	0.01 ± 0.01
(20:5n-3)	0.03 ± 0.03
(22:5n-3)	0.02 ± 0.01
(22:6n-3)	0.14 ± 0.15

MJ – megajoule; SFA – short chain FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA

Table 3 shows the results of the plasma FA composition. Mean FA (±SD) compositions of plasma are shown. The FA intake is expressed as absolute intake (mg/ml) and percentage of total FA in the plasma. No C18:3n3 was detected in the plasma of any of the participants.

Table 3 Plasma FA composition (n=5)

FA	Plasma FA Absolute value (mg/ml)	Plasma FA Percentage of total FAs
SFA		
(14:0)	0.0152 ± 0.0156	0.998 ± 1.110
(16:0)	0.5104 ± 0.0965	34.36 ± 8.47
(18:0)	0.1738 ± 0.0780	11.10 ± 2.68
MUFA		
C18:1n7	0.1532 ± 0.1657	9.26 ± 9.86
C18:1n8	0.2076 ± 0.1761	15.16 ± 13.14
C18:1n9	0.0968 ± 0.1661	5.31 ± 8.12
n-6 PUFA		
Linoleic (18:2n-6)	0.272 ± 0.0995	17.97 ± 6.25
Arachidonic (20:4n-6)	0.092 ± 0.0463	5.84 ± 2.54

FA – FA; SFA – short chain Fas; MUFA – monounsaturated Fas; PUFA – polyunsaturated FAs

Correlations between the FA composition of plasma and dietary intake were calculated and are presented in **Table 4**. None of the dietary FAs and plasma FAs correlate significantly.

Table 4 Correlation coefficients between dietary intake (g/day) vs plasma FAs (mg/ml) and percentage TFA between dietary intake and plasma

FA	Pearson's correlation				Spearman's correlation			
	g/day vs mg/ml	p value	Percentage of TFA	p value	g/day vs mg/ml	p value	Percentage of TFA	p value
SFA								
C14:0	-0.46	0.43	-0.01	0.99	-0.15	0.80	-0.36	0.5
C16:0	-0.63	0.25	-0.43	0.47	-0.80	0.10	-0.60	0.28
C18:0	-0.72	0.17	-0.48	0.41	-0.10	0.87	-0.30	0.62
PUFA								
C18:2	-0.03	0.96	0.25	0.69	0.05	0.93	0.10	0.87
C20:4	0.69	0.19	0.26	0.67	0.67	0.22	0.30	0.62

TFA – total Fas; SFA – short chain Fas; PUFA – polyunsaturated FAs

Reliability testing

Demographic details of the participants for the reproducibility study are shown in **Table 5**. A different six participants were involved in this study and all were women. The average BMI fell within the overweight range.

Table 5 Demographics of reproducibility study population (n = 6)

Demographic	Mean ± SD
Age (years)	45.79 ± 17.32
Height (cm)	1.62 ± 0.06
Body weight (kg)	68.67 ± 6.12
BMI (kg/m^2)	26.39 ± 0.20
Smokers (percentage current)	33% (n = 2)

BMI – Body mass index

The dietary data of selected key nutrients (mean ± SD) and p value for paired T-test is shown in Table 6. The assessed nutrient intake resulting from the second interview does not differ significantly from that of the first interview, except for percentage energy from protein (12.33 ± 1.29 % vs 17.48 ± 3.18, p = 0.015). The dietary data indicates good reliability in this group.

Table 6 Dietary data of reproducibility group (n=6)

Dietary component	Test (1)	Retest (2)	p value (paired T-test)
Energy intake (MJ/day)	6.94 ± 1.88	5.98 ± 1.84	0.386
Total fat intake (g/day) (percentage of total energy)	68.25 ± 16.90 37.64 ± 4.69	55.87 ± 19.80 35.63 ± 4.82	0.395 0.451
Saturated fat (g/day)	21.00 ± 5.16	18.48 ± 6.39	0.560
Monounsaturated FAs (g/day)	20.95 ± 4.69	19.37 ± 8.26	0.747
Polyunsaturated FAs (g/day)	19.79 ± 7.76	11.45 ± 5.21	0.141
Cholesterol (mg/day)	203.99 ± 163.53	189.43 ± 65.12	0.858
Protein intake (g/day)	51.29 ± 17.84	59.41 ± 15.23	0.396

(percentage of total energy)	12.33 ± 1.29	17.48 ± 3.18	0.015*
Carbohydrate intake (g/day) (percentage of total energy)	194.67 ± 55.40 47.59 ± 3.64	154.32 ± 48.95 43.61 ± 5.32	0.079 0.451
Total dietary fiber (g/day)	13.28 ± 6.86	12.65 ± 9.33	0.892
Insoluble dietary fiber (g/day)	2.88 ± 1.62	2.71 ± 1.71	0.807
Soluble fiber (g/day)	2.32 ± 1.30	1.92 ± 1.11	0.420
Alcohol intake (g/day)	0.400 ± 0.980	2.22 ± 4.34	0.366
Vitamin A (RE/mcg/day)	495.95 ± 193.42	629.01 ± 608.90	0.506
Vitamin C (mg/day)	37.61 ± 21.91	56.83 ± 29.36	0.082
Folate (mcg/day)	172.89 ± 79.27	210.88 ± 113.29	0.439
Calcium (mg/day)	559.32 ± 241.95	488.92 ± 291.70	0.595
Iron (mg/day)	8.98 ± 2.46	10.90 ± 6.55	0.439

* < 0.05

DISCUSSION

This study has shown good reproducibility and poor validity of dietary intake in a group of IBS patients using three day estimated food records and as such rejects the first part of the null hypothesis (that poor reliability would be demonstrated with food records) and accepts the second part of the null hypothesis that the validity as assessed by plasma fatty acids was poor. Many IBS patients report restricting their dietary intake or eliminating certain provocative dietary agents in order to reduce their symptoms. The restriction of certain trigger foods could potentially distort macro- and micronutrient intake and place IBS individuals at risk of low nutrient intake [18]. This study has shown that with a fairly short assessment tool (a three day food record) dietary data is reliable in IBS in spite of them

reducing some dietary items when necessary. In the literature the validity of an estimated food record is primarily determined by comparing this method with a weighted three day or longer food record. However this study used plasma FAs to validate dietary FA intake and showed poor validity and indicates that larger numbers are needed to assess the use of this biomarker in IBS patients.

Biomarkers of dietary fat intake have been more difficult to identify than for other nutrients. The ideal biomarker of dietary fat would be able to reflect both quantity and quality, but currently no marker of total fat intake exists [9]. FA composition is regulated leading to typical profiles that reflect the function and origins of the fraction. Blood FAs, because they are mainly derived from the diet, are useful biomarkers of dietary intake and PUFAs have been shown to be the most useful as biomarkers of dietary intake [9]. Competition between metabolic pathways may lead to changes in FA composition not directly related to diet [19]. Biomarker level measured in biological samples takes into account any effects of absorption, influences of microbiota (e.g. bioconversion, release of bioactive dietary compounds, enterohepatic circulation), interactions between nutrients, tissue turnover, metabolism and turnover [20]. Additional considerations are issues pertaining to nutrient bioaccessibility and bioavailability [21].

The effects of genetic variability in FA biochemistry pathways, direct/indirect determinants of fat absorption, or gene-diet/nutrient or gene-gene interactions in FA profiles as dietary markers is largely unexplored [20]. For example, FA profiles may be altered by variations in genes encoding for enzymes in the elongase/desaturase pathway of n-3 and n-6 FA metabolism [22] or by interactions between high intake of dietary fat, obesity and variations

in FA binding protein 2 gene which may result in modulation of insulin resistance [23]. These examples indicate that genetic factors that affect FA metabolism may also affect the utility and application of these compounds as dietary biomarkers – although this remains to be validated [20]. The current understanding of dietary biomarkers is limited by a very incomplete comprehension of how genetics, diet and nutrients interact to affect metabolism [20].

The strength of the association between diet and biomarker depends on the FA analysed. FAs not synthesised endogenously such as 18:2 n-6, tend to correlate well with diet. In cross–sectional studies, PUFA content of the diet has shown to correlate well with FA composition in all adipose and all blood lipid fractions. Correlations are weaker for other SFA and MUFA [19], but SFAs are more subjected to hepatic elongation. Homeostatic feedback systems involved in storage and metabolic mechanisms help to keep plasma FA levels as close to normal as possible. This was illustrated by Poudyal *et al*. showing that chronic supplementation with chia seed (a rich source of α-linolenic acid (ALA), increased docosahexaenoic acid (DHA) accumulation in both the heart and liver, although no such change was seen in the plasma [24]. For some FAs, a possible explanation may be that it is difficult to modify the proportion of a FA that is already present in a relatively high percentage in the diet or in the body. Therefore large numbers are needed for cross-sectional or epidemiological studies.

The sample size in validation studies is usually small because they are logistically difficult and costly to carry out [25] and reproducibility and validity studies are generally conducted in a subsample of a population being investigated as a part of a larger investigation. Cade *et al.*

in their reviews of food frequency questionnaires undertaken found a wide range of sample sizes ranging from 6 to 3750, with a median sample size of 110 subjects [26] and mean sample size of 255 subjects [27]. They found that the sample size of the validation study did not seem to greatly affect the study results. Cade *et al.* reviewed the test and dietary intake reference method correlation coefficients and found sample sizes of between 100 -200 subjects to be sufficient. However, they indicated that some studies to not manage to come anywhere close to these sizes and that sample size inevitably depends on the available resources [26]. In terms of percentages, subsample sizes have ranged from between 0.25% to 4.1%, although in terms of absolute amounts these were large studies by Astorg *et al.* and Sun *et al.* respectively [28, 29]. Differences between the sample used for the validity study and the overall study population with regard to education level, sex and age may influence the validity of the dietary assessment tool [30]. A limitation of the present study is the small sample size, which may mean that the results may be a result of random error. As this was a very small study population the results cannot be used to estimate the gain in power which would result from the larger study [31].

The plasma linoleic acid level in this study was 17.97%, this is comparable to those found in Japan [32], Australia [33] and America [34], reflecting the high intakes of linoleic acid in most countries (4-6% of energy) [35]. In a systematic review by Hodson *et al.* plasma total FA composition in 472 men and 510 women from 9 studies was reported. The most abundant FAs were 16:0 (23.0 mol%), 18: 1n-9 (19.5 mol%) and 18: 2 n-6 (30.4 mol%), the latter was the most variable [19]. This differs from our study where 16:0 was the most abundant (34.36 mol%), followed by 18:2 n-6 (17.97 mol%) and 18: 1 n-8 (15.16 mol%). This study did not detect plasma linolenic acid. This may be because the proportion of FAs in the dietary lipid pool and not the diet plays a significant role in responses to the long chain

n-3 PUFA [24]. Surette *et al.* also raised the possibility that dietary omega-3 FAs compete with the omega-6 family of dietary polyunsaturated FAs for incorporation into all cell membranes and are preferentially incorporated [36].

Regardless of the level of detail obtained from participants, all assessments of nutrient intake are limited by the available food composition database. The availability of a wide range of manufactured and processed foods, coupled with variations in the fats and oils used in the industry, increases the difficulty associated with the estimation of the FA composition of foods and subsequent estimation of intakes [37, 38]. The difficulties associated with incomplete food composition databases have been highlighted [39, 40]. The present study used the most complete FA food composition database currently available for the South African food supply, The South African Food Composition Database System (SAFOODS). However, there are often situations where alternative food items are coded because a specific food item is not available in SAFOOD. In such cases, the coder searched for an item that best fitted the food item consumed [40]. Relative intakes of individual FAs in the diet are therefore, extremely difficult to estimate from reported dietary intakes.

A limitation of this study is that no correction was considered or accounted for the effect of plasma lipoprotein concentrations. The major lipoproteins in plasma (very low density lipoprotein, low density lipoprotein and high density lipoprotein) contain different proportions of phospholipids, triacylglycerol and cholesterol esters. Because phospholipids, triacylglycerol and cholesterol esters have a distinctive FA content and composition, a change in the concentration of one or more of the lipoproteins could potentially make a difference in the total plasma FA composition [19]. Generally there are low concentrations of n-3 PUFAs

and careful analysis is required to accurately measure this. It is also important to consider factors that may influence the relationship between diet and biomarker such as total dietary fat, blood lipid concentrations, recent dietary changes, smoking habits, exercise and in this study the effect of IBS, dybiosis in GI microbiota and low-grade inflammation, the influence of which is unknown. Measurement error is inherent in any biomarker [41].

This study demonstrates a good reproducibility of the three day estimated food record. The time elapsed between the two administrations in this study was eight weeks. It is recommended to administer the second questionnaire within a fairly short time 4-8 weeks, long enough so respondent is not simply remembering what they answered before and short enough to minimise real dietary changes [30]. There is a paucity of data evaluating the reliability and validity of three day food records in adults. One study by Trumble-Waddell *et al*. showed both good reliability and validity using estimated three day food records, a test-retest (6 week interval) and weighted three day food record for reliability and validity testing was used respectively in a group of preschool children, the data was provided by parents or caregivers [42].

In conclusion the results of this study indicate that a three day estimated food record gave reproducible results for estimations for energy, macro and micronutrient intakes in a small group of IBS patients. The dietary data as provided by three day food records showed poor validity when validated against plasma FA using a very small subsample of 6.2% (n = 5). This suggests that linoleic acid is possible not a good biomarker for validity testing for IBS dietary data.

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Competing interests

The authors declare they have no competing interests

Author's contributions

C Stevenson, R Blaauw, E Fredericks, J Visser and S Roux designed the research; C Stevenson and E Fredericks performed the research; C Stevenson analysed the data and C Stevenson, R Blaauw, E Fredericks, J Visser and S Roux wrote the paper.

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Chapter 6

The effect of nutrient intakes and a probiotic on gastrointestinal microbiota in irritable bowel syndrome patients

Manuscript

The effect of nutrient intakes and a probiotic on gastrointestinal microbiota in irritable bowel syndrome patients

Cheryl Stevenson, Renée Blaauw, Ernst Fredericks, Janicke Visser, Saartjie Roux

Introduction: Nutrient intakes and probiotics modulate the gastrointestinal (GI) microbiota and influence symptoms of irritable bowel syndrome (IBS). The extent to which these factors influence the microbiota is still relatively unknown.

Aim: Investigate the influence of nutrient intakes and a probiotic on the faecal microbiota and GI symptoms of IBS subjects

Methods This study formed part of a larger randomised controlled trial (RCT) assessing the efficacy of an eight-week treatment regime of *Lactobacillus plantarum* 299v on IBS symptoms and quality of life (QoL). During the RCT nutrient intake was recorded by three day estimated food records. Faecal samples were collected at three time points: 1) baseline (A), 2) after supplementation (B) and 3) following a two week washout period (C). Total *Bacteroides* spp., *Bifidobacteria bifidum* and *Lactobacillus plantarum* were quantified by quantitative polymerase chain reaction (qPCR).

Results: Fifty-two IBS patients were recruited [28 diarrhoea predominant IBS (D-IBS); 24 constipation predominant IBS (C-IBS)]. There was a significant difference between the C-IBS (includes treatment and placebo) and D-IBS (includes treatment and placebo) groups and the presence of *Lactobacillus plantarum* at baseline (point A) (-0.956±1.239 vs. -1.700±1.239; p=0.024). There was no significant change in bacterial counts after completion of the trial (B) and following the washout period (C) between C-IBS and D-IBS. There were strong inverse correlations in the total IBS (combined C-IBS and D-IBS) groups for *Bacteroides* and total (r=-0.424; p=0.019), insoluble (r=-0.406; p=0.023) and soluble fiber

(r=-0.466; p=0.008). A direct correlation was found for the same fiber fractions [(total (r=0.529; p=0.002), insoluble (r=0.465; p=0.008) and soluble fiber (r=0.433 p=0.015)] and *Lactobacillus plantarum*. In the D-IBS group correlations were found for protein intake and *Bifidobacteria* (r=0.497; p=0.036) and *Lactobacillus plantarum* (r=0.487; p=0.041). *Bifidobacteria* was correlated with insoluble fiber (r=0.523; p=0.026) intake and *Bacteroides* inversely correlated with total (r=-0.528; p=0.024) and soluble dietary fiber (r=-0.571; p=0.013). A strong correlation was found in the D-IBS group for percentage energy from fat and *Bacteroides* (r=0.617; p=0.006) and linolenic acid and *Bifidobacteria* (r=0.516; p=0.028). In the C-IBS group *Lactobacillus plantarum* correlated well with total dietary fiber (r=0.584; p=0.036). There was no difference in symptom severity scores between the treatment and placebo groups, (treatment group 272.89±93.76 to 209.08±110.63 vs. 277.56±88.12 to 185.30±96.52; p=0.800) over the trial period.

Discussion: No significant beneficial effects of the probiotic were observed on the severity of GI symptoms in IBS patients. The probiotic did not stabilise or exert changes on the GI microbiota analysed. Certain dietary agents (e.g. protein and fiber) strongly correlate to certain bacterial profiles, highlighting that nutrient intakes are undoubtedly a factor that significantly influences the GI microbiota composition.

Introduction

Irritable bowel syndrome (IBS) is a common multifactorial functional gastrointestinal (GI) disorder of unknown aetiology [1]. It is characterised by a variable combination of chronic and recurrent symptoms including abdominal pain or discomfort, irregular bowel movements, flatulence and constipation or diarrhoea. According to the stool consistency, IBS subjects can be divided into three subcategories predominant in diarrhoea (D-IBS), constipation predominant (C-IBS) or mixed, alternating (A-IBS) [1,2]. The mechanisms of pathogenesis behind IBS are only partly understood and cannot be traced to one factor. Proposed mechanisms include visceral hypersensitivity, abnormal motor function, low-grade mucosal inflammation, food intolerance, altered GI microbiota as well as psychosocial and genetic factors [3-5].

The human GI microbiota constitutes a complex ecosystem that is beneficial to the host under normal conditions [6]. GI infection or administration of antibiotics perturbs the GI microbiota composition and has been linked to the expression of dysfunctional GI symptoms [7]. One study showed that mice treated with antibiotics have a perturbed GI microbiota composition which was normalised with the administration of probiotics [8]. Clinical trials have demonstrated an improvement in IBS symptoms with the administration of probiotics in humans [9-11]. It therefore seems that the composition of GI microbiota plays an important role in IBS symptoms [12].

Although much has been discovered in the last decade about the GI microbiota, there are biases and limitations to the current knowledge related to trial study design, sample collection

and confounding variables, such as diet [13]. Diet is a factor that undoubtedly influences the composition of GI microbiota.

There have been relatively few randomised controlleed trials (RCT) that have assessed the effects of a probiotic on IBS symptoms and GI microbiota [10] and to the authors' knowledge none that have assessed the effect of diet on the GI microbiota in IBS subjects. Whether a disease-prone microbial composition can be transformed into a healthier composition by a probiotic or dietary interventions and improve patient sense of well-being remains fundamentally an unanswered question. The aim of the present study was to investigate the influence of nutrient intakes and *L.plantarum* 299v on the i) faecal microbiota and ii) GI symptoms of IBS subjects. More specifically the two null hypotheses being tested were: i) nutrient intakes do not have an influence on faecal microbiota and ii) *Lactobacillus plantarum* 299v does not have an influence on the faecal microbiota of both C-IBS and D-IBS patients.

Materials and Methods

Subjects

A total of 52 IBS subjects participated in this study which formed a part of a larger probiotic RCT (Clinical Trials Registry number NCT01886781) evaluating the efficacy of an eight-week treatment regime of *Lactobacillus plantarum* 299v for IBS [14]. The study was approved by the Research Ethics Committee at Stellenbosch University (reference number: N10-08-270) and was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki. Written informed consent to participate was obtained

from each participant on enrolment. Twenty-four C-IBS and 28 D-IBS patients were included. Probiotic treatment was given to 19 D-IBS patients and 16 C-IBS patients while 17 patients from both D-IBS and C-IBS groups received placebo. This was a double blind study and therefore patients were randomly given either the probiotic treatment or placebo. The groups were therefore not matched for severity of symptoms or for other demographics. Participants were screened by a gastroenterologist and recruited according to the study inclusion criteria and their willingness to participate. The diagnosis of IBS was made using Rome II criteria. Participants underwent physical measurements, including weight and height, which were used to calculate body mass index (BMI kg/m^2).

Intervention

The RCT was 12 weeks in duration, the active treatment phase eight weeks. During the intervention, all subjects received once daily either *L. plantarum* 299v or placebo. The study product contained 5 x 10^9 cfu (colony forming units) of *L. plantarum 299v* and it was tested against placebo capsules, filled with micro crystalline cellulose powder, of identical taste, texture and appearance by the manufacturer (Ferlot Manufacturing and Packaging (PTY) Ltd). The test product was analysed for viable units and this confirmed packaging quantity details. The dose was two capsules taken orally every morning.

Dietary Assessment

A registered dietitian explained and trained each participant on the procedure for completing a prospective, three day estimated dietary record. Portion sizes were estimated using household food measures like spoons, cups and bowls and a ruler. The importance of food

recording immediately after it was eaten was emphasized. The results were analysed by FoodFinder^{TM} III – a computer-based data evaluation system for South African foods [15].

Faecal sampling

Faecal samples were collected at three time points: baseline (A), after supplementation (B) and following a two week washout period (C). Samples were collected with disinfected plastic equipment after defecation and immediately frozen, kept at -20^{0}C for up to a month before being stored at -80^{0}C until analysis. Not all participants provided a stool sample at each time point (A, B and C).

DNA extraction

Total DNA was extracted using the QIAmp DNA stool extraction mini kit (Qiagen, Hilden GmbH, Germany). Manufactures instructions were followed, lysozyme enzyme to a final concentration of 10mg/ml, was introduced during cell lysis to assist with degradation of gram positive peptidoglycan cell wall layers, thus ensuring that genomic DNA extracted was representative of the entire bacterial population. The DNA concentration and integrity were determined using a Nanodrop spectrophotometer (Nanodrop Technologies). Only samples with integrity between 1.8 and 2.2 were used.

qPCR bacterial analysis

Total *Bacteroides* spp. *and Bifidobacteria bifidum* as well as total *Lactobacillus plantarum* were quantified using The PrimerDesign™ genesig Kits (Primer Design, UK) and quantitative real-time polymerase chain reaction (qPCR) amplification and detection. These kits were designed to have the broadest detection profile possible for *in vitro* quantification of all *Bacteroides* species and all *Bifidobacterium bifidum* genomes. A detection kit was specifically developed for *Lactobacillus plantarum* (Primer Design, UK).

IBS symptom severity score

The severity of GI symptoms was assessed by a validated questionnaire for use in IBS patients, the Francis Severity Score (FSS) [16]. The FSS questionnaire was completed at six different time points over the 12 week trial. The questionnaires were self-administered.

Statistical methods

In qPCR analyses, some of the target organisms remained below the detection limit. These values may not have been truly zero or missing values, but caused by technical limitations of the qPCR technique. Therefore, for data analysis, the undetected samples were given a value, which corresponded to the limit of detection of the respective qPCR assay. The data were not normally distributed per treatment groups (i.e. placebo vs. probiotic) and per IBS (C-IBS vs. D-IBS) groups. Thus the variables were transformed with a log transformation to yield more normally distributed data. The analyses showed that the log-transformed data were still not normally distributed. Therefore the ANOVA comparisons were confirmed with Mann-Whitney U tests. Correlations among the continuous variables were done with Pearson

and Spearman rank correlation coefficients. Repeated measures ANOVAs were done with the assumption of compound symmetry (i.e. equal-correlation among the FFS responses over time). The statistical analyses were done with STATISTICA Version 11 (2012) and a significance level of 5% was used for all analyses.

RESULTS

A total of 52 IBS participants fulfilling Rome II criteria were included in this study. Demographic detail and clinical characteristics are shown in **Table 1.** Participant's BMIs fell either into the overweight (25.0 – 29.9kg/m^2) or obese (>30kg/m^2) categories. IBS was longstanding i.e.> five years for most of the participants.

Table I Patient demographic and clinical characteristics (n = 52)

	D-IBS		C-IBS	
	Treatment	Placebo	Treatment	Placebo
Number of subjects	19	9	16	8
Average age (years) (range)	52.2 ± 16.2 (24.9 - 75)	42.5 ± 7.2 (31.9 - 51.2)	51.5 ± 9.9 (35.9 – 69)	49.4 ± 13.9 (33.0 – 72)
Gender (female/male)	18/1	9/0	16/0	8/0
BMI (kg/m^2) (range)	29.4 ± 7.4 19.26 – 42.0	33.07 ± 9.3 17.19 – 48.9	30.9 ± 7.2 20.2 – 49.0	27.8 ± 6.3 21.33 – 40.0
Duration of IBS symptoms (years)	7.3 ± 11.7	7.9 ± 7.1	12.7 ± 9.9	12.4 ± 8.2

There was a significant difference between the C-IBS (includes treatment and placebo) and D-IBS (includes treatment and placebo) groups and the presence of *Lactobacillus plantarum* at baseline (point A) (-0.956 ± 1.239 vs. -1.700 ± 1.239; p = 0.024), the D-IBS group had significantly lower counts of *Lactobacillus plantarum.* *Bacteroides* spp. and *Bifidobacteria* spp. were not different at baseline (A) between the C-IBS and D-IBS groups (data not in Table II). The probiotic had no significant effect on bacterial profiles between those on treatment and those receiving the placebo from baseline (A) to end of treatment (B) in both C-IBS and D-IBS groups, see **Table II**. There was no significant change in bacterial counts after completion of the trial (B) and following on into the washout period (C).

Table II Bacterial counts in stool at baseline(A), after supplementation(B) and after washout(C). Mean ± SD

		D-IBS			C-IBS		
		Mean count ± SE (log transformed per nanogram DNA)			Mean count ± SE (log transformed per nanogram DNA)		
Bacteria	Group	Before (A)	After (B)	Washout (C)	Before (A)	After (B)	Washout (C)
Bacteroides	Test group	2.16 ± 2.49 (n = 15)	2.30 ± 2.85 (n = 15)	2.48 ± 2. 91 (n = 14)	3.21 ± 2.61 (n = 12)	2.69 ± 2.60 (n = 12)	3.10 ± 2.52 (n = 13)
Bacteroides	Placebo group	1.12 ± 2.13 (n = 7)	2.16 ± 2.93 (n = 7)	1.54 ± 2.39 (n = 7)	2.49 ± 2.99 (n = 7)	2.65 ± 3.13 (n = 8)	2.74 ± 2.94 (n = 7)
Bifidobacteria	Test group	-0.69 ± 1.50 (n = 15)	-0.67 ± 1.67 (n = 17)	-0.88 ± 1.36 (n = 14)	-0.16 ± 1.66 (n = 13)	-0.53 ± 1.44 (n = 13)	-0.24 ± 1.82 (n = 12)
Bifidobacteria	Placebo group	-1.14 ± 1.14 (n = 7)	-1.12 ± 1.21 (n = 7)	-0.95 ± 1.59 (n = 7)	-0.37 ± 1.66 (n = 7)	-0.59 ± 1.85 (n = 8)	-0.46 ± 1.54 (n = 6)
Lactobacillus plantarum	Test group	-1.88 ± 0.00 (n = 12)	-1.14 ± 1.18 (n = 14)	-1.34 ± 0.90 (n = 13)	-0.96 ± 1.31 (n = 8)	-0.44 ± 1.21 (n = 10)	-0.89 ± 1.39 (n = 12)

Lactobacillus plantarum	Placebo group	-1.34 ± 0.82 (n = 6)	-1.80 ± 0.17 (n = 6)	-1.60 ± 0.58 (n= 5)	-0.95 ± 1.27 (n = 5)	-0.96 ± 1.41 (n = 7)	-1.66 ± 0.48 (n = 5)

No significant differences

When the data of only those that provided all three stool samples were analysed, no significant differences were found between the C-IBS and D-IBS groups.

Table III gives an overview of the participant's dietary intake at baseline (time point A). There were no significant differences for any nutrients between the D-IBS and C-IBS groups. The C-IBS group had a higher intake of energy and macronutrients, fat, protein and carbohydrate, as well as a slightly higher fiber intake compared to the D-IBS group. This data was used to correlate to the findings of the faecal microbiota **Table IV**. There were significantly strong but inverse correlations in the total IBS (combined C-IBS and D-IBS) groups for *Bacteroides* and total (r = -0.424; p = 0.019), insoluble (r = -0.406; p = 0.023) and soluble fiber (r = -0.466; p = 0.008). A direct correlation was found for the same fiber fractions ((total (r =0.529; p = 0.002), insoluble (r = 0.465; p = 0.008) and soluble fiber (r = 0.433 p = 0.015)) and *Lactobacillus plantarum*. In the D-IBS group correlations were found for protein intake and *Bifidobacteria* (r = 0.497; p = 0.036) and *Lactobacillus plantarum* (r = 0.487; p = 0.041). *Bifidobacteria* was correlated with insoluble fiber (r = 0.523; p = 0.026) intake and *Bacteroides* inversely correlated with total dietary fiber (r = -0.528; p =0.024) and soluble dietary fiber (r = -0.571; p 0.013). A strong correlation was found in the D-IBS group for percentage energy from fat and *Bacteroides* (r= 0.617; p = 0.006) and linolenic acid (C18:3) intake and *Bifidobacteria* (r = 0.516; p = 0.028). In the C-IBS group *Lactobacillus plantarum* correlated well with total dietary fiber (r = 0.584; p = 0.036).

Table III: Dietary intake of participants (n = 52), mean ± SD

	All groups	D-IBS (n = 28)	C-IBS (n = 24)
Energy (MJ)	7.25 ± 1.95	7.02 ± 1.76	7.53 ± 2.22
Total fat (g)	68.30 ± 22.93	66.13 ± 20.29	70.84 ± 26.29
% energy from fat	35.72 ± 6.78	36.15 ± 7.35	35.21 ± 6.05
Total protein (g)	61.04 ± 17.34	59.35 ± 17.09	63.02 ± 18.35
% energy from protein	14.88 ± 4.57	14.77 ± 3.91	15.00 ± 5.14
Total carbohydrate (g)	198.16 ± 69.29	191.49 ± 67.30	205.93 ± 71.13
% energy from carbohydrate	45.83 ± 7.34	45.64 ± 7.65	46.05 ± 7.34
Total dietary fiber (g)	14.14 ± 7.92	13.66 ± 7.69	14.72 ± 8.03
Insoluble dietary fiber (g)	4.07 ± 2.62	3.99 ± 2.59	4.16 ± 2.50
Soluble dietary fiber (g)	3.26 ± 2.41	3.28 ± 2.70	3.23 ± 1.77
C18:2 (g)	16.11 ± 7.80	15.91 ± 7.67	16.34 ± 8.00
C18:3 (g)	0.40 ± 0.19	0.39 ± 0.17	0.42 ± 0.20

Table IV Correlations between faecal microbiota and dietary components

	All groups			D-IBS			C-IBS		
	Bacteroides	*Bifidobacteria*	*Lactobacillus plantarum*	*Bacteroides*	*Bifidobacteria*	*Lactobacillus plantarum*	*Bacteroides*	*Bifidobacteria*	*Lactobacillus plantarum*
Total fat (g)	0.012	-0.126	0.201	0.207	0.292	-0.161	-0.167	-0.382	0.503
% energy from fat	0.333	-0.087	-0.097	**0.617***	0.099	-0.160	0.095	-0.021	0.179
Total protein (g)	-0.235	0.109	**0.382***	-0.202	**0.497***	**0.487***	-0.334	-0.288	0.313
% energy from protein	0.008	0.188	0.031	-0.175	0.106	**0.568***	0.127	0.162	-0.289
Total carbohydrate (g)	-0.288	-0.181	0.284	-0.289	0.089	-0.183	-0.312	-0.445	0.551
% energy from carbohydrate	-0.250	-0.161	-0.011	-0.313	-0.126	-0.235	-0.193	-0.245	0.089

Table IV. continued									
Total dietary fiber (g)	**-0.424***	-0.057	**0.529****	**-0.528***	0.175	0.240	-0.443	-0.309	**0.584***
Insoluble dietary fiber (g)	**-0.406***	0.083	**0.465****	-0.417	**0.523***	0.253	-0.481	-0.338	0.539
Soluble dietary fiber (g)	**-0.466****	0.080	**0.433***	**-0.571***	0.306	0.319	-0.493	-0.277	0.421
C18:2 (g)	0.056	-0.196	0.126	0.038	-0.784	-0.277	0.081	-0.332	0.407
C18:3 (g)	-0.150	-0.035	0.756	0.218	**0.516***	-0.265	-0.503	-0.379	0.279

$^{*}p < 0.05$, $^{**}p < 0.01$.

There was no statistical difference in symptom severity score between the treatment and placebo groups, (treatment group 259,54 ± 104,59 to 197,56 ± 114,74 vs. placebo group 258,71 ± 110,88 to 180,00 ± 96,1; $p = 0.599$) over the trial period. The groups were also further divided into C-IBS vs. placebo and D-IBS vs. placebo, no significant differences were found. Both the study group and placebo group had a significant improvement in (FSS) scores over the study period, from an average of 259,27 to 191,71 ($p < 0.0001$) indicating a large placebo effect. A strongly significant positive correlation was found in D-IBS patients receiving placebo at time point B, higher symptom severity score correlated with higher *Lactobacillus plantarum* ($r = 0.892$, $p < 0.05$). A strongly significant inverse correlation was seen in the C-IBS placebo group at time point A, lower *Lactobacillus plantarum* counts translated to a higher symptom severity score ($r = -0.907$, $p < 0.05$). No other significant correlations were found between FSS and microbiota.

DISCUSSION

This study investigated the effects of single strain probiotic supplementation, *L.plantarum* 299v, and nutrient intake on GI microbiota and symptoms. No significant beneficial effects of the probiotic were observed on severity of GI symptoms nor on GI microbiota composition. However, nutrient intakes were shown to have significant impact on GI microbiota composition. Therefore the first hypothesis that nutrient intakes do not have an influence on faecal microbiota is rejected and the second hypothesis that *L.plantarum* 299v does not have an influence on the faecal microbiota anlaysed is accepted.

The microbiota of the GI tract plays an important role in the maintenance and function of the GI ecosystem. Dysbiosis has been associated with the development of inflammatory disorders such as Crohn's disease [17] and ulcerative colitis [18]. GI microbiota alterations are increasingly being recognised as an important factor in the pathogenesis and pathophysiology of IBS [19]. In recent years, many research groups have focused on identifying the GI microbiota composition in IBS patients, using modern culture-independent techniques [20]. No single deviance has been identified in IBS microbiota, but various alterations in the bacterial composition have been characterised [21-24]. Work by Jeffrey *et al*. found that clustering by microbiota composition revealed subgroups of IBS patients, one of which showed normal-like microbiota composition compared with healthy controls. The other IBS samples were defined by large microbiota-wide changes characterised by an increase of *Firmicutes*-associated taxa and a depletion of *Bacteroidetes*-related taxa [25]. A further study that divided IBS patients according to subtype showed that D-IBS patients had lower numbers of *Lactobacilli* spp. while C-IBS patients had increased amounts of *Veillonella* spp. [26]. In this study a significantly lower level of *L.plantarum* in the D-IBS group compared to the C-IBS group at baseline (time point A) was found. This is similar to the findings of Malinen *et al*. [26]. Previous studies also demonstrate that D- IBS is associated with significant increases in detrimental bacteria like *Proteobacteria* [27,28], decreases in beneficial bacteria such as *Lactobacillus* spp. [26,29], *Actinobacteria* and *Bacteroidetes* [30], as well as an overall reduction in microbial diversity [31].

To date there have been very few RCT on IBS and probiotics that have investigated possible modifications of the microbiota by the probiotic. Knowledge on the role of microbiota modulation in symptom relief is therefore limited [9,10]. Nobaek *et al.*

examined the effect of *L.plantarum* DSM 9843 (299v) on faecal microbiota and IBS symptom relief. They used culture-dependent techniques and investigated *L.plantarum* DSM 9843 (299v), *Enterobacteriaceae*, Sulphite-reducing clostridia and *Enterococci* counts before and after treatment. There were no significant changes in *Enterobacteriaceae*, Sulphite-reducing clostridia and *Enterococci* counts following supplementation, although the *Enterococci* count remained the same in the test group, whereas there was a small increase in the placebo group at the end of supplementation. The *L.plantarum* DSM 9843 faecal count increased significantly in the test compared to placebo group ($p < 0.001$). Flatulence was rapidly and significantly reduced in the test group compared with the placebo group and abdominal pain was reduced in both groups [9]. Kajander *et al.* showed a significant improvement in composite IBS scores with a multispecies probiotic in the treatment versus placebo group. At the same time they demonstrated a stabilization of the microbiota, as the microbiota similarity index increased with the probiotic supplementation, it decreased in the placebo group, the difference between the two groups was significant ($p = 0.0015$) [10]. In another study, Ki Cha *et al*. evaluated the effects of a multispecies probiotic on IBS symptoms and the composition of faecal microbiota in D-IBS patients. The proportion of responders was significantly higher in the probiotic vs. placebo group, for the primary outcome measure of adequate relief of overall IBS symptoms (48% vs. 12%, $p = 0.01$). Comparison of denaturing gradient gel electrophoresis (DGGE) profiles of faceal flora showed that the concordance rate (similarity) between bacterial compositions before and after treatment was significantly higher in the probiotc vs. placebo group (69.5% vs. 56.5%, $p = 0.0005$)[32]. One recent study comparing the composition and temporal stability of intestinal microbiota between IBS and healthy controls by PCR-DGGE revealed a greater temporal instability in IBS

patients (43% instability) than in the control group (29% instability)[21]. These results suggest that the pathophysiology of IBS may be associated with temporal instability in the composition of intestinal microbiota. However in this study we found that a probiotic exerted no beneficial changes on the GI microbiota and no consistent correlations were found between GI symptom severity and total *Bacteroides*, *Bifidobacterium bifidum* or *Lactobacillus plantarum* counts. As studies suggest an association between microbes and symptoms in IBS and the relative importance of different taxa for IBS symptoms has been found to be inconsistent between existing studies [19].

Data indicates that the composition and activity of the GI microbiota is affected by the genetic background, age, diet and health status of the host [33]. Dietary factors have effects on the GI microbiota composition and this might be of considerable importance for symptoms of IBS patients. This study has clearly demonstrated strong correlations between certain dietary agents and the resulting GI microbiota. It seems as though lower fiber diets predispose towards an increased *Bacteroides* and decreased *Bifidobacteria*, seen in both C-IBS and D-IBS groups. Higher fiber intake was strongly associated with increased *Lactobacillus plantarum* counts in both groups. In the D-IBS group a higher percentage energy from fat and low fiber intake correlated to high *Bacteroides* counts. These findings are in agreement with a previous study by Wu *et al*. By combining detailed nutritional analysis and microbiome determination in 98 individuals, Wu *et al*. sought to indentify nutrients that substantially affect abundances of microbial species. They found that a higher fat intake and lower fiber intake were associated with the *Bacteroides* enterotype [34]. The dietary associations seen here parallel a recent study by De Filippo *et al*. They compared European children, who eat a typical Western diet high in animal protein and fat, to children in Burkinda Faso, who eat high-carbohydrate diets low in animal protein. The European

microbiome was dominated by taxa typical of the *Bacteroides* enterotype, whereas the African microbiome was dominated by the *Prevotella* enterotype [35].

A significant positive correlation was also found for both C-IBS and D-IBS groups in this study for *Lactobacillus plantarum* and total protein intake, as well as *Bifidobacteria* and protein intake in the D-IBS group. De Palma *et al*. assessed the effects of a gluten free diet (GFD) in ten healthy subjects and analysed the the change in faecal microbiota by fluorence *in situ* hybridisation (FISH) and qPCR. *Bifidobacterium, Lactobacillus* and *Bifidobacterium longum* counts decreased ($p = 0.020$, $p = 0.001$ and $p = 0.017$, respectively) after the GFD (a mean decreased intake of 72.99 ± 15.69 to 68.48 ± 13.19 g/day, $p > 0.05$, insignificant decrease) assessed by qPCR [36]. These findings are similar to those found in this study.

Recent research has highlighted that dietary intervention that aimed at decreasing fermentable carbohydrates and FODMAPS, and as a result improved IBS symptoms, also resulted in the decrease of beneficial *Bifidobacteria* [37]. This opens the question as to whether probiotic supplementation is needed in addition to dietary advice to restrict fermentable carbohydrate. Our research has demonstrated that low fiber intake decreased *Bifidobacteria* but this did not translate over to improved GI symptoms.

Based on the available data, differences in the compositions of the GI microbiota are demonstrable between groups of people living on different diets. These diet associated changes in composition can lead to changes in the metabolic activity of the GI microbiota, which, in turn, may provoke changes in inflammatory responses. Although attempts to

change the diet composition of the GI microbiota by varying the diet have been successful in mice, there is a relative paucity of human dietary intervention studies. Moreover, mechanisms that link dietary changes to microbial composition alterations remain poorly defined and need to be investigated further [13]. Large, well-controlled trials are required to determine the impact of altering long term dietary patterns on the human GI microbiota and well controlled trials are needed to determine the extent to which (and in which IBS subpopulation) certain probiotics are useful therapeutic strategies in the management of IBS symptoms [38] and how GI microbiota modulation can have a positive impact on symptom management.

Strengths of the present study include the simultaneous assessment of microbiota, IBS symptoms and dietary intake. We also divided IBS subjects according to bowel habit sub-type. This study is not without limitations, we quantitatively analysed only a few major groups of bacteria that occur in the faeces and there may have been quantitative shifts between different factions within groups that were not detected in this analysis. The small size of the study population may have failed to detect significant changes in the microbiota.

Conclusion

This study has shown that GI *Lactobacillus plantarum* differs between IBS phenotypes. An eight week course of the single stain probiotic *L. plantarum* 299v did not have any significant changes on the GI microflora or GI symptoms. Certain dietary agents strongly correlate to certain bacterial profiles and this provides an attractive explanation that dietary nutrients are a factor that influences the composition of GI microbiota.

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Chapter 7

Concluding discussion and summary

1. Discussion

Gastrointestinal (GI) symptoms and gut dysfunction caused by irritable bowel syndrome (IBS) affect up to one fifth of the adult population worldwide. IBS is multi-faceted in its aetiology and is seen as a complex condition in which a number of major mechanisms at the central and peripheral level interact. These mechanisms are poorly understood and there is no single curative treatment. Therapy is aimed at reducing the symptoms, often with very little success. Previous studies propose the involvement of diet and GI microbiota in the pathophysiology of the condition as well as a beneficial effect of certain probiotics in the alleviation of IBS symptoms. This study investigated the nutrient intakes, GI microbiota and the impact of a probiotic, *L.plantarum* 299v as part of a randomised controlled trail (RCT) in IBS patients.

2. Methodological considerations

This section contains a discussion on the methodology and limitations thereof.

1.1 Subjects

IBS patients were recruited from a single centre, private referral hospital by an experienced gastroenterologist. Significant differences in treatment response may exist between primary and referred patients.[1,2] While the current study was being conducted, data on the same probiotic strain in a group of patients from India was published.[3] Their study consisted of a multi-centre trial with patients attending primary care centres and significant superiority of *L. plantarum* 299v was demonstrated over placebo. This is in contrast to our findings and may indicate that the benefit of the probiotic is effective in primary health care patients but not those of referred patients and that the type of patient we enrolled was experiencing a greater

severity of symptom or more resistant conditions than those experienced by patients attending primary health care facilities.

All subjects fulfilled the Rome II criteria for IBS, and the number of patients needed for the clinical trial (Article 2, Chapter 3), was based on statistical power calculations, which is in line with current recommendations for trials in IBS.[4] The majority (97.5%) of patients studied were women, which may reflect the higher prevalence of IBS in women[5] or the fact that women in general are more prone to seek medical advice for any health problems.[6] For the RCT (Article 2, Chapter 3), D-IBS vs. C-IBS vs. placebo data was compared, no statistically significant differences between the C-IBS and D-IBS groups were found and the data was grouped together as the total IBS vs. placebo group. Healthy subjects were recruited as controls in Article 3 (Chapter 4). A limitation of this part of the study is that the control subjects were not age or gender matched with the patients. To comply with ethical requirements, the control group had to be patients referred for a colonoscopy for specific clinical reasons, but otherwise as healthy as possible (generally controls were persons coming for screening as there was a family history of colorectal cancer). All potential patients came from the same referral pool, but only those that met the study inclusion criteria were enrolled, the rest were controls. Overall, it was considered that the controls represented the general, healthy adult population well. The subjects recruited for Article 4 (Chapter 5) were recruited on a "first come, first serve" basis. In total 11 patients were used for the reliability (7.4%, n= 6) and validity testing (6.2%, n = 5 of the total study population, n = 81) of the dietary records. This is considered a good percentage of the sample for a validity and reliability study, 4.1% and 0.25% used by Astorg *et al.* and Sun *et al.* respectively.[7,8] However, in terms of absolute numbers, it falls short of the golden standard of n= 50 for validity testing,

using 50 in our study would have been 62% of the total study sample. The aim of this thesis and part of this study was however not to conduct a validation study but to validate the dietary information collected. Fifty-two people were included in the GI microbiota analysis (Article 5, Chapter 6), they formed part of the same group involved in the RCT (Article 2, Chapter 3) and this sample size is comparable to other studies (**Table 5** of Introduction). A total of 124 out of the 186 (67%) stool samples collected were analysed.

2.1 Study designs

RCTs are the gold standard in clinical research.[9] This trial was conducted as a double-blind, randomised, placebo-controlled study, which is the recommended trial design in IBS.[4] Unfortunately, many RCTs with probiotics and IBS, vary considerably in design,[10-13] some of the older studies are of poor quality, and few attempt to define the mechanism of action or assess whether symptomatic improvement is accompanied by a change in the GI microbiota. A recent systematic review reported that studies of poorer quality tended to show larger effects and published data indicate a publication bias, with non-reporting of negative effects in small trials.[13] The exceptionally high placebo response, estimated to vary between 16 and 71%,[14] makes the placebo treatment arm essential in all IBS trials. The current guidelines differentiate between short-term studies with a minimum treatment of four weeks, and long term studies lasting at least six months.[4] Enck *et al*. demonstrated in their RCT that a mean response time of four- five weeks for active treatment and more than eight weeks for placebo was needed when a combination probiotic was used for treatment in IBS.[14] The current study (Article 2, Chapter 3) was of a longer duration than the previous four trials using the same strain probiotic in IBS.[3,15-17] The dosage in this study was the same as most recently published data, using the same strain probiotic in primary-level IBS patients.[3] We

investigated whether there was an improvement in this study at week four and whether this possible mimicked Ducrotte *et al.* however, this was not a finding. This highlights the importance of follow-up over longer periods of time and maybe also explains why the results of this study differ from previous studies. It seems from our study as though the effect of *L. plantarum* 299v becomes stable fairly quickly and is best seen over the short term. Similar findings were reported by Nobaek *et al*., Niedzielin *et al*. and Sen *et al*.[15-17]

2.3 Selection of probiotic

Different strains of organisms have very different and specialised metabolic activity. The level of specificity in describing a probiotic is important as effects can be and are strain-specific.[18,19] The value of using a single strain probiotic over a combination of probiotic strains or species remains a continuing debate (Article 1, Chapter 2). Micro-organisms may behave differently when administered in combinations compared to in isolation. The use of combinations or cocktails concerns some investigators as attempts to classify the mechanism of action are hard to define.[20] The reasons for the selection of *L.plantarum* 299v has been explained in greater detail in Article 1, Chapter 2 and Article 2, Chapter 3.

2.4 Questionnaires

The use of validated measurement instruments are recommended in IBS.[4] Fortunately these tools exist and approval has been granted for use in this study. In this study the Francis Severity Score (FSS)[21] and IBS quality-of-life (QOL)[22] were used (Article 2, Chapter 3). The IBS-QOL is a well- tested and validated questionnaire that has been used extensively

around the world as a tool for assessing quality of life. The FSS questionnaire is also a validated questionnaire for use in IBS. A three day estimated food record was used for the collection of dietary data. The method of choice for the collection of dietary intake data in intervention studies/clinical trials is the weighted or estimated dietary intake record method.[23] This method is also usually used as the reference method when other questionnaires e.g. quantified food frequency questionnaire, needs to be validated. There are inherent limitations to using an estimated three day food record, e.g. incorrect portion size estimation. This prospective method of dietary assessment may be influenced by the presence of active IBS symptoms and may distort usual dietary intake and symptom severity would need to be taken into account. Changes in the dietary intake of patients based on symptom severity would be expected over time, however Article 4 (Chapter 5) shows good reliability of dietary data and indicates that patients did not change their diets as symptoms fluctuated. The reliability and validity of the three day food records gathered (Article 4, Chapter 5) was assessed by a test-retest design and plasma biomarker respectively. The validity of dietary data as assessed by plasma fatty acids was poor. This suggests that further research and testing are needed in a larger grouping of IBS patients as plasma fatty acid levels may be influenced by factors causing IBS symptom generation (GI dysbiosis or altered fatty acid metabolism; Article 4, Chapter 5) hitherto unexplored.

2.5 Microbiological and biochemical analysis

In Article 5 (Chapter 6) quantitative polymerase chain reaction time (qPCR) was used detecting16S ribosomal RNA (16S) of *Bacteroides* spp. *Bifidobacteria bifidum* and *Lactobacillus plantarum* in bacterial DNA of patients' stool samples. Kits which included

positive controls were provided by Primer Design (United Kingdom, UK). A kit was specifically developed by Primer Design to detect *Lactobacillus plantarum* in this study.

2.6 Plasma fatty acid analysis and short comings

Total lipids were extracted from serum samples in 2 ml chloroform methanol (2:1) and 1ml 0.05% sulfuric acid. Lipids were derivatised in 3% methanoic sulphuric and methyl esters (FAMEs) extracted in hexane and analysed by gas chromatography and mass spectroscopy (GC-MS). A highly polar HP-88 column (Agilent) was used and could detect long chain fatty acids, C12 to C26 and their different isomers.

2.7 Protocol deviations

There were five protocol deviations during the course of this research and they were:

- One of the initial objectives was to establish whether a course of probiotics may alter stool short chain fatty acids (SCFA). Unpublished results (from within the same study group) obtained at the end of 2012 on IBS patients entitled, "An investigation of short-chain fatty acid profiles and influential GI microbiota associated with irritable bowel syndrome"[24] did not show a direct correlation between *Bacteroides, Bifidobacteria* and SCFA in stool samples. The conclusion was that the transit time which is the time available for SCFA to be taken up by the colon, differs between the different groups. C-IBS patients showed the least SCFA, but have the longest transit time. A *post hoc* analysis of the current study showed no change in patient's constipation or diarrhoea frequency as compared to controls. These results only became available at the end of

2012. To do the SCFA analysis would therefore in all likelihood prove to be a waste of time and money, because the probiotic has already shown to have no significant improvement on clinical outcomes. More importantly however was that the researchers realised that the conclusions drawn from such an analysis would be invalid and could be misleading. SCFA in the stool do not reflect whether probiotics may influence SCFA production by the microbiota. This is because SCFA do not take into account factors like transit time, available butyrate transporters and other possible role players. Leaving out the investigation into SCFA would not influence the aim of the study, since the quantification and effects of *Bacteroides* spp. *Bifidobacterium bifidum and Lactobacillus plantarum* were still determined.

- Initially only patients requiring a colonoscopy as a part of their diagnostic work up were eligible for study inclusion. An amendment was approved by the Health Research Ethics Committee (HREC) (Ethics reference number N10/08/270) in August 2011 allowing the use of the gastroenterology practice's existing data base to recruit patients and use those that have had a colonoscopy within the last three years.
- A further amendment was approved by the HREC (Ethics reference number N10/08/270) in August 2011 allowing a decrease in the total sample size. The initial power calculation was with 90% power. However, current guidelines[4] recommend 80% power (ß error or type II error of 20%) and α (type I) error of 5% using a two-sided test. The sample size was adjusted accordingly from n= 123 to n = 81. The sample size calculation was based on the expected behaviour of the primary outcome measure, i.e. abdominal pain relief at the end of week 8 on the visual analogue scale [(VAS), 0-100%] pain severity score. The minimally clinically important difference (MCID) based on the primary outcome measure with this instrument is 50 points.[21]

- Plasma linolenic acid (C18:3n3), an essential fatty acid was to be used to correlate with analysed C18:3 (linolenic acid) dietary intake. However we used a wider spectrum of fatty acids, and not solely linolenic acid (Article 4, Chapter 5).
- Total *Bifidobacteria*, total *Lactobacilli*, total *Bacteroides*, total *Endobacteria*, *L. plantarum* 299v, total anaerobes, gram negative anaerobes, total aerobes and clostridia were to be measured in the stool samples. The costs associated with the microbiota analyses have also increased substantially since the protocol was compiled. Due to cost and time constraints only *Bifidobacteria bifidum*, total *bacteroides* and *Lactobacillus plantarum* were analysed. There is no available gene sequencing for *L. plantarum* 299v and so indigenous *Lactobacillus plantarum* was assessed, following the development of the assay by Primer Design (UK).

2.8 Study limitations

- Patients were recruited from a single centre to ensure uniform selection by a gastroenterologist. The gastroenterologist is a referral specialist, and generally sees more complicated patients. Therefore more exclusion factors were applied when patients were selected.
- The cost for the analysis of the microbiota escalated dramatically over the course of the research and because of this not all the desired microbiota could be analysed. This study showed significant correlations between the diet and the microbiota analysed.
- Rome II criteria for patient recruitment were used, however for the rest of the RCT Rome III guidelines were strongly adhered to. The prevailing trend at the beginning of this research was to use Rome II criteria for diagnosis.
- Participants were not age and gender matched in Article 3 (Chapter 4) (already

discussed).

- There are inherent limitations associated with the used of an estimated three day food record, Article 4, Chapter 5 (already discussed).
- The drop- out (20%), although within the acceptable range[4] for the RCT, could have clouded the interpretation of data (Paper 2, Chapter 3). The study power was reduced through this level of drop-outs, and it therefore remains a possibility that an effect of the active product was missed.
- The diet methodology used in Table II (Article 3, Chapter 4) was revised (Addendum 1 of Chapter 4). The methodology as primarily outlined by the Institute of Medicine was adhered to and is attached as Addendum 1 of Chapter 4.

In summary, the methods used in the current study are considered to be of a good quality, suitable for the study settings and comply with current recommendations. A range of diverse methods have been utilized. A newly developed *L. plantarum* detection kit has been used in conjunction with well-established techniques. As for the clinical trial, the duration of the trial was sufficiently long and the number of patients included considered sufficient. The study shows that the search is still on for a biomarker that can be used to confirm dietary intake using only small subsets of participants.

Bearing in mind the study limitations as stated in section 2.8 and in chapters 2-6, the core conclusions of this study on the relationship between nutrient intakes, GI microbiota and IBS as detailed as the research aims in Chapter 1 Section 5.2, are discussed below, furthermore the specific contribution from each area are detailed in **Figure 1** and illustrates the contribution to knowledge that this body of work presents:

1.1. Literature review: To update healthcare professionals on current probiotic information and provide an overview of probiotic treatment approaches, with special emphasis on IBS (Article I, Chapter 2).

Article 1 updated the health care professional on probiotic usage as relates to IBS. There are a number of systematic reviews and meta-analysis on probiotics usage in adult IBS patients.[10-13,25-28] The meta-analysis of RCTs in adult IBS patients and probiotics indicated a beneficial effect of different probiotics on global symptoms, abdominal pain, and flatulence, whereas the influence on bloating was equivocal.[29] To date the most commonly studied probiotic species remain *Lactobacillus* and *Bifidobacteria.* A number of RCTs have been performed on investigating the effectiveness of probiotics in IBS, a common theme remains suboptimal study design. More appropriately powered studies of longer duration are required to assess efficacy. Treatment options for IBS remain limited in both number and efficacy and a therapeutic trial of probiotics is therefore reasonable for patients interested in this approach, especially considering probiotics' good safety profile.

1.2. Probiotic supplementation: To conduct a well-designed randomised, double blind, placebo-controlled clinical trial with *L. plantarum* 299v as part of an intervention and establish whether a course of probiotics may alleviate undesirable symptoms of IBS and improve quality of life (Article 2, Chapter 3).

Article 2 presents the results of a RCT in adult IBS patients with probiotic *L. plantarum* 299v. Self-analysis on the trial's methodology as found in Brenner *et al*. indicates that this RCT was well designed,[10] **Table 1.** Further methodological considerations relevant to this RCT are given in Sections 2.1-2.4 – Methodological Considerations.

Table 1. Methodology score for this RCT assessing *L.plantarum* 299v in IBS

Study	***L. plantarum* 299V**
ROME criteria used to define IBS	*
Randomization	*
Parallel study design	*
Double-blinding	*
Complete follow-up (intention-to-treat)	*
No placebo run-in	*
Baseline observation period before trial initiation	*
Treatment duration of 8 – 12 weeks or longer	*
Follow-up after treatment to assess symptoms	*
Treatment compliance measured	*
Sample size calculation is provided/ adequate sample enrolled	*
Primary outcome = improvement in global IBS symptoms	
Primary outcome based on patient assessment	*
Validated scale used to measure improvement of IBS symptoms	*
Rome methodology score	**13/14**

Adapted from Brenner[10]

The RCT as a part of an intervention in this study, contributed towards the field of *original* research in the field of IBS and probiotics. Unfortunately, the single strain probiotic supplementation with *L. plantarum* 299v, did not significantly alleviate abdominal pain (primary outcome measure), as measured with the FSS. The FSS, which incorporates both the severity and frequency of abdominal pain, was reduced by 23.3% (from 259.66 to 199.13) with the probiotic supplementation and by 21.1% (from 256.04 to 201.98) with the placebo supplementation. According to recent guidelines on clinical trial design in functional GI disorders,[4,30] global symptom measures that integrate IBS symptoms into a single numerical index are one of the recommended outcomes. There is no consensus on what constitutes a clinically meaningful improvement in IBS, but an approximately 50%

improvement in the primary endpoint has been suggested as a reasonable definition of a responder and a 10-15% improvement of the global outcome measure over placebo as a clinically significant gain.[30] The minimally clinically important difference (MCID) based on the primary outcome measure with the FSS was 50 points. The integration of Quality of Life (QoL) monitoring into the treatment trials for IBS is strongly encouraged.[4] In Article 2, no effect on QoL was seen as assessed by the IBS-QOL questionnaire for probiotic supplementation vs. placebo. This IBS-specific questionnaire was developed and validated by Patrick *et al*.[22] and further validated in terms of responsiveness to treatment in a referral-based clinical population of patients with mild to moderate FBD.[31] The participants of this study responded very well to the probiotic therapy, however they also responded very well to placebo. The high placebo effect trend continues among trials, using a variety of endpoint measures.[32] It is known that IBS is a condition with few outcomes that are able to be objectively measured and a high degree of subjectivity in many outcome measures in IBS clinical trials exists.

1.3. Nutrient intake: To assess nutrient intake in patients with IBS compared to dietary recommendations. This is with the hypotheses that a condition in which subjects insist that diet or trigger foods play a part in symptom generation, may lead to risk of nutrient inadequacy (Article 3, Chapter 4).

Food and diet are central issues that concern patients with IBS and food is one of the most commonly reported triggers of IBS symptoms. Few studies have *prospectively* analysed dietary intake in IBS.[33] Article 3 (Chapter 4) aimed to determine the nutrient intake in South African IBS patients in comparison to Dietary Reference Intakes (DRI), assess nutritional differences between IBS subgroups and evaluate nutrient intake in IBS patients

in comparison to the general population. We hypothesized that IBS patients, or at least a substantial proportion of the patients, would demonstrate reduced intake of certain nutrients compared with the general population. This may be due to patient's food alterations and restrictions because to gastrointestinal symptoms. In the current study, patients with IBS demonstrated no major differences in energy and nutrient intake compared with a control group from the general population (Table III, Article 3, Chapter 4), which is in accordance with earlier findings.[33-37] Moreover, we were unable to find a relationship between nutrient intake and IBS subtype based on the predominant bowel habit (Table III, Article 3, Chapter 4). When the IBS (combined C-IBS and D-IBS groups) was assessed for risk of nutrient inadequacy using the EAR cut-point method (Addendum 1 of Chapter 4), the patients in this study were at risk for inadequate nutrient intakes of protein, fiber, calcium, iron, vitamin C, folate and vitamin A (Table 1, Addendum 1 of Chapter 4).

There are currently very few randomized controlled trials on dietary treatment of IBS patients. Instead the current recommendations are primarily based on studies assessing physiological function in relation to dietary components.[38] Common dietary advice to reduce IBS symptoms are to reduce intake of lactose, FODMAPS, fat, gas-producing food items and also to decrease or increase the intake of dietary fiber depending on symptom profile.[39,40] All this dietary advice implicates a risk of an inadequate nutrient intake. For example, a decreased milk intake may imply a decreased intake of vitamin B12, calcium, riboflavin and vitamin D, if it is not replaced with a mineral, and vitamin –enriched substitute.[33]

It is possible that the IBS patients in our study, to decrease symptoms, avoided some of the

above-mentioned food items, and consumed less milk products, which would result in a smaller intake of calcium, as usual intake places the patients at risk of inadequacy according to the EAR cut-point method (Table 1 in Addendum 1 of Chapter 4). Unfortunately, we have no information if the IBS patients had altered their food intake to reduce IBS symptoms, or due to other reasons.

Despite the fact that food is central to patients with IBS, and patients often complain of postprandial worsening of their symptoms,[41] few studies exist, which have characterized nutrient intake in IBS patients. An American population-based, case-control study compared patients with functional gastrointestinal disorder (FGID) with control subjects and found no difference in intakes of nutrients, calories, dietary fiber, vitamins and minerals between the groups, similar to our findings. However only a subgroup completed a prospective food diary in that study and the information reported mainly comes from a retrospective food frequency questionnaire, with the obvious risk of recall bias.[34]

Food items exacerbate symptoms of IBS and exclusion of some food items form the diet is considered common,[42] and it seems as though the nutrient intake in IBS patients in this study places them at risk of inadequacy. This is in contrast to previous studies which show that IBS patients consume food in accordance to current nutritional recommendations[33-35] but similar to the findings of McCoubrey *et al*. and Irvine *et al*. where they concluded that IBS patients may be at risk of low micronutrient intakes.[43,44] However, symptoms for poor micronutrient intake (e.g. calcium, iron, folate) do not correspond with clinical symptoms in

our study. In summary, we have used a dietary assessment tool aimed at estimating nutrient intakes in adult IBS patients. There are no reported studies, which have used this strategy in the South African IBS population. Our data suggests that this group is at risk of inadequate nutrient intakes.

1.4. Validation and reproducibility of dietary data: To validate and assess the reproducibility of food records in irritable bowel syndrome patients (Article 4, Chapter 5).

In Article 4 (Chapter 5) we examined the reliability and validity of three day estimated food records in a subgroup of IBS patients. Actual nutrient intake in IBS, has not been extensively reported. An even littler known fact is how reliable and valid the dietary data is. To the author's knowledge there has been no published data on the validity and reliability of IBS dietary data. This is pertinent information to know in a condition where diet is thought to play such a significant role. This study has shown that with a fairly short assessment tool (a three day food record) dietary data is reliable in IBS. However, using plasma fatty acids as biomarkers for validating nutrient intake data showed weak correlations, suggesting that further research and testing are needed in a larger grouping of IBS patients. The fundamental advantage of using a biomarker is that measurement errors are unrelated with errors in any dietary assessments, e.g. do not rely on memory, self-reported information or interviewer bias.[43] Studies have confirmed the use of plasma fatty acids as biomarkers of dietary intakes in healthy subjects.[7,45,46] Their use in patients with disease –profiles are lesser known. The effects of genetic variability in fatty acid biochemistry pathways, direct/indirect determinants of fat absorption, or gene-diet/nutrient or gene-gene interactions in fatty acid profiles as dietary markets is largely unexplored.

1.5. Characterisation of probiotic action and the nutrient intake on GI microbiota: Identify possible nutrient risk components for establishing GI microbiota involved in IBS and as part of an intervention, determine whether a course of probiotics may alter stool microbiota (Article 5, Chapter 6).

Article 5 (Chapter 6) contributes to a growing body of knowledge on the role of probiotic modulation and diet on GI microbiota. This study has clearly demonstrated strong correlations between certain nutrients (e.g. protein, fiber) and the resulting GI microbiota. Overall dietary changes can explain 57% of the total structural variation in GI microbiota whereas changes in genetics accounted for no more than 12%.[47] This indicates that diet has a dominating role in shaping GI microbiota and changing key populations may transform healthy GI microbiota into a disease-inducing entity.[48] The altered microbiota resulting from diet-induced dysbiosis found in this study, may be a factor contributing towards symptom generation in IBS. This is a field of study very little explored to date.

No published work has been conducted on the faecal microbiota community of IBS in the South African population. A better understanding of the prevalent bacterial community in faecal samples might be useful for either eliminating or restoring certain species to improve one's health. This is of particular importance in IBS patients where deviations in the GI microbiota as compared to healthy controls are well documented.[49-52] Although findings vary between studies, many show that patients with IBS have lower *Lactobacilli* and *Bifidobacteria* (two organisms frequently used in probiotic preparations).[53] Furthermore differences in IBS phenotype are also documented.[54,55] In this study we looked at the GI microbiota between C-IBS and D-IBS patients for *Bacteroides* spp., *Bifidobacteria bifidum*

spp. and *Lactobacillus plantarum*. The *Lactobacillus plantarum* was found to be significantly lower in D-IBS patients as compared to C-IBS (Article 5, Chapter 6). No other differences were found between the two groups.

Relatively few RCT on IBS and probiotics have investigated possible modifications of the GI microbiota by the probiotic. Knowledge on the role of microbiota modulation in symptom relief is therefore limited.[15, 56-58] Pervious trials have indicated that the probiotic seemed to exert a balancing effect on the microbiota.[58] In this study the bacterial counts were analysed by genus and strain-specific qPCR assays. We found that a probiotic exerted no beneficial changes on the GI microbiota (Article 5, Chapter 6). Furthermore, no consistent correlations were found between GI symptom severity and the microbiota. Paradoxically, this confirms previous studies where probiotics did influence symptom severity and GI microbiota. We found no improvement in GI symptoms and no change in the microbiota following a course of probiotics. This may be due to the specific probiotic used in the trial. It does seem then that GI symptoms improve only if GI microbiota improve in favourable response to probiotic administration. Studies suggest an association between microbes and symptoms in IBS, however, these findings are inconsistent between existing studies.[59] **Figure 1** shows the contribution to knowledge achieved by this body of research.

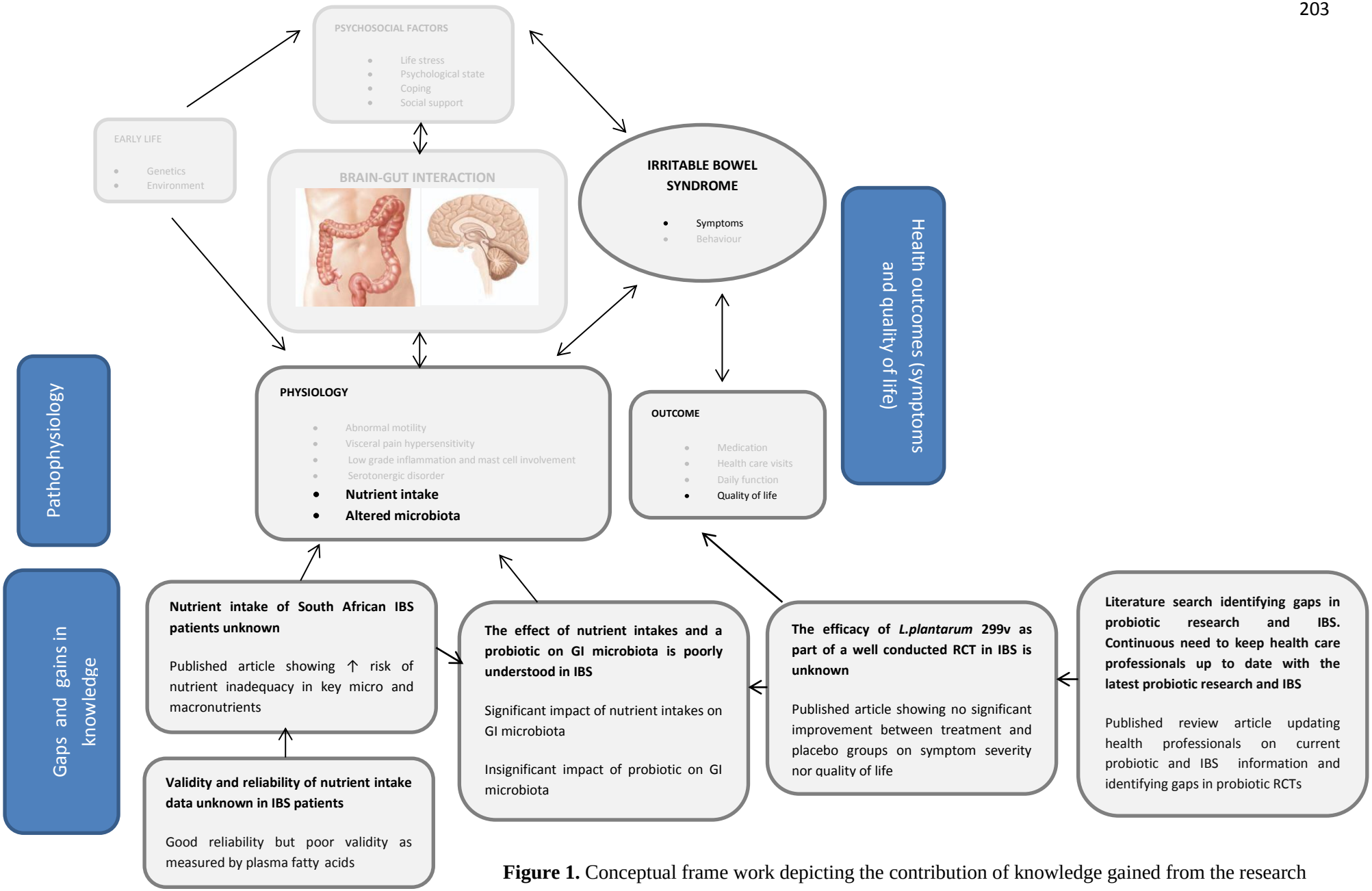

Figure 1. Conceptual frame work depicting the contribution of knowledge gained from the research

The study hypotheses (as discussed on page 57) are therefore accepted / rejected as follows:

- An intervention of the probiotic, *L.plantarum* 299v at a dose of two daily 5 X 10^9 colony forming units (cfu), will not alleviate the undesirable i) symptoms of IBS nor ii) improve quality of life (Article 2, Chapter 3) in C-IBS and D-IBS subjects. Accepted point i) and ii) of the null hypothesis
- The nutrient intakes of IBS (C-IBS and D-IBS) subjects placed them at risk nutrient of inadequacy compared to Dietary Reference Intake (DRI) recommendations (Article 3, Chapter 4). Accept the null hypothesis.
- Three day estimated food records demonstrate poor i) reproducibility and ii) validity in IBS (C-IBS and D-IBS) subjects (Article 4, Chapter 5). Reject point i) and accept point ii) of the null hypothesis.
- Nutrient intakes do not have an influence on the faecal microbiota (i.e. *Bifidobacterium bifidum, Lactobacillus plantarum* and *Bacteroides* spp.) of IBS (C-IBS and D-IBS) subjects (Article 5, Chapter 6). Reject the null hypothesis.
- *L.plantarum* 299v does not have an influence on the faecal microbiota (i.e. *Bifidobacterium bifidum, Lactobacillus plantarum* and *Bacteroides* spp.) (Article 5, Chapter 6). Accept the null hypothesis.

Finally, it can be conclusively stated that this study provides original insights into i) the efficacy of *L.plantarum* 299v as a treatment option in IBS, ii) nutrient intakes in a population of South African IBS patients, iii) the validity and reliability of dietary data in IBS patients, iv) the modulatory effect of nutrients and a probiotic on GI microbiota and GI symptoms.

Recommendations for future research include the following: i) As no study has reliably assessed fluctuations over time in food intake in IBS patients and their relationship with fluctuations in the symptom pattern, this is a future research recommendation that would be of great value. Prospective studies are needed to assess the association between fluctuations in symptom severity over time and changes in intake of nutrients and ii) as IBS is chronic in nature, longitudinal studies assessing GI microbiota during remission, and symptom flare-ups, stress, infection or following dietary manipulation and the use of probiotics are warranted.

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Addenda

ADDENDUM 1

PATIENT STUDY CODE

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT: The relationship between dietary intake, gut microflora and irritable bowel syndrome

PRINCIPAL INVESTIGATOR: Cheryl Stevenson

ADDRESS: Suite G2, Netcare Greenacres Hospital, Port Elizabeth

CONTACT NUMBER: 041 581 0034 / 082 751 4411

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the dietitian (Cheryl Stevenson) or doctor (Dr Ernst Fredericks) any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee (HREC) at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the International Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- This research study is looking at the relationship between food intake, probiotics (good bacteria that are normally found in the body) and irritable bowel syndrome. We suspect that there may be an imbalance of certain healthy bacteria in the gut of patients with irritable bowel syndrome (IBS). We would like to investigate if a certain probiotic can rectify the imbalance and as a result decrease the symptoms (e.g. pain and bloating) of IBS.
- The study will only be conducted at Suite G2, Netcare Greenacres Hospital, Port Elizabeth. The total number of participants to be recruited for this study is 81. Only patients that suffer from IBS will be eligible for inclusion in this study.

- In this project we are aiming to:
  - Find out if there is a relationship between the bacteria found in your colon and food intake.
  - Establish whether a course of probiotics may alleviate undesirable symptoms of IBS and improve quality of life.
- Most of the procedures are routine for IBS patients consulted by Dr Fredericks and are required regardless of the study. These include:
  - Your personal and relevant medical and surgical history (routine data).
  - The other procedures for which you will receive full instructions prior to the study include:

    The collection of three stool samples, before, during and after a course of probiotics

    The completion of a three day estimated food record

    The completion of irritable bowel syndrome symptoms and quality of health questionnaires (At **six** time points during the duration of the trial)
- You will be required to take either an eight week course of probiotics or a placebo (an inactive substance that looks like a medicine but contains no active ingredient). You will be unaware of whether you are receiving the probiotics or the placebo during the course of the trial. You will be required to take the probiotics/placebo in the form of two daily capsules. This will be given to you free of charge.

Why have you been invited to participate?

- You have been asked to participate in this study as you have irritable bowel syndrome.

What will your responsibilities be?

Your responsibility will be:

- To collect three stool samples at home and deliver them to the practise at Netcare Greenacres Hospital.
- Record your food intake for three days and return this information on the day of your second visit.
- Complete an eight week course of probiotics or placebo.
- Complete questionnaires on your IBS symptoms and Quality of Lifestyle at intervals during the trial.
- Attend all five scheduled visits.
- Be available for one telephonic consultation with the dietitian.

Will you benefit from taking part in this research?

- If you are selected to receive the probiotic treatment, you will hopefully experience an alleviation of IBS symptoms, with specific regards to abdominal pain. Future IBS patients will be given improved dietary advice based on the findings of this research.

Are there in risks involved in your taking part in this research?

- There are no risks involved with your taking part in this research. Probiotics are safe for human consumption and should not cause any discomfort. In previous research this probiotic had no side-effects. In studies with other types of probiotics, some patients have complained of the taste of the product, nausea, headache and heartburn. In the unlikely event of an adverse effect you will be covered by the University of Stellenbosch Insurance for Clinical Trials.

If you do not agree to take part, what alternatives do you have?

- This study will not affect your medical assessment or treatment both now or in the future.

Who will have access to your medical records?

- The information collected will be treated as confidential and protected. If it is used in a publication or thesis, the identity of you, the participant, will remain anonymous. The doctor, nursing sister and principal investigator of this project will have access to the information.

What will happen in the unlikely event of some form of injury occurring as a direct result of your taking part in this research study?

- The probiotic forms part of normal gut flora and we suspect that IBS patients have insufficient available numbers of this gut flora. The probiotic has medical approval for human consumption and should therefore not be harmful. If you experience any worsening of your symptoms and you suspect it may be due to the probiotic please contact Dr Fredericks to discuss your symptoms with him.

Will you be paid to take part in this study and are there any costs involved?

- You will receive R80 per visit for transport costs for all visits to the practise. There are no other additional expenses.

Is there any thing else that you should know or do?

- It is your right to be told any new relevant information that arises during the course of the trial and this will be done so by the investigator.
- You can contact Dr Fredericks on tel. 041 3632870 or after hours at 082 859 6354 if you have any further queries or encounter any problems regarding your medical care.

- You can contact Cheryl Stevenson (the dietitian) at tel. 041 581 0034 or 082 751 4411 with regards to dietary related questions.
- You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled: *The relationship between dietary intake, gut microflora and irritable bowel syndrome.*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.
- I have no objections that data collected during the course of this study can be used for research purposes as stated and that my anonymity will be guaranteed.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

....

Signature of participant Signature of witness

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above

Signed at (*place*) .....….....…….. on (*date*) ……...…….. 2013.

....

Signature of investigator Signature of witness

ADDENDUM 2

PASIËNT STUDIEKODE

DEELNEMERINLIGTINGSBLAD EN -TOESTEMMINGSVORM

TITEL VAN DIE NAVORSINGSPROJEK: Die verhouding tussen dieetinname, derm mikroflora en prikkelbare derm sindroom *("The relationship between dietary intake, gut microflora and irritable bowel syndrome").*

HOOFNAVORSER: Cheryl Stevenson

ADRES: Suite G2, Netcare Greenacres Hospitaal, Port Elizabeth

KONTAKNOMMER: 041 581 0034 / 082 751 4411

U word genooi om deel te neem aan 'n navorsingsprojek. Lees asseblief hierdie inligtingsblad op u tyd deur aangesien die detail van die navorsingsprojek daarin verduidelik word. Indien daar enige deel van die navorsingsprojek is wat u nie ten volle verstaan nie, is u welkom om die dieetkundige (Cheryl Stevenson) of die dokter (Dr Ernst Fredericks) daaroor uit te vra. Dit is baie belangrik dat u ten volle moet verstaan wat die navorsingsprojek behels en hoe u daarby betrokke kan wees. U deelname is ook **volkome vrywillig** en dit staan u vry om deelname te weier. U sal op geen wyse hoegenaamd negatief beïnvloed word indien u sou weier om deel te neem nie. U mag ook te eniger tyd aan die navorsingsprojek onttrek, selfs al het u ingestem om deel te neem.

Hierdie studie is goedgekeur deur **die Komitee vir Mensnavorsing van die Universiteit Stellenbosch** en sal uitgevoer word volgens die etiese riglyne en beginsels van die Internasionale Verklaring van Helsinki, Suid-Afrikaanse riglyne vir Goeie Kliniese Praktykvoering en die Mediese Navorsingsraad (MNR) se etiese riglyne vir navorsing.

Wat behels hierdie navorsingsprojek?

- Die studie ondersoek die verband tussen voedselinname, probiotika (goeie bakterieë wat normaalweg in die liggaam gevind word) en prikkelbare derm sindroom (PDS). Ons vermoed dat daar 'n wanbalans van sekere gesonde bakterieë in die derms van pasiënte met PDS is. Ons wil graag ondersoek of 'n sekere probiotika die wanbalans reg kan stel en gevolglik die simptome (bv. pyn en winderigheid) van PDS verminder.
- Die studie sal uitgevoer word by Suite G2, Netcare Greenacres Hospitaal, Port Elizabeth. Die totale aantal persone wat genooi gaan word om deel te neem aan hierdie studie is 81. Slegs PDS pasiënte sal in aanmerking kom vir insluiting in hierdie studie.
- In hierdie projek wil ons:
    - Uitvind of daar 'n verband is tussen die bakterieë in jou derm (kolon) en voedsel inname.

- Vasstel of 'n kursus van probiotika die PDS ongewensde simptome kan verlig en kwaliteit van lewe kan verbeter.

- Die meeste van die prosedures is roetine vir PDS pasiënte gekonsulteer deur Dr Fredericks en is nodig, ongeag van die studie. Dit sluit in:

  - U persoonlike en relevante mediese en chirurgiese geskiedenis (roetine data).

  - Die ander prosedures waarvoor u volledige instruksies sal ontvang voor die studie begin, sluit in:

    Die versameling van drie stoelgangmonsters, voor, tydens en na 'n kursus van probiotika.

    Die voltooiing van 'n drie-dag voedsel rekord.

    Die voltooiing van ‘n PDS-simptome en -kwaliteit van gesondheidsorg vraelyste (op **ses** tydspunte tydens die studie).

- U sal gevra word om ‘n agt week kursus van probiotika óf ‘n plasebo (‘n onaktiewe stof wat lyk soos 'n medisyne, maar wat geen aktiewe bestandele bevat) te neem. U sal nie weet of u die probiotika óf die plasebo neem nie. Daar sal van u verwag word om die probiotika/plasebo in die vorm van twee kapsules per dag te neem. Dit sal gratis aan u gegee word.

Hoekom is jy genooi om deel te neem?

- U is gevra om deel te neem aan hierdie studie omdat want u prikkelbare derm sindroom het.

Wat sal u verantwoordelikhede wees?

U verantwoordelikheid sal wees:

- Om drie stoelgang monsters by die huis te versamel en dit af te lewer by die praktyk by Netcare Greenacres-hospitaal.
- Om rekord van u voedselinname oor drie dae te hou en dit in te handig op die dag van u kolonoskopie.
- Om 'n agt week kursus van probiotika óf plasebo te volg.
- Om vraelyste te voltooi oor u PDS simptome en die kwaliteit van lewe met tussenposes gedurende die studie.
- Om al vyf geskeduleerde besoeke na te kom.
- Om beskikbaar wees vir een telefoniese konsultasie met die dieetkundige.

Sal u voordeel trek deur deel te neem aan hierdie navorsingsprojek?

- As u gekies word om die probiotika te ontvang, sal u hopelik verligting van PDS simptome ervaar, met spesifieke betrekking tot abdominale pyn. Toekomstige PDS

pasiënte sal moontlik beter dieetriglyne ontvang op grond van die bevindinge van hierdie studie.

Is daar risiko's verbonde aan u deelname aan hierdie navorsing?

- Daar is geen risiko's verbonde aan u deelname aan hierdie navorsing nie. Probiotika is veilig vir menslike gebruik en behoort nie enige ongerief te verskaf nie. In die onwaarskynlike geval van enige newe-effekte, sal u gedek wees deur die Universiteit Stellenbosch Assuransie vir Kliniese Proewe.

Watter alternatiewe is daar indien u nie instem om deel te neem nie?

- Hierdie studie sal nie u huidige of toekomstige mediese assessering of behandeling beïnvloed nie.

Wie sal toegang hê tot u mediese rekords?

- Die inligting wat versamel word, sal as vertroulik hanteer en beskerm word. Indien dit gebruik word in 'n publikasie of tesis, sal u identiteit anoniem bly. Slegs die dokter, verpleegsuster en hoofnavorser van hierdie projek sal toegang hê tot die rekords.

Wat sal gebeur in die onwaarskynlike geval van 'n besering wat mag voorkom as gevolg van u deelname aan hierdie navorsingsprojek ?

- Die probiotika vorm deel van die normale dermkanaal flora en ons vermoed dat PDS pasiënte onvoldoende hoeveelhede van die derm flora het. Die probiotika het mediese goedkeuring vir menslike gebruik en behoort dus nie skadelik te wees nie. In vorige navorsing het hierdie probiotika geen newe-effekte getoon nie. In studies met ander vorme van probiotika, het sommige pasiënte gekla oor die smaak van die produk, naarheid, hoofpyn en sooibrand. Indien u enige verergering van simptome ondervind en u vermoed dat dit moontlik te wyte aan die probiotika kan wees, kontak asb Dr Fredericks om die simptome met hom te bespreek.

Sal u betaal word om deel te neem in hierdie studie en is daar enige koste verbonde?

- U sal R80 per besoek ontvang. Daar is geen ander addisionele uitgawes nie.

Is daar enige iets anders wat u moet weet of doen?

- Dit is u reg om te weet van nuwe relevante inligting wat beskikbaar kom gedurende die uitvoering van die studie en dit sal gedoen word deur die navorser.
- U kan Dr Fredericks kontak by tel. 041 3632870 of na ure by 082 859 6354 indien u enige verdere navrae of probleme het aangaande u mediese sorg.
- U kan Cheryl Stevenson (die dieetkundige) kontak by tel. 041 581 0034 of 082 751 4411 met betrekking tot voedingverwante vrae.
- U kan die Komitee vir Mensnavorsing kontak by 021-938 9207 indien u enige bekommernis of klagte het wat nie bevredigend deur u studiedokter hanteer is nie.
- U sal 'n afskrif van hierdie inligtings- en -toestemmingvorm ontvang vir u eie rekords.

Verklaring deur deelnemer

Met die ondertekening van hierdie dokument onderneem ek …… om deel te neem in 'n navorsingsprojek getiteld: *Die verband tussen dieetinname, derm mikroflora en prikkelbare derm sindroom.*

Ek verklaar dat:

- Ek hierdie inligtings- en toestemmingsvorm gelees het of aan my laat voorlees het en dat dit in ’n taal geskryf is waarin ek vaardig en gemaklik mee is.
- Ek geleentheid gehad het om vrae te stel en dat al my vrae bevredigend beantwoord is.
- Ek verstaan dat deelname aan hierdie navorsingsprojek **vrywillig** is en dat daar geen druk op my geplaas is om deel te neem nie.
- Ek te eniger tyd aan die navorsingsprojek mag onttrek en dat ek nie op enige wyse daardeur benadeel sal word nie.
- Ek gevra mag word om van die navorsingsprojek te onttrek voordat dit afgehandel is indien my dokter of navorser van oordeel is dat dit in my beste belang.
- Ek geen besware het dat die data wat versamel word gedurende die studie gebruik kan word vir navorsingdoeleindes soos uiteengesit is en dat my anonimiteit gewaarborg sal word nie.

Geteken te *(plek)* ..... op *(datum)* …… 2013.

……... ……

Handtekening van deelnemer **Handtekening van getuie**

Verklaring deur die navorser

Ek, *(naam)*……. verklaar dat:

- Ek die inligting in hierdie dokument verduidelik het aan…….

- Ek hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek tevrede is dat hy/sy al die aspekte van die navorsingsprojek soos hierbo bespreek, voldoende verstaan .

Geteken te *(plek)*…… op *(datum)*……2013

…….. …….

Handtekening van navorser **Handtekening van getuie**

ADDENDUM 3

INTERVENTION CHECKLIST – TO BE INITIALED ON COMPLETION BY INVESTIGATORS

Patient code:

Contact number:

VISIT 1: (First consultation) WEEK -2 – 0 of trial (Run in period) DATE:……

Screening of patient by Dr for trial – Rome II criteria	Informed consent doc. for study given to patient – dietitian/Dr	Francis Severity Score (no.1) completed, if score < 75 can't enrol in study - dietitian	3 day dietary record explained - dietitian	Stool sample collection (no. 1) explained – nursing sister
Colonoscopy prep explained – nursing sister				

VISIT 2: (Colonoscopy procedure day) WEEK 0 of trial (Baseline) DATE:……**.....**

Signed informed consent doc. handed in – to receptionist/nursing sister	Stool sample (no. 1) handed in – to receptionist/nursing sister	Dietary info completed and handed in	Francis Severity Score questionnaire (no. 2) completed at practise – dietitian/nursing sister	Complete IBS-QOL questionnaire (no.1) at practise – dietitian/nursing sister
Randomisation to treatment product or placebo – dietitian/nursing sister	Patient to take home capsules and start treatment (weeks 0 - 4 of trial) - dietitian/nursing sister	Colonoscopy - Dr		

VISIT 3: TELEPHONIC CALL: WEEK 2 of trial (Treatment) DATE:…….

Francis Severity Score Questionnaire (no.3) completed by dietitian via telephone call	Ask questions on tolerability/adverse events and compliance (see questions directly below)			

Please ask the patient the following:

Tolerability of test product during weeks 0-2?: Good/poor, why?

Adverse health event (this could be anything that happens in the time whilst on the test product e.g. angina or something that might be caused by the test product e.g. dyspepsia), weeks 0-2? Yes/no, what?

Compliance in using test product during weeks 0-2?

Do you have any leftover tablets Yes/No

If yes, why were you unable to take the tablet (s)?

How many left over tablets do you have?

VISIT 4: WEEK 4 of trial (Treatment) DATE:…….

Stool sample collection (no. 2) explained, give specimen bottle (same as for sample 1) – nursing sister/dietitian	Francis Severity Score questionnaire (no.4) completed at practise – nursing sister/dietitian	Ask questions on tolerability/adverse events and compliance (see questions directly below) – nursing sister/dietitian	Patient to take home capsules (weeks 4-8 of trial) – nursing sister/dietitian	Give participant travel money (R80)

Please ask the patient the following:

Tolerability of test product during weeks 3-4?: Good/poor, why?

Adverse health event (this could be anything that happens in the time whilst on the test product e.g. angina or something that might be caused by the test product e.g. dyspepsia), weeks 3-4? Yes/no, what?

Compliance in using test product during weeks 3-4?

Do you have any leftover tablets Yes/No

If yes, why were you unable to take the tablet (s)?

How many left over tablets do you have?

Please hand tablets back:

VISIT 5: WEEK 8 of trial (End of treatment) DATE:……..

Stool sample (no. 2) handed in – nursing sister	Francis Severity Score questionnaire (no.5) completed at practise – nursing sister/dietitian	Complete IBS-QOL questionnaire (no.2) at practise – nursing sister/dietitian	Ask questions on tolerability/adverse events and compliance (see questions directly below) – nursing sister/dietitian	Stool sample collection (no. 3) explained (same as for no 1 & 2), give specimen bottle- nursing sister/dietitian
Give participant travel money (R80)				

Please ask the patient the following:

Tolerability of test product during weeks 4-8?: Good/poor, why?

Adverse health event whilst on test product, weeks 4-8? Yes/no, what?

Compliance in using test product during weeks 4-8?

Do you have any leftover tablets Yes/No

If yes, why were you unable to take the tablet (s)?

How many left over tablets do you have?

Please hand back:

VISIT 6: WEEK 10 of trial (Wash – out period) DATE:……

Stool sample (no 3) handed in – nursing sister	Francis Severity Score questionnaire (no.6) completed at practise – nursing sister/dietitian	Complete QOL questionnaire (no.3) at practise – nursing sister/dietitian	Ask questions on tolerability/adverse events and compliance (see questions directly below) – nursing sister/dietitian	Give participant travel money (R80)

Please ask the patient the following:

Adverse health event during weeks 8-10? Yes/no, what?

ADDENDUM 4

PATIENT STUDY CODE

DEMOGRAPHIC QUESTIONNAIRE

1. Initials_____

2. DOB: _____

3. Height: _____

4. Weight: _____

5. BMI: _____

6. Gender: Male

Female

7. Population group:

Caucasian

Coloured

Black

Indian

8. Smoke: Y/N

If yes: how many____/day

9. Background medical history

_____

_____

_____

_____

10. Current medication:

_____

_____

_____

_____

11. Previous bowel surgery:

_____

_____

_____

_____

12. Recent antibiotic use (name and date):

_____

_____

_____

_____

13. Family history of CRC: Y/N If yes,

Relationship_____

Age at diagnosis: _____

Relationship_____ Age at diagnosis: _____

Relationship_____ Age at diagnosis: _____

Relationship_____ Age at diagnosis: _____

14. Family history of IBD: Y/N If yes,

Relationship_____ Diagnosis: _____

Relationship_____ Diagnosis: _____

15. Family history of metabolic syndrome: Y/N If yes,

Relationship_____ Diagnosis: _____

Relationship_____ Diagnosis: _____

Relationship_____ Diagnosis: _____

Relationship_____ Diagnosis: _____

16. Family history of IBS: Y/N. If yes,

Relationship_____

Relationship_____

17. Type of IBS
Constipation pre-dominant

Diarhhea pre-dominant

Alternating (if alternating, what is the predominant symptom currently?)

18. Frequent antibiotic use during childhood: Y/N

19. Trigger of IBS:

None

Emotional

Gastroenteritis

Antibiotic treatment

Other

20. Lactose intolerance: Y/N

If yes, was this a self-diagnosis or medical (doctor) diagnosis?

Futhermore was the diagnosis lactose intolerance or lactose malabsorption?

21. Use of anti-IBS treatment: Y/N

If yes, what and for how long?

22. Duration of IBS:

o----------O----------o

ADDENDUM 5

THREE DAY FOOD RECORD

PATIENT STUDY CODE [ ] (official use)

(Please record all the food/drink eaten in the three days prior to the preparation for operational procedures)

Name:

Date of Birth: Age:

Male/Female

Telephone no. (H): (W): (cell):

Please contact dietitian Cheryl Stevenson with any queries on 082 751 4411

PLEASE REMEMBER:

- COMPLETE FOR TWO WEEK DAYS AND ONE WEEKEND DAY
- DO NOT COMPLETE ON COLON PREPERATION DAY PRIOR TO COLONOSCOPY

PROCEDURE FOR COMPLETING THE ESTIMATED DIETARY RECORD

The participant is required to record all foods and drinks eaten/drank on three days (one weekend and two week days). The dietary record RECORDING SHEET consists of a cover page and several recording pages.

Steps to follow:

Step 1: From the time you wake up in the morning until you go to bed at night you need to record everything you eat and drink on the recording sheets. **DO NOT** change the way you eat. Remember to record the day of the week every time you start a new day.

Step 2: Every time you eat and drink something, remember to record the time of day in the first column.

Step 3: In the next column describe the food item in as much detail as possible and according to the method of preparation, examples follow below:

Liquids

An average sized small or large glass can be filled to different levels, please specify. If even smaller or larger glasses were used (diagrams have been shown), record the height and width of the glass in centimeters if possible (a ruler should be available for these purposes)

Dairy

The type of milk used is important e.g. condensed, evaporated milk (e.g. *Ideal Milk*), fresh milk (e.g. skim milk (0% fat), low fat (2%), full cream (4% fat)), milk powder (e.g. *Nespray*), non-dairy creamer (not a dairy powder but is made from plant oils e.g. *Cremora* or *Ellis Brown*). How much milk is used if a little milk is used – tea/coffee only slightly cloudy, see through. If medium amount is used tea/coffee is cloudy/lighter and not see through. If a lot of milk is used the tea/coffee is very light. If a little milk is added to porridge/cereal the milk is equal to about ¼ the volume of the porridge/cereal. If a medium amount of milk is added to porridge this is equal to ½ the volume of the porridge. If a lot of milk is added the milk is equal to the total volume of the porridge.

Fats

Determine type of fat used (e.g. Lard, tallow, butter, white margarine like *Wooden Spoon* or normal margarine) Tub vs. brick margarine and state if possible whether it was light or regular. How is fat spread on bread? If it is thinly spread it hardly covers the bread and the bread can still be clearly seen. If it is medium spread it covers the bread well but not yet thick. If it is thickly spread – shows teeth marks when a bite is taken. If a sandwich is eaten is bread spread on both sides?

Fruit juice

Is it fresh, sweetened, unsweetened, nectar, whole juice (pure) or reconstituted concentrate. E.g. of pure fruit juices which are unsweetened include *Appetizer*, *Grapetiser*, tomato juice (actually a vegetable juice), *Liquifruit* and *Ceres*. Nectars are fruit juices that have 50% or less pure juice, e.g. *Capri-Sonn*, *Minute Maid* and *Ice*.

Meat

Determine if the cut of the meat was fatty/lean, with/without bones. Determine the cooking/preparation method used to prepare foods e.g.

- Roasting – cooking in an oven with the addition of little or no fat
- Pan frying – cooking in a pan in shallow fat
- Deep fat frying – cooking in deep oil
- Grilling or boiling – cooking in the oven, over hot coals (braai) in an electric griller or heavy metal grilling pan where fat can drain off into the grooves
- Pot roasting – cooking in an iron pot or saucepan on top of stove or fire
- Braising – cooking by browning on its own or with a little fat and then adding small amounts of boiling water or other liquid.
- Stewing – cooking in sufficient liquid to cover food
- Boiling – food is placed in cold water that is brought to the boil, once boiling the heat is reduced and food allowed to cook until tender.

Chicken

Determine if light or dark meat was eaten and the preparation method used.

Fish

Fish is classified based on the fat content:

- Fatty fish – Kipper, herring, butterfish, mackerel and salmon
- Moderately fat fish – Yellow fish, trout, cod, galjoen, harder, pilchards, shad, snoek, trout and tuna
- Low fat/white fish – Stockfish, sole, cob, kabeljou, kingklip, hake and geelbak.

Protein sources

Pieces of meat, cheese and fish can be expressed as number of matchboxes (1, 2, 3 etc).

Fruit

Fresh, canned or home-cooked (with/without sugar), whether it was peeled or unpeeled. Fruit salad or stewed fruit may be a combination of different fruits. Describe the fruits that were included and whether ±equal amounts of all fruits were included or whether some fruits 'dominated'. Determine if sugar was added to fruit salad/stewed fruit.

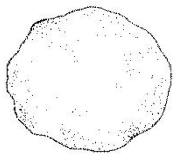
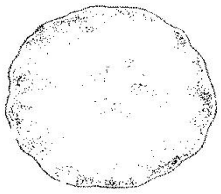
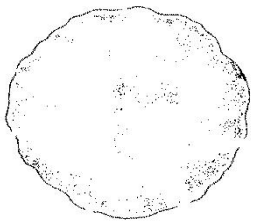
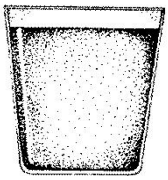
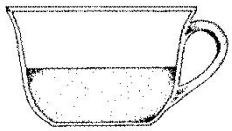
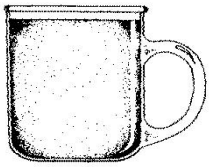
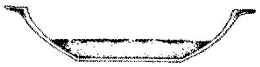
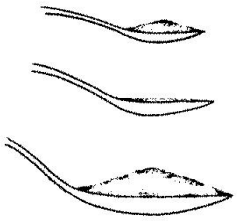
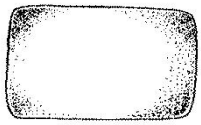
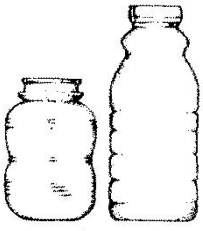
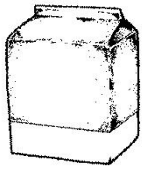
Vegetables

Vegetables may be purchased fresh, frozen, sun-dried or canned. They may be eaten raw or cooked. Fat (butter, margarine) sugar, mayonnaise, sour cream may be added to the vegetables.

Indicate if you are not sure what you ate. Make use of brand names if possible. State whether homemade or commercial products were used (e.g. biscuits, cakes, puddings)

Step 4: Describe the portion size of each item consumed according to the guidelines provided by the dietitian. Also see attached sheet of generic food portions.

Generic life-size sketches found in the Food Photo Manual on pages 1-15

¼ cup amorphous-top view 	½ cup amorphous-top view 	1 cup amorphous-top view 
1 cup amorphous-side view 	Small glass 	Large glass 
Teacup 	Mug 	Shallow bowl 
Deep bowl 	Spoons 	2 x matchboxes (top view) 
2 x matchboxes (side view) 	Bottles 	Carton 

DAY 1

Date:

Day: M T W Th F S S

Time	Food Item	Detail	Detail of portion size
e.g. 06:30	Bread	Brown Margarine (Flora Light)	3 thick slices Thinly spread on all 3 slices
	Coffee	Instant – caffeinated Milk – 2% fresh milk White sugar	1 cup (250ml) 2 Tablespoons (30ml) 1 teaspoon (5ml)

DAY 2

Date:

Day: M T W Th F S S

Time	Food Item	Detail	Detail of portion size
e.g. 06:30	Bread	Brown Margarine (Flora Light)	3 thick slices Thinly spread on all 3 slices
	Coffee	Instant – caffeinated Milk – 2% fresh milk White sugar	1 cup (250ml) 2 Tablespoons (30ml) 1 teaspoon (5ml)

DAY 3

Date:

Day: M T W Th F S S

Time	Food Item	Detail	Detail of portion size
e.g. 06:30	Bread	Brown Margarine (Flora Light)	3 thick slices Thinly spread on all 3 slices
	Coffee	Instant – caffeinated Milk – 2% fresh milk White sugar	1 cup (250ml) 2 Tablespoons (30ml) 1 teaspoon (5ml)

ADDENDUM 6

IBS QUESTIONNAIRE

Name: _____ **G.P. Name:** _____

Address: _____ **Address:** _____

_____ _____

_____ _____

Telephone: _____ **Telephone:** _____

Date of birth: _____

Marital status: Single / Married / Divorced / Widowed / Co-Habit

Occupation: _____ **Sex:** M F

Ethnic background: Caucasion (white) / Afro-Carribean / Asian / Oriental

Fathers Occupation (even if retired): _____

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (ie over the last 10 days or so). All information will be kept in strict confidence.

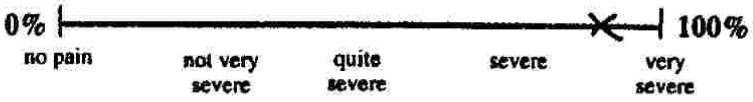
1. For questions where a number of different responses are a possibility please circle the response appropriate to you.
2. Some questions will require you to write in an appropriate response.
3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem:

For example:

How severe was your pain?

Please place your cross (X) anywhere on the line between 0-100%
in order to indicate as accurately as possible the severity of your symptom.

This example shows a severity of approximately 90%.



PART 1 : SEVERITY SCORE

For office use only

SCORE

1. ***a) Do you currently suffer from abdominal (tummy) pain?*** **YES** **NO**

Circle appropriate box

b) If yes, how severe is your abdominal (tummy) pain?

0% ——— 100%

no pain | not very severe | quite severe | severe | very severe

c) Please enter the number of days that you get the pain in every 10 days.

For example if you enter 4 it means that you get pain 4 out of 10 days. If you get pain every day enter 10

Number of days with pain _____ x10

2. ***a) Do you currently suffer from abdominal distension* (bloating, swollen or tight tummy)*** **YES** **NO**

Circle appropriate box

*(*women, please ignore distension related to your periods)*

b) If yes, how severe is your abdominal distension/tightness

0% ——— 100%

no distension | not very severe | quite severe | severe | very severe

3. ***How satisfied are you with your bowel habit?***

0% ——— 100%

very happy | quite happy | unhappy | very unhappy

4. ***Please indicate with a cross on the line below how much your Irritable Bowel Syndrome is affecting or interfering with your life in general***

0% ——— 100%

not at all | not much | quite a lot | completely

IBS SEVERITY SCORE: _____

PART 2 : OTHER IBS DATA

BOWEL HABIT

5. a) ***What is the most number of times you open your bowels per day/week/month?***

Number of times ☐ per day / week / month *(Circle appropriate)*

Note: For some people the answer to part a and b could be the same

b) ***What is the least number of times you open your bowels per day/week/month?***

Number of times ☐ per day / week / month *(Circle appropriate)*

6. In the following questions you may circle more than one answer:

Are your motions ever:

a)	***normal***	often / occasionally / never	*(Circle appropriate)*
b)	***hard***	often / occasionally / never	*(Circle appropriate)*
c)	***very thin (like string)***	often / occasionally / never	*(Circle appropriate)*
d)	***in small pieces (like rabbit pellets)***	often / occasionally / never	*(Circle appropriate)*
e)	***mushy (like porridge)***	often / occasionally / never	*(Circle appropriate)*
f)	***watery***	often / occasionally / never	*(Circle appropriate)*

7. In the following questions you may circle more than one answer:

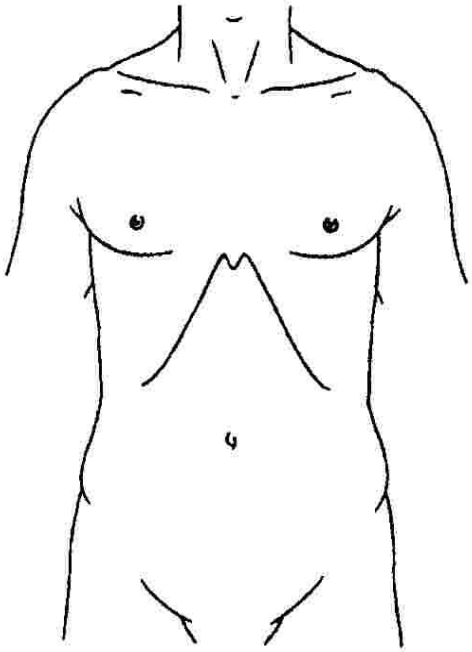
Do you ever:

		Circle appropriate box	
a)	***pass mucus (or slime or jelly) with your motions***	YES	NO
b)	***pass blood with your motions***	YES	NO
c)	***have to hurry/rush to the toilet to open your bowels***	YES	NO
d)	***strain to open your bowels***	YES	NO
e)	***feel you haven't emptied your bowel completely after you have passed a motion***	YES	NO

PART 2 : Continued

SITE OF PAIN

Please mark with a cross (x) on the diagram below where you get your pain
(use more than one x if necessary)



8. *Do you ever:*

   a) *notice your stools are more frequent or loose when you get pain* — YES / NO
   Circle appropriate box

   b) *notice whether the pain is frequently eased by opening your bowels* — YES / NO
   Circle appropriate box

9. *In the last year on approximately how many weeks were you:*

   i) *absent from work due to IBS*
   (enter 52 if you have given up completely work because of IBS) _____

   ii) *at work suffering from IBS* _____

ADDENDUM 7

PARTICIPANT/PATIENT ID:

PLEASE WRITE IN

TODAY'S DATE: _____ _____ _____

DAY MONTH YEAR

PLEASE READ THIS CAREFULLY

ON THE FOLLOWING PAGES YOU WILL FIND STATEMENTS CONCERNING BOWEL PROBLEMS (IRRITABLE BOWEL SYNDROME) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE CHOOSE THE RESPONSE THAT BEST APPLIES TO YOU

AND **CIRCLE** THE NUMBER OF YOUR RESPONSE.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT, PLEASE GIVE THE BEST RESPONSE YOU CAN. **THERE ARE NO RIGHT OR WRONG RESPONSES.**

YOUR RESPONSES WILL BE KEPT STRICTLY CONFIDENTIAL.

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT:

SITE ADDRESS AND PHONE NUMBER TO BE PLACED HERE

About Your Feelings

Please think about your life over the **past month (last 30 days)** and look at the statements below. Each statement has five possible responses. For each statement, please circle the one response that best describes your feelings.

1. I feel helpless because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL

   2 SLIGHTLY

   3 MODERATELY

   4 QUITE A BIT

   5 EXTREMELY

2. I am embarrassed by the smell caused by my bowel problems. *(Please circle one number)*

   1 NOT AT ALL

   2 SLIGHTLY

   3 MODERATELY

   4 QUITE A BIT

   5 EXTREMELY

3. I am bothered by how much time I spend on the toilet. *(Please circle one number)*

   1 NOT AT ALL

   2 SLIGHTLY

   3 MODERATELY

   4 QUITE A BIT

   5 A GREAT DEAL

4. I feel vulnerable to other illnesses because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

5. I feel fat or bloated because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

6. I feel as though I am losing control of my life because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

7. I feel that my life is less enjoyable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

8. I feel uncomfortable when I talk about my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

9. I feel depressed about my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

10. I feel isolated from other people because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

11. I have to be careful about the amount of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

12. Because of my bowel problems sexual activity is difficult for me. *(Please circle one number)*
(If not applicable, please circle "NOT AT ALL")

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

13. I feel angry that I have bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

14. I feel as though I irritate others because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

15. I worry that my bowel problems will get worse. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

16. I feel irritable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

17. I worry that people think I exaggerate my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

18. I feel that I get less done because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

19. I have to avoid stressful situations because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

20. My bowel problems reduce my sexual desire. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

21. My bowel problems limit what I can wear. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

22. I have to avoid strenuous activity because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

23. I have to be careful about the kind of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

24. Because of my bowel problems I have difficulty being with unfamiliar people. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

25. I feel sluggish because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

26. I feel "unclean" because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

27. Long trips are difficult for me because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

28. I feel frustrated that I cannot eat when I want to because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

29. It is important to be near a toilet because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

30. My life revolves around my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

31. I worry about losing control of my bowels. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

32. I am afraid that I won't be able to have a bowel movement. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

33. My bowel problems are affecting my closest relationships. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

34. I feel that no one understands my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY