Exploring the link between mental health and functional gastrointestinal disorders

---

Simone L. Peters

BSc Psychophysiology (Hons)

Thesis submitted for the degree of Doctor of Philosophy at Monash University in 2015.

Faculty of Medicine, Nursing and Health Sciences, Monash University.

Department of Gastroenterology, Central Clinical School, The Alfred.

Copyright notice

To my son Harvey,

I hope you look to me and see that with hard work and dedication anything is possible.

Dream big my little prince.

Love Mum xx

Abstract

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder (FGID) characterised by abdominal pain and associated with altered bowel habits. There is no cure for IBS and treatment is limited to symptom management strategies. Common approaches to control symptoms associated with IBS include pharmacological agents, dietary therapies and psychological treatments. There is high-quality evidence of efficacy for the low FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols) diet but the gluten-free diet is also being increasingly applied. In fact, non-coeliac gluten sensitivity (NCGS), defined by the worsening of symptoms when consuming gluten and the improvement of symptoms on a gluten-free diet, is now considered a distinct clinical entity. Psychological treatments are also being increasingly sought but how their efficacy compares with that of dietary approaches is unknown.

This thesis contains a series of studies which aimed to 1) gain a greater understanding of the differences, similarities and possible overlap between IBS and NCGS; 2) fill gaps in evidence regarding effects of gluten ingestion on extraintestinal symptoms in patients with NCGS; 3) develop a method for the large-scale isolation of gliadin suitable for human feeding trials; and 4) examine the efficacy of gut-directed hypnotherapy compared to that of the low FODMAP diet.

Initial results from a double-blind, placebo-controlled, randomised, cross-over trial in 22 participants were suggestive of gluten-specific changes to current feelings of depression in patients with NCGS but more detailed analysis in a different cohort of patients with NCGS failed to reproduce this effect. Notably, additional investigations showed no evidence of gluten-specific worsening of any psychological indices, quality of life, fatigue or

gastrointestinal symptoms. The only exception was a small increase in response times on the Subtle Cognitive Impairment Test (SCIT). Overall, these studies failed to support a specific entity of NCGS in patients who would otherwise be classified as having IBS or another FGID.

A method for the large-scale isolation of gliadin suitable for human consumption was successful. However, the lack of specific gluten-mediated effects precluded the planned study of purified gliadin in defining its role in symptom induction in patients with NCGS.

How effective psychological therapies might be for IBS was addressed in a randomised un-blinded study in 78 participants with IBS comparing the relative efficacy of gut-directed hypnotherapy with that of the more established 'gold standard', the low FODMAP diet, alone or in combination. In terms of effect on gastrointestinal symptoms and quality of life, both therapies were similarly efficacious over 6 months, but without additive effects. Gut-directed hypnotherapy had additional psychological benefits. These results indicate that gut-directed hypnotherapy should be considered a viable modality and applied as a primary therapy for patients with IBS.

In conclusion, this thesis has made a considerable contribution to our understanding of the association between psychological health and FGID and has fulfilled its aims. First, it provides high-quality evidence that gluten ingestion is unlikely to be associated with the worsening of psychological (or gastrointestinal) manifestations in patients with self-reported NCGS and questions the existence of the latter as a specific entity. Secondly, a procedure was developed to enable future study of the responsible moiety for gluten-mediated effects. Thirdly, it provides compelling evidence that gut-directed hypnotherapy is a viable therapeutic option for the treatment of IBS. Further research into mechanisms of action and predictors of response, as well as subtle effects of gluten on cognition, is warranted.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

The length of this thesis does not exceed 100,000 words.

Simone Louise Peters

List of publications

The following papers were published during my PhD tenure (2011-2015) and relate to the individual studies or concepts discussed in this thesis:

- **Peters SL**, Yao CK, Shepherd S, Philpott H, Yelland G, Muir JG, Gibson PR. Gut-directed hypnotherapy and a low FODMAP diet are similarly efficacious in patients with irritable bowel syndrome: A randomised controlled non-inferiority trial. *Gastroenterology*. 2015; 148: S487-88

  (0 citations at 8/12/15)

- **Peters SL**, Muir JG, Gibson PR. Review article: Gut-directed hypnotherapy in the management of irritable bowel syndrome and inflammatory bowel disease. *Aliment Pharm Ther*. 2015; 41: 1104-15

  (2 citations at 8/12/15)

- **Peters SL**, Biesiekierski JR, Yelland G, Gibson PR, Muir JG. Randomised clinical trial: Gluten causes depression in subjects with non-coeliac gluten sensitivity – An exploratory clinical study. *Aliment Pharm Ther*. 2014; 39:1104-12

  (35 citations at 8/12/15)

- **Peters SL**, Muir JG, Gibson PR. Gluten sensitivity without coeliac disease – A new twist. *Argo FOOD Indust Hi Tech.* 2014; 25: 38-42

  (0 citations at 8/12/15)

- Biesiekierski JR, **Peters SL**, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-coeliac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology*. 2013; 145: 320-28

(213 citations at 8/12/15)

Acknowledgements

The research contained within this thesis was completed with advice and support from many people. I wish to acknowledge the important contributions made by the following people for their involvement in this work:

- Dr Jane Muir (principle supervisor) for her caring and nurturing approach. You have taught me that in the world of research, it always good to step slightly outside of my comfort zone. I have learnt so much, thanks to you
- Professor Peter Gibson (secondary supervisor) for providing me with limitless support and encouragement. Your guidance and immense knowledge have shaped me to be the researcher I am today. I am eternally grateful for the opportunity of being your student
- Dr Greg Yelland (secondary supervisor) who provided me with statistical and psychological measurement advice throughout the term of my candidature. Your understanding and patience was invaluable
- Dr Sue Shepherd (secondary supervisor) for your expertise on designing dietary trials. Your cheerful nature brightened many a day
- Dr Jessica Biesiekierski for her help co-ordinating the study reported in Chapter 4. It was a pleasure working by your side
- Debbie King for her assistance in food preparation
- Dr Evan Newnham for his involvement in patient screening in Chapter 4
- Chu Yao for her help with recipe formulation in Chapter 5 and for her dietetic expertise in Chapter 7. I feel blessed to have been able to soak in some of your extensive knowledge
- Dr Mark Ward and Dr Avik Majumdar for their involvement in patient screening in Chapter 5

- Judy Moore who happily stepped in and helped to conduct the study reported in Chapter 5 in my absence. I wholeheartedly thank you
- Dr Ferenc Békés for his ongoing support as a protein expert and for the development of the wheat gluten isolation methodology. This work could not have been done without you
- Dr John Pearce and Amy Barrie for helping with the design and application of the wheat gluten isolation project
- Dr Andrew Lawrence for his assistance freeze-drying the end-products obtained from the wheat gluten isolation project
- Dr Hamish Philpott for his involvement in patient screening in Chapter 7
- To my colleagues for the stimulating discussions, the fun we have had together in the last four years and for the encouragement to push on writing this thesis to the very end
- To the Department of Gastroenterology, Monash University, Central Clinical School who has provided me with the support I have needed to complete this thesis and for providing the funding to assist in travel and accommodation for conferences which enabled me to present locally in Melbourne; interstate in Sydney, Brisbane, Adelaide and Hobart; and internationally in Chicago, United States of America; Prato, Italy and Manchester, United Kingdom. These travels were also funded with assistance from scholarships received from The Nutrition Society of Australia. Finally, to the Japan Society for the Promotion of Science who funded my attendance at the 14th HOPE Conference in Tokyo, Japan
- To Yvonne and the late Geoffrey Wilson for awarding me with the Andrea Joy Logan Scholarship in loving memory of their daughter
- Dr Emma Halmos for her never ending love and support. You laughed and cried with me through the rollercoaster of this PhD. I couldn't have survived without you

- The study participants who’s dedication made this research possible

I would also like to thank Mum, Dad, Danielle and the rest of my family whose upmost patience, love and inspiration allowed me to pursue my dreams and give my best efforts. Without you none of this would have been possible. Lastly to John, my soulmate and best friend, thank you for always being on my team. Your love and support is invaluable.

Table of contents

List of tables

List of figures

List of abbreviations

5-HT	5-hydroxytryptamine
5-HT_3	5-hydroxytryptamine-3
ACTRN	Australian Clinical Trials Registry
ANS	Autonomic nervous system
APC	Antigen-presenting cells
ATI	α-amylase trypsin inhibitors
CBT	Cognitive behavioural therapy
CD	Compact disc
CI	Confidence interval
CNS	Central nervous system
CPRS	Composite Primary Reduction Score
CRF	Corticotrophin releasing factor
CRF_1	Corticotrophin releasing factor-1
DASS	Depression Anxiety Stress Scale
D-FIS	Daily Fatigue Impact Scale
DSM-V	Diagnostic and Statistical Manual of Mental Disorders
EMG	Electromyography

ENS	Enteric nervous system
FDR	False discovery rate
FGID	Functional gastrointestinal disorder
fMRI	Functional magnetic resonance imaging
FMT	Faecal microbiota transfer/transplant
FODMAP	Fermentable oligosaccharides, disaccharides, monosaccharides and polyols
g	Gram
GDH	Gut-directed hypnotherapy
GI	Gastrointestinal
GOS	Galacto-oligosaccharides
GP	General Practitioner
GSRS	Global Symptom Rating Scale
HADS	Hospital Anxiety and Depression Scale
HLA	Human leukocyte antigen
HMW-GS	High molecular weight gluten subunits
HPLC	High-performance liquid chromatography
IBS	Irritable bowel syndrome
IBS-IS	Irritable bowel syndrome impact scale
IBS-QOL	Irritable bowel syndrome quality of life

IFN-γ	Interferon γ
IgE	Immunoglobulin E
IgG	Immunoglobulin
IL	Interleukin 13
L	Litres
LFD	Low FODMAP diet
LMW-GS	Low molecular weight gluten subunits
mg	Milligram
MHC	Major histocompatibility complex
Min	Minute
MJ	Mega joule
ml	Millilitre
mm Hg	Millimetres of mercury
mm	Millimetre
MRI	Magnetic resonance imaging
NA	Not applicable
NCGS	Non-coeliac gluten sensitivity
NICE	National Institute for Health and Clinical Excellence
NK	Natural killer

nm	Nanometre
NNT	Number needed to treat
PVDF	Polyvinylidene fluoride
QOL	Quality of life
RAST	Radioallergosorbent testing
RCT	Randomised control trial
SCIT	Subtle Cognitive Impairment Test
SCL-90R	Symptom Checklist–90 Revised
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SF-36	Short Form-36
SIBO	Small intestinal bacterial overgrowth
SPSI	Stress-related Physical Symptoms Inventory
SSRI	Selective serotonin reuptake inhibitors
STPI	State Trait Personality Inventory
TCA	Tricyclic antidepressants
tTG	Tissue transglutaminase
UK	United Kingdom

US	United States
USA	United States of America
VAS	Visual analogue scale
WGA	Wheat lectin agglutinin
η_p^2	Effect size
μg/dl	Micrograms per decilitre
μl	Microliter

Chapter 1 – Introduction

Functional gastrointestinal disorders (FGID) are a broad spectrum of different disorders that affect various parts of the gastrointestinal tract. Irritable bowel syndrome (IBS) is the most common FGID and is the primary focus of this thesis.

1.1 Irritable Bowel Syndrome: An Overview

IBS is characterised by recurrent abdominal pain and changes in stool consistency and form in the absence of any pathological abnormality.[1] Clinical features including abdominal bloating, excessive wind and nausea are also frequently described by patients. IBS is the most common functional gastrointestinal disorder affecting the small and large intestine. It is likely to be of heterogeneous pathogenesis and aetiology and its treatment involves multiple modalities, reflecting the clinical difficulty in achieving satisfactory outcomes. A detailed analysis of all treatments is beyond the scope of this thesis, but a brief overview will follow. Areas of specific interest and relevance to the research contained within this thesis will be discussed in more detail in later sections.

1.1.1. Diagnosis

Several sets of diagnostic criteria have been developed for the use in IBS. The first, the Manning criteria were developed in the 1970s where IBS was identified by comparing symptoms in patients with abdominal pain who either had or did not have organic disease. In 1988, however, an international working group, The Rome Foundation, created a new set of guidelines to aid in the diagnosis and treatment of all FGID and to ensure homogeneity in the classification of patients.[2] The latest version of the criteria, the Rome III criteria, were published in 2006 and includes six major domains for adults: esophageal, gastroduodenal,

bowel, functional abdominal pain syndrome, biliary and anorectal.[2] Each category contains several disorders with each having relatively specific clinical features.[2] The functional bowel disorders include IBS, functional bloating, functional constipation and functional diarrhoea which are anatomically attributed to the small and large intestine and rectum[2] and are outlined in Table 1.1. Application of the Rome III criteria is not limited to clinical trials but is also used in clinical practice.

1.1.2. Epidemiology

IBS is common with current data indicating a prevalence of between 5 and 12% in Western countries,[3, 4] but with wide variations between countries (Figure 1.1).[5] The condition mainly occurs between the ages of 15 and 65 and is more common in women.[5] Diagnostic criteria are associated with varying prevalences and bowel habit sub-types including diarrhoea, constipation and alternating habits.[3] IBS has a considerable impact on sufferer's lifestyle and health care.[3] Forty percent of patients formally diagnosed as having IBS have been diagnosed for 10 years or more and approximately 60% report experiencing ongoing symptoms despite continuing care.[3] Levels of co-morbid disease are also high and can include other gastrointestinal complaints, non-gastrointestinal related disorders such as chronic fatigue syndrome or fibromyalgia and psychiatric illness.

Table 1.1. Rome III Functional Bowel Disorder diagnosis criterion

Functional Bowel Disorders	Diagnostic criterion
Irritable Bowel Syndrome	Recurrent abdominal pain or discomfort at least 3 days/month in the last 3 months associated with two 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
Functional Bloating	1. Recurrent feeling of bloating or visible distention at least 3 days/month in the last 3 months 2. Insufficient criteria for a diagnosis of functional dyspepsia, irritable bowel syndrome, or other functional GI disorder
Functional Constipation	1. Must include two or more of the following: a. Straining during at least 25% of defecations b. Lumpy or hard stools in at least 25% of defecations c. Sensation of incomplete evacuation for at least 25% of defecations d. Sensation of anorectal obstruction/blockage for at least 25% of defecations e. Manual manoeuvres to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor) f. Fewer than three defecations per week 2. Loose stools are rarely present with the use of laxatives 3. Insufficient criteria for irritable bowel syndrome
Functional Diarrhoea	Loose (mushy) or watery stools without pain occurring in at least 75% of stools
Unspecified Functional Bowel Disorder	Bowel symptoms not attributed to an organic aetiology but that do not meet criteria for the previously defined categories

1.1.3. Pathogenesis of irritable bowel syndrome

The pathogenesis of IBS seems to be multifactorial with intestinal motility, visceral sensitivity, microbiota and brain-gut interactions playing a central role. Specifically, alterations in motility have been proposed to underlie irregularities in bowel habits.[6, 7] Increased perception of visceral stimuli may contribute to a heightened sense of abdominal symptoms resulting from changed central or enteric nervous system signalling.[8, 9] An altered intestinal microbiota could also contribute to symptoms of IBS, yet a causative role remains to be established.[10-13] Given the association between symptoms of IBS and various psychological states and the responsiveness of symptoms in many patients to therapies directed at the central nervous system (CNS), altered brain-gut interactions could also play a role. The relationship between psychological states, gastrointestinal symptoms and altered gut-brain interactions will be discussed in detail within this thesis.

1.1.4. Treatments for irritable bowel syndrome

There is no known cure for IBS and treatment is limited to symptom management strategies. Common approaches to control symptoms associated with IBS include pharmacological agents, dietary therapies and psychological treatments.

1.1.4.1. Pharmacological agents

Due to the lack of effective pharmacological agents for the overall improvement of multiple symptoms in IBS, medications are usually aimed at treating the patient's most troublesome symptom/s. The most commonly used symptom-based pharmacological agents include laxatives, anti-diarrhoeal agents, antispasmodics, antidepressants and probiotics, prebiotics and antibiotics, as detailed below.

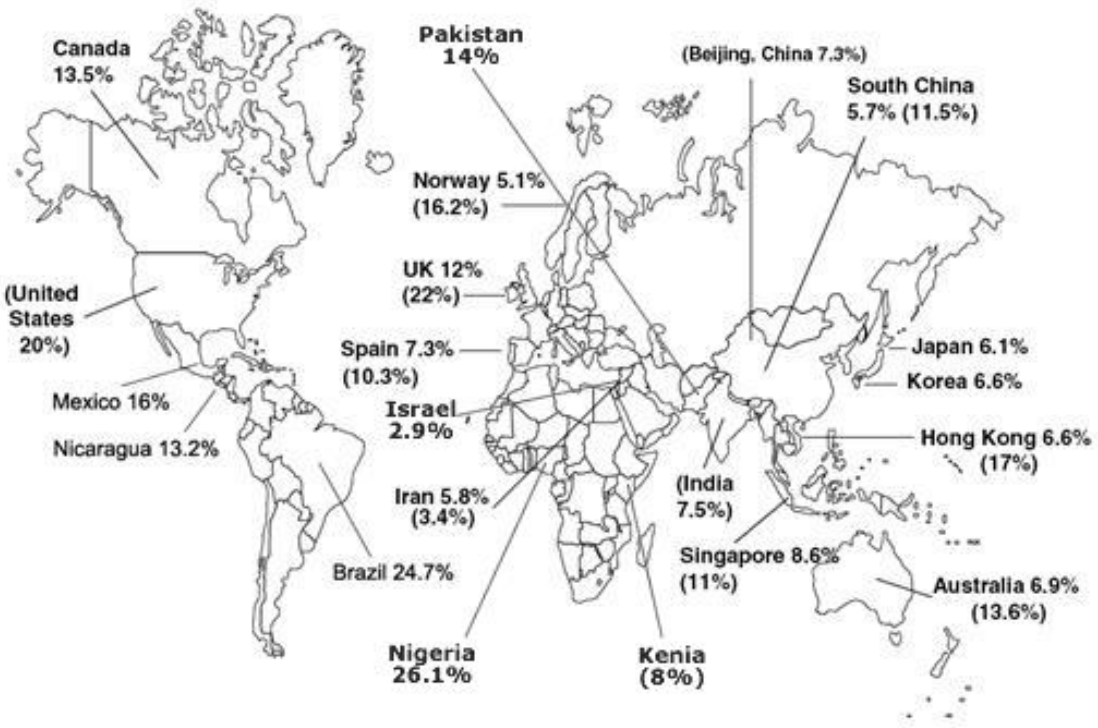


Figure 1.1. World map of IBS prevalence based on Rome II and Rome III criteria, with figures for the Manning criteria in brackets where available. Taken from WGO Practice Guidelines, 2009.[5]

1.1.4.1.1. Laxatives

Laxatives increase the frequency of bowel actions and include several classes such as fibre, osmotic laxatives, stimulant laxatives and emollients. They are often used in patients with constipation. Fibre is not digested in the small intestine and moves largely unchanged into the large intestine where is it fermented by bacteria. Patients with constipation are initially recommended to increase their daily dietary intake of fibre to 25-30 g per day over several weeks. If results from this increase are not satisfactory, then commercially-packaged bulking agents can be used. In general, increasing dietary fibre has not been shown to be more effective than placebo in managing IBS symptoms, with the notable exception of ispaghula

(psyllium).[14] Osmotic laxatives can also be used and are poorly absorbed compounds that cause an influx of water into the small and large intestine, thereby increasing stool bulk. These laxatives are safe to take for chronic constipation and can be used long-term. Stimulant laxatives should only be used when osmotic laxatives have been inefficient as they have a direct stimulating effect on the network of nerves in the large intestine and reduce the absorption of water from gastrointestinal content and should only be recommended for short-term use. Finally, emollients act as stool softeners or lubricants and can be used to provide moisture to the stool making it easier to pass.

1.1.4.1.2. Anti-diarrhoeal agents

Anti-diarrhoeal agents, notably loperamide and diphenoxylate, are effective in patients with both acute and chronic diarrhoea. They work by slowing down the movement of the gut, are beneficial with respect to frequency of defecation, stool form and overall pain intensity and have evidence of efficacy in IBS populations.[15-17]

1.1.4.1.3. Antispasmodics

Antispasmodics relax the smooth muscles of the gastrointestinal tract, helping to prevent or reduce pain. While many studies suggest that antispasmodics are of benefit the degree of benefit is largely dependent on the type of antispasmodic used.[14] The best evidence for an individual compound seems to be for hyoscine, but other antispasmodics should be considered if this fails.[14] Peppermint oil has also has evidence for its superiority over placebo.[14]

1.1.4.1.4. Antidepressants

Antidepressant drugs are often used in patients with IBS and have shown good benefit over placebo.[18] The most commonly prescribed antidepressants include tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). Most studies have employed

TCAs at daily doses that are below the psychiatric range for antidepressant effects.[19] With TCAs, the onset of action is rapid, effects appear to be sustained and the benefits are unrelated to change in measures of anxiety and depression thus supporting a mechanism of action that is distinct from recognised psychiatric effects of the medications.[19] Evidence for more contemporary antidepressants, including SSRIs, is much less robust. These medications are typically used in full psychiatric dosages and generally produce a slow onset of effect on IBS symptoms. Benefits of antidepressants may be related more to a reduction in associated anxiety or depressive symptoms, and so have an indirect effect on IBS symptom reporting.[19] The mechanistic difference between TCAs and SSRIs in IBS symptom management has not been completely determined, but clinical experience favours important distinctions between antidepressant types.[19] While concerns regarding adverse events in the minority have limited the widespread use of antidepressants, reasonable evidence that both TCAs and SSRIs reduce abdominal pain and alter bowel habits in both diarrhoea- and constipation-predominant IBS is now available.[20] Vigilance for adverse effects and ongoing weighting of treatment benefits against any adverse effects is necessary when considering long-term antidepressant use.

1.1.4.1.5. Probiotics, prebiotics and antibiotics

There is a growing body of evidence to implicate a potential role of intestinal bacteria in the pathophysiology of IBS, and dysbiosis of gut microbiota has now been described by several groups.[21-23] As such, manipulation of the gut microbiota via probiotics, prebiotics, antibiotics and, more recently, faecal microbiota transfer/transplant (FMT) is becoming increasingly popular in IBS symptom management.

Probiotics are defined as being live micro-organisms that when administered in adequate amounts, confer a health benefit on the host.[24] Commonly used probiotics include bacteria from the genera, lactobacillus and bifidobacteria, or the yeast, saccharomyces. In IBS, it is believed that probiotics may have some impact on the hosts' microbial ecosystem, immune

function or on colonic fermentation[25] and may have immunomodulary effects.[26] The effectiveness of probiotics in IBS has been subject to several systematic reviews and meta-analysis[27-29] and despite the growing popularity as a therapy, results from well-designed clinical trials are inconclusive. Some studies have reported symptomatic improvement while others have failed to demonstrate any beneficial effect. Perhaps the best evidence is for the probiotic *Bifidobacterium infantus 35624*,[30, 31] but more work is needed to establish whether other species and strains are efficacious, the optimal dose to recommend and the subgroups of patients who are most likely to benefit.

Prebiotics are defined as non-digestible, fermentable food components that result in the selective stimulation of growth and/or activity of one or a limited number of microbial species in the gut microbiota that confer health benefits.[32] The most commonly used prebiotics are inulin-type fructans (inulin, oligofructose, fructo-oligosaccharides) and galacto-oligosaccharides (GOS). There are only four randomised control trials (RCT) of prebiotics in IBS.[25] The first, resulted in an increase in symptoms,[33] the second no difference in symptoms[34] and the third and fourth revealed a global improvement in IBS symptoms.[35, 36] The main difference between these studies was the dose of prebiotic provided. This suggests that the dose of prebiotic is important in determining any clinical benefit in IBS, with lower doses being effective and larger doses having a negative impact on symptoms.[25]

Antibiotics have been hypothesised to be both causative of IBS and efficacious as a treatment, presumably for the same reasoning of altering the gut microbiome. Antibiotics can cause acute diarrhoea.[37] This is presumably due to changes in the gut microbiota, although in many patients the specific changes responsible are not understood. However, in some, this is due to colonisation with pathogenic bacteria such as *Clostridium difficile*. There is also some evidence for an association between antibiotics and IBS symptoms where subjects who are given a course of antibiotics are more than three times as likely to report more bowel

symptoms four months later than controls.[37] Rifaximin, a non-absorbable antibiotic, is the only antibiotic that has shown a consistent benefit of improving symptoms associated with IBS.[38, 39] This may be the result of reducing small intestinal bacterial overgrowth (SIBO), a condition characterised by an increase in the number of bacteria and or species present in the small intestine, and may be associated with changes in stool consistency and abdominal pain. However, SIBO exists within an anatomically normal small bowel (as in IBS) and is controversial at best due to the difficulties with diagnosis. Breath hydrogen testing after lactulose if often used, but this has poor performance characteristics.[40] Whether correction of SIBO is the mechanism by which rifaximin works or not, clinical practice has indicated that recurrent courses are needed to continue efficacy.[41] While serious adverse events are notably rare, longer-term efficacy and safety trials of cyclic treatments of rifaximin are needed.

1.1.4.2. Dietary therapies

Dietary strategies are often employed to help control or reduce symptoms associated with IBS. In fact, most IBS patients will try to modify their diet as either a primary self-help therapy or following the advice given by their General Practitioner (GP), gastroenterologist, or alternative health care provider. Common dietary restrictions include, but are not limited to, the reduction of fat to influence motility, avoidance of caffeine due to its presumed bioactive nature, alteration of dietary fibre to improve the quality of colonic contents and reduce constipation and the elimination of suspected dietary triggers with a purely trial-and-error approach.[42] There have also been several complex dietary strategies published via books and on the internet, such as the removal of all sugar from the diet, the 'specific carbohydrate diet' or 'palaeolithic diet.'[42] Unfortunately, these strategies have little or no scientific evidence to validate their claims of efficacy, although this does not mean that they have no efficacy. Perhaps of more importance, they may challenge nutritional adequacy because of their major change to dietary intake and, for many, restrict foods across multiple food groups. Diets with

some evidence of efficacy, such as the restriction of short-chain carbohydrates or of wheat and gluten containing foods, are also commonly applied and are discussed in detail within Sections 1.2 and 1.3, respectively.[43, 44]

1.1.4.2.1. Dietary fibre

Fibre has been used, usually in the form of dietary supplements for many years in the treatment of IBS. Initially it was believed that a diet low in fibre may be the cause of symptoms, although, more recently, caution has been expressed in the increased use of fibre as it may exacerbate certain symptoms in some patients. Despite this, a recent systematic review of 14 RCTs determined that fibre supplementation is effective in improving global IBS symptoms.[45] The effect of fibre, however, appears to be limited to soluble (as opposed to insoluble) fibre.[45]

1.1.4.2.2. Elimination diets

An elimination diet is aimed at identifying foods that may be causing an allergy or other symptoms. It usually involves the removal or reduction of the suspected food from the diet to see if the symptoms disappear and then adding them back one at a time to see if any make the symptom reoccur.

Removing foods according to serum immunoglobulin (IgG) antibodies to food antigens is growing in popularity. RCT support of this approach has been demonstrated, but clinically significant benefits have only been seen in a small number of patients[46] with no long-term follow-up. Levels of IgG have not been shown to correlate specifically with symptom severity.[47] It must be noted that IgG antibodies to food antigens are detected in healthy individuals as well as those with IBS and may represent a normal physiological response to food ingestion.[48]

Symptoms resulting from food intake can also be due to allergy or an immunoglobulin E (IgE)-mediated response. This occurs most commonly to food proteins such as eggs, milk, fish, crustacean shellfish, tree nuts, peanut, soya and wheat. Diagnosis is usually made though skin-prick tests or radioallergosorbent testing (RAST), although, both tests have poor predictive values and false positive tests are frequently seen in healthy controls.[42] Furthermore, there is some suggestion that IgE reactions to food are related to IBS.[42]

A scientifically more satisfying approach has been not to target whole foods, but specific dietary components with putative pathogenic effects on the enteric nervous system (ENS).[42] Limited evidence for the removal of naturally-occurring food chemicals has been identified[49] but a greater degree of evidence for targeting short-chain carbohydrates and gluten now exists.

Food chemicals have been hypothesised to induce gastrointestinal symptoms in some IBS patients with food chemical sensitivities. As such, the removal of these naturally occurring food chemicals (salicylates, amines and glutamate) and food additives (colours, preservatives and flavour enhancers) is becoming more widespread in Australia, despite only anecdotal evidence for efficacy. Application of this diet is also difficult considering the effects of these compounds are dose-related and vary depending on the individual's recent intake from a variety of food sources.[49] As such, a particular food might not produce the same reaction on different occasions.[49] This, together with the fact that reactions may be delayed for many hours, means patients become easily confused or mistaken about which food (if any) causes symptoms.[49]

1.1.4.3. Psychological treatments

Psychological factors may interfere with brain processing of visceral signals as well as with gastrointestinal physiology (through the autonomic nervous system [ANS] and secretion of soluble factors such as corticotropin-releasing factor[CRF]) in the generation of IBS

symptoms.[50] Of psychotherapeutic techniques, cognitive behavioural therapy (CBT) is one of the most extensively studied psychological treatments showing benefits within IBS populations. Psycho-education and various forms of biofeedback are also promising treatment options. A further psychological treatment worthy of consideration is gut-directed hypnotherapy where it is explored within this thesis.

1.1.4.3.1. Cognitive behavioural therapy

CBT is based on the idea that the autonomic arousal caused by unhelpful thoughts and avoidance behaviour triggers gastrointestinal symptoms in IBS patients, creating vicious cycles.[50] Interventional work includes educating the patient on symptom self-monitoring techniques, the relationship between IBS symptoms and stress, relaxation skills, restructuring of cognitions, attentional control skills, and (interoceptive) exposure.[50] Numerous RCTs have shown CBT to be efficacious in the long term in both individual and group settings when compared to no treatment, standard medical care or other psychological intervention (Table 1.2).

1.1.4.3.2. Psycho-education

Patients with IBS have to cope not only with their symptoms but also with the social stigma that is often attached to the condition.[50] The diffuse symptomatology, unclear aetiology, and lack of medical diagnosis and clear-cut treatment strategy often make dealing with these patients difficult for physicians.[50] In return, IBS patients often feel misunderstood and as if their problems have not been taken seriously.[50] Psycho-education has proven to be pivotal to addressing patient concerns and improving treatment outcomes.

Bengtsson and colleagues found that educating women with IBS on medical care, physical activity, stress-management, diet and health insurance leads to significant improvements in vitality and abdominal pain as well as to a reduced number of visits to dietitians and

physicians.[51] Similarly, other work has confirmed the effectiveness of psycho-education treatment relative to usual IBS care.[52, 53] These studies highlight the importance of patient education as part of a multicomponent treatment program.[50]

Table 1.2. Randomised control trials using cognitive behavioural therapy (CBT) studies in irritable bowel syndrome (IBS) patients

Study	N	Intervention	Control	Duration (weeks)	Results
Greene 1994[54]	20	CBT	Symptom monitoring wait-list control	8	Greater reduction in gastrointestinal symptoms following CBT when compared to symptom monitoring. At post-treatment, 80% of the CBT group and 10% of the symptom monitoring group showed clinically significant improvement. Results maintained 3-months post-treatment
Payne 1995[55]	34	CBT	Self-help support group or symptom monitoring wait list control	8	Greater reductions in individual and overall gastrointestinal symptoms following CBT when compared to self-help support and symptom monitoring wait list control. Results maintained 3-months post-treatment
Vollmer 1999 [56]	32	CBT	Group based CBT; symptom monitoring wait list control	10	Greater reduction in gastrointestinal symptoms following CBT when compared to symptom monitoring. No observable difference was noted between individual and group CBT. At post-treatment, 64% of the group CBT, 55% of the individualised CBT and 10% of those receiving symptom monitoring showed clinically significant improvement. Results maintained 3-months post-treatment

Heymann-Monnikes 2000[57]	24	CBT plus standard medical treatment	Standard medical treatment	10	Greater improvement following CBT and standard medical treatment compared to standard medical treatment alone. Improvement maintained at 3 and 6-months post-treatment
Boyce 2003[58]	105	CBT	Standard care or relaxation training	8	No difference in improvement between groups
Drossman 2003[59]	215	CBT	Education	12	Greater reduction in overall gastrointestinal symptoms following CBT compared to education control. Responder rate 70%
Tkachuk, 2003[60]	28	Group CBT	Symptom monitoring	10	Greater reduction in overall gastrointestinal symptoms following CBT when compared to symptom monitoring. Improvements maintained 3-months post-treatment
Kennedy 2006[61]	149	CBT plus mebeverine	Mebeverine alone	6	Greater reduction in overall gastrointestinal symptoms following CBT plus mebeverine when compared to mebeverine alone. Improvements maintained at 3 but not 6 or 12-months post-treatment
Sanders 2007[62]	16	Self-administered CBT	Wait-list control	8	Greater reduction in overall gastrointestinal symptoms following self-administered CBT compared to wait-list controls. Participants in the treatment reported a 25% improvement compared to those in the control who reported a 32% worsening of symptoms. Improvements maintained 3-months post-treatment

Blanchard 2007[63]	210	Group CBT	Psychoeducational support or stress monitoring control	10	Greater reductions in overall gastrointestinal symptoms following CBT and psychoeducational support compared to stress monitoring. No difference in improvement was observed between the CBT and psychoeducational support groups. Improvements maintained 3-months post-treatment
Hunt 2009[64]	54	CBT delivered via the internet	Wait-list	5	Greater reduction in overall gastrointestinal symptoms following CBT when compared to the wait-list control. Improvements maintained 3-months post-treatment
Ljotsson 2010[65]	68	CBT delivered via the internet	Online discussion forum	10	Greater reduction in overall gastrointestinal symptoms following CBT compared to the online discussion forum. Improvement maintained 3-months post-treatment
Moss-Moris 2010[66]	64	Self-administered CBT	Treatment as usual	8	77% of patients who received CBT reported a reduction in gastrointestinal symptoms compared to 21% in the treatment as usual control across three time-points (end of treatment and 3 and 6-months post-treatment)
Ljotsson 2011[67]	61	CBT delivered via the internet	Wait-list	10	Greater reduction in overall gastrointestinal symptoms following CBT when compared to the wait-list control. Improvement maintained 12-months post-treatment. A high drop-out rate was associated with severe symptoms and large impairment

Oerlemans, 2011[68]	76	CBT	Standard care	4	Greater reduction in abdominal pain following CBT when compared to standard care alone. Improvement not maintained 3-months post-treatment
Mahvi-Shirazi 2008[69]	50	CBT plus medical treatment	Medical treatment	8	Greater reduction in overall gastrointestinal symptoms in the CBT plus medical treatment group when compared to the medical treatment group alone

1.1.4.3.3. Biofeedback

Biofeedback is a conditioning treatment in which physiological signals are measured and converted into a simple auditory or visual cue.[70] The cue enables patients to recognise their own body signals and to influence them by the use of operant conditioning principles, first by the use of biofeedback equipment, and later without (by process of internalisation).[50] The ultimate goal is that patients can integrate and generalise the biofeedback modulated skills into their daily life.[50]

The first attempts to treat IBS with biofeedback were aimed at modifying colonic motility in patients with diarrhoea predominant IBS.[71] All patients reported symptom improvement, which was said to be well correlated with learning control over bowel sounds.[71] However, other investigators have not been able to replicate these findings.[72] Other authors have used forehead electromyography (EMG) biofeedback and thermal biofeedback as nonspecific relaxation training techniques in IBS patients.[73-77] These too have been shown to be effective in reducing overall gastrointestinal symptoms but have been limited by low numbers of participants and are plagued by complex protocols. Future biofeedback studies with improved research methodologies are warranted.

1.2. Short-chain Carbohydrates in Irritable Bowel Syndrome

1.2.1. Dietary FODMAPs

Short-chain carbohydrates that are indigestible or slowly absorbed in the small intestine are a large group of dietary sugars found in many common foods (Table 1.3). They have been

grouped together into the collective term, FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols). FODMAPs individually or in various combinations have been shown to induce gastrointestinal symptoms in the majority of patients with IBS. As such, IBS patients will often benefit from the reduction or restriction of these FODMAP containing foods from their diet.

Table 1.3. Dietary foods with high FODMAP content from each corresponding short-chain carbohydrate. GOS = Galacto-oligo saccharide

Fructans	GOS	Lactose	Excess Fructose	Polyols
Wheat, Rye, Onions, Garlic, and Artichokes	Legumes	Milk	Honey, Apples, Pears, Watermelon, and Mango	Apples, Pears, Stone fruit, Sugar free mints/gums, Mushrooms, and Cauliflower

1.2.2. Summary of evidence of the low FODMAP diet

Dietary management of IBS has improved substantially since the development of the low FODMAP diet. Restricting single carbohydrates from the diet, such as lactose or fructose, was first documented decades ago, but this form of singular restriction is rarely effective as a therapy for IBS itself.[78] As such, manipulating the intake of multiple short-chain carbohydrates from the diet has been of clinical and research interest. Initial retrospective work reported that 74% of selected patients with both IBS and fructose malabsorption reported a positive response to dietary restriction of fructose and fructans.[79] This retrospective

study was then followed-up with a double-blinded, randomised, quadruple arm, placebo-controlled re-challenge trial in patients with IBS and fructose malabsorption.[80] All patients who had improved on a low fructose/fructan diet, had significant exacerbation of symptoms by re-challenge of fructose or fructans, further exacerbated by a combination of fructose and fructans.[80] This observed causal association between fructose and fructan ingestion and symptomatic exacerbation in patients with IBS and fructose malabsorption led to extending the restriction to all short-chain carbohydrates that are known to be slowly absorbed or indigestible and to all patients with IBS, irrespective of fructose absorptive status. Thus, the low FODMAP diet was developed. There is now a strong body of evidence that demonstrates its efficacy (Table 1.4). To date, several RCTs have investigated the effect of FODMAP restriction on IBS symptoms, three in adult[1, 81-83] and one in paediatric[84] populations. Two of those in the adult populations were controlled feeding studies and two were based upon dietary advice given in a clinical setting. The first controlled feeding study compared the effect of provided diets that were either very high (50 g/day) or low (9 g/day) in FODMAPs over 2 days and found that composite symptoms were significantly reduced during carbohydrate restriction.[1] The second assessed the effects of two provided diets in a cross-over study design, comprising of a diet low in FODMAPs and a diet aimed to provide FODMAP content of a typical Australian diet.[82] Improvement in overall and individual gastrointestinal symptoms was observed in 70% of participants to a symptom level arbitrarily considered to represent good symptom control.[82] Improvements were seen across all IBS subtypes and results were specific to IBS subjects as symptoms in the healthy control group remained low and unaltered by the provided diets.[82] Controlled feeding, however, does not mimic the real-life challenges associated with sustained restricted diets. As such, a further study assessed the effect of dietary advice given in a clinical setting showed that educating patients to follow a low FODMAP diet was superior to the existing standard dietary advice

based on the United Kingdom (UK) National Institute for Health and Clinical Excellence (NICE) guidelines (satisfaction with symptom response seen in 76% of patients in the low FODMAP group compared to 54% in the NICE group).[81] The final study similarly reported improvements on the low FODMAP diet but interestingly these improvements were not superior to a traditional IBS diet where patients were taught principles of how and when to eat rather than on what foods to ingest.[83] Of note, however, was that participants in the traditional IBS diet group were instructed to reduce their intake of onions, cabbage and beans and to avoid sweeteners that end with –ol. These foods are restricted on a low FODMAP diet and thus participants in the traditional IBS diet group were potentially on a lower FODMAP intake than usual. The diet was also taught by dietitan with only recently acquired knowledge of the low FODMAP diet and thus emphasises the importance of the diet being taught by dietitians well trained in this area. Finally, the data from the original retrospective study, whereby interviewed patients were restricting fructose and fructans for up to 40 months highlighted that the effects of restricting dietary short-chain carbohydrates were maintained and the diet was easily applied.[79] This long-term effectiveness of the low FODMAP diet has recently been confirmed where 71% of 100 participants with IBS who were followed from baseline (pre-FODMAP restriction), through to the elimination and re-challenge phases, and for 1 year thereafter, reportedly maintained improvement.[85] The low FODMAP diet has, therefore, a high level of efficacy based not only on RCTs but also comparative and observational studies where symptomatic improvement is observed across all bowel habit subtypes.[82] Taken together, these studies support the use of the low FODMAP diet as the first-line dietary therapy within IBS populations.

Table 1.4. Evidence for efficacy of the low FODMAP diet in treatment of functional gastrointestinal symptoms

Study	Population	N	Type of trial	Duration	FODMAP analysis method	Results
Biesiekierski 2013[86]	IBS with self-reported NCGS	37	Double-blind, cross-over RCT	2 weeks low FODMAP followed by gluten and whey challenges (placebo)	Symptoms assessed prior to and after low FODMAP education	Reduction of abdominal symptoms in all participants. No induction of symptoms in gluten over placebo
Ong 2010[1]	Unselected IBS	15 IBS 15 healthy	Cross-over RCT	2 days each diet	Provided very high versus low FODMAP diets	High FODMAP increased breath hydrogen in all and increased symptoms in IBS but not controls
Staudacher 2011[87]	Unselected IBS	82	Non-randomised comparative	2-6 months	Self-assessed response to past low FODMAP education versus NICE guidelines	Satisfaction with symptom response in 76% of participants on low FODMAP compared to 54% NICE
Staudacher 2012[81]	Unselected IBS	35	Parallel RCT	4 weeks	Low FODMAP education versus habitual diet	Adequate symptom control in 81% low FODMAP compared to 26% habitual diet

De Roest 2013[88]	Unselected IBS	90	Observational	6 weeks	Low FODMAP education	Improvement of 37-60% of participants in specific IBS symptoms
Halmos 2014[82]	Unselected IBS	30 IBS 8 healthy	Cross-over RCT	21 days per treatment	Provided low FODMAP versus typical Australian diets	Reduction in overall symptoms while on low FODMAP compared to typical Australian diet. Improvement observed in 70% on low FODMAP
Chumpitazi 2015[84]	Unselected paediatric IBS	33	Cross-over RCT	2 days each diet	Provided low FODMAP versus typical American childhood diet	Reduction in abdominal pain while on low FODMAP compared to typical American childhood diet. Improvement on the low FODMAP diet only observed in 30%
Böhn 2015[83]	Unselected IBS	75	Parallel RCT	4 weeks	Low FODMAP education versus traditional IBS diet	Symptom severity reduced in both groups during the intervention with no difference

		between the two groups. Improvement on the low FODMAP diet observed in 50% compared to 46% on the traditional IBS diet

1.2.3. Applying the low FODMAP diet

The application of the low FODMAP diet involves removing foods with high or moderate FODMAP content and replacing them with low FODMAP foods from the same food categories. Patients will usually respond symptomatically to the low FODMAP diet within 1 week[82] but the diet is commonly applied strictly for 4-6 weeks. Implementation of the low FODMAP diet is well tolerated by the majority and easily incorporated into patients' lives.[88] If good symptomatic response is achieved during the initial 4-6 weeks, patients are instructed to reintroduce previously excluded foods back into the diet with the aim of liberalising the diet whist maintaining good symptomatic control. Tolerance levels for each patient and to each FODMAP will vary according to individual sensitivities. Patients who fail to experience any improvement on the low FODMAP diet can return previously excluded foods back into their diet without following the re-introduction phase. It is recommended that patients follow the low FODMAP diet under the guidance of a dietitian to ensure that restricted foods are replaced with suitable alternatives and nutritional adequacy is met and to guide the patient through the reintroduction phase.

1.2.4. Mechanisms of symptom induction from FODMAP ingestion

FODMAPs are believed to exacerbate symptoms associated with IBS though various mechanisms (Figure 1.2). Firstly, some fermentable short-chain carbohydrates are osmotically active. This osmotic effect results a greater small intestinal water volume. Ileostomy recovery work has revealed a correlation between FODMAP intake and increase ileostomy output[89-91] and has been confirmed in studies using magnetic resonance imaging (MRI) where small intestinal water volume was found to be greater following ingestion of various short-chain carbohydrates.[92, 93] Secondly, FODMAPs are rapidly fermented by the colonic microbiota. This rate of fermentation varies according to chain length with short-

chain carbohydrates being fermented at a much more rapid rate than longer chain carbohydrates. The increased fermentation and associated gas production results in luminal distension, thereby exacerbating symptom severity in those with visceral hypersensitivity.[94] Finally, short-chain carbohydrates have an effect on motility.[95, 96] The previously described osmotic effect of FODMAPs has been shown to accelerate small intestinal and colonic transit.

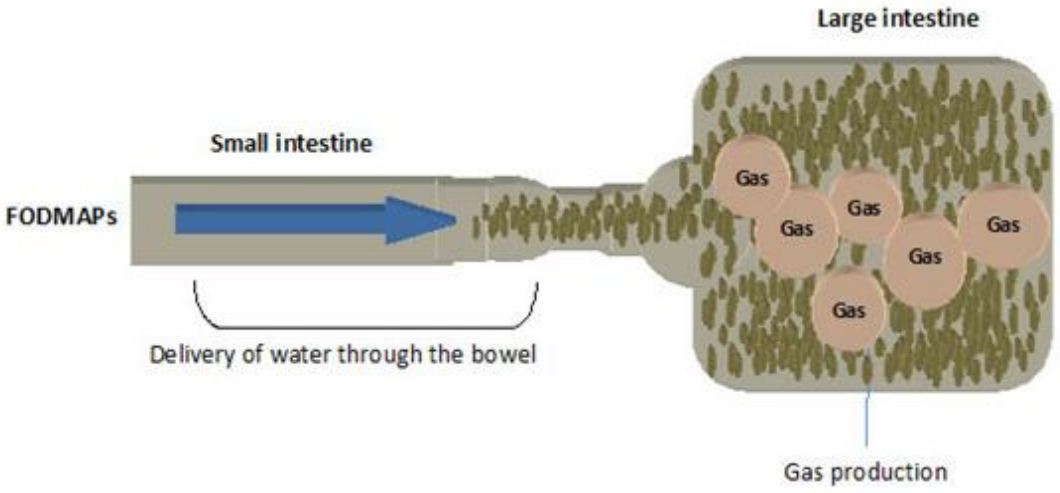


Figure 1.2. Illustration representing the mechanism of FODMAPs.[97] FODMAPs are poorly absorbed in the small intestine, increase water delivery through the small and large intestine, are fermented by bacteria in the large intestine producing gas thereby resulting in distention of the large intestine and contributing to gastrointestinal symptoms

1.2.5. Limitations associated with the low FODMAP diet

One possible limitation is that patients following a low FODMAP diet may unnecessarily restrict their diet. This, in turn, raises concern over nutritional adequacy of their diet and the potential alterations in the gut microbiota (due to the reduction in natural prebiotic intake). Predictors of response have also been poorly characterised, where to date, no markers of

response have been identified. Moreover, as it currently stands, the low FODMAP diet has not been compared to other IBS treatments.

1.2.6. Applicability and acceptability of the low FODMAP diet

Despite the abovementioned limitations, following the low FODMAP diet is generally well-accepted by patients where adherence rates are high.[88] A recent prospective observational study revealed that 60% of participants found the diet easy to follow and 44% were able to incorporate the diet into their life easily.[88] Considering the current high quality evidence for efficacy, short-term strict implementation before reintroduction and tolerability by patients, the low FODMAP diet is an effective approach to the management of patients with IBS.

1.3. Gluten

1.3.1. Gluten avoidance

Adoption of the gluten-free diet is growing exponentially. Indeed, the demand for specialised gluten-free products has fuelled a global market of gluten-free products approaching $2.5 billion (US) in global sales annually.[97] This movement is supported by the belief that gluten is contributing to a wide range of health-care concerns. The best studied and well understood gluten-related disorder is coeliac disease. However, in recent years, there have been a growing number of reports of individuals experiencing reactions to gluten-containing food where the clinical markers of coeliac disease cannot be identified. These people are defined as having non-coeliac gluten sensitivity (NCGS).

1.3.2. Structure of wheat gluten

Wheat is cultivated worldwide and is considered a highly nutritious and useful grain. It is the largest crop used for human consumption, followed closely by maize and rice. Wheat kernels are complex structures comprising an outer bran layer, germ and endosperm and contain multiple components including starch, proteins (gluten and non-gluten), moisture, lipids and ash. Figure 1.3 denotes the approximate breakdown of the wheat component in a typical wheat kernel.[98]

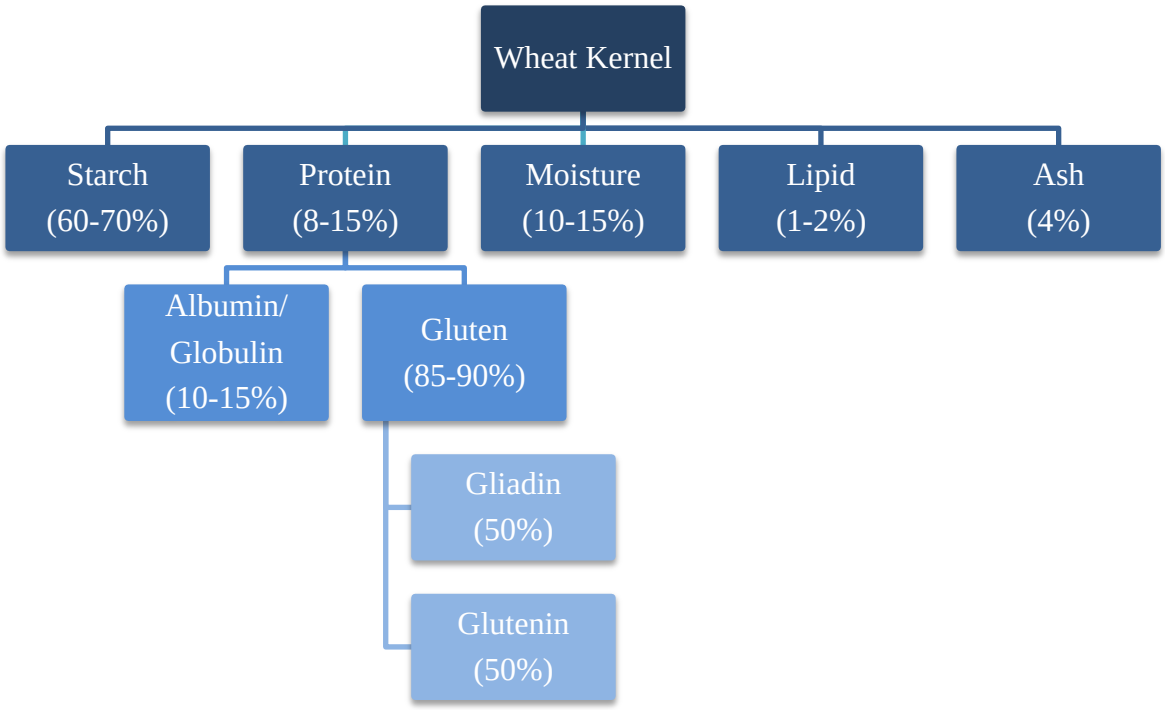


Figure 1.3. Approximate breakdown of wheat components[99]

1.3.2.1. Proteins

The protein content of wheat is a complex mix of different, but related proteins that include albumin, globulin, gliadin and glutenin classes. Gliadin and glutenin protein classes are the

main storage proteins of wheat and together contribute to 80-85% of the total wheat protein. Storage proteins similar to gliadins (generally termed prolamins) are also found in rye (secalins), barley (hordeins) and oats (avenins). Derivatives of these grains such as triticale and malt and other wheat varieties such as spelt and kamut also contain gluten.

1.3.2.1.1. Glutenin

Glutenins contain low and high molecular weight subunits, ranging from approximately 500,000 to more than 10 million.[99] It is this weight distribution that acts as a main determinant of dough strength, elasticity and overall baking performance. The largest polymers termed 'glutenin macropolymer' make the greatest contribution to dough properties and their amount in wheat flour (~20-40 mg/g) is strongly correlated with dough strength and loaf volume. Both glutenins and gliadins display a high content of the amino acids glutamine (32-56%) and proline (15-30%), and due to their cysteine content, glutenins can form complex homopolymers and heteropolymers with gliadins.[100]

1.3.2.1.2. Gliadin

Gliadins contribute mainly to the viscosity and extensibility of the dough system.[99] They have little elasticity and are less cohesive than glutenins but they form the most diverse group of wheat prolamins.[101] Gliadins are usually characterised as monomeric with a molecular range between 30 and 75kDa.[101] Based on their electrophoretic mobility on PAGE analysis, they can be differentiated into four categories, alpha-, beta-, gamma- and omega-gliadins.[101] Alpha-gliadins are the fastest-moving group and omega-gliadins the slowest.[101] Despite this, alpha- and beta-gliadins share common characteristics and are often grouped together.[101, 102] Gliadin subunit categories are detailed in Figure 1.4.

1.3.2.1.3. Albumin/globulin

Albumins and globulins are principally metabolic proteins involved with enzymatic or enzymatic-inhibiting functions important for providing nutrients and energy during germination and for protecting against pests and disease.[103] Specifically, α-amylase trypsin inhibitors (ATIs) and small glycoproteins are present in high quantities in wheat grains as they confer survival advantages to the host.

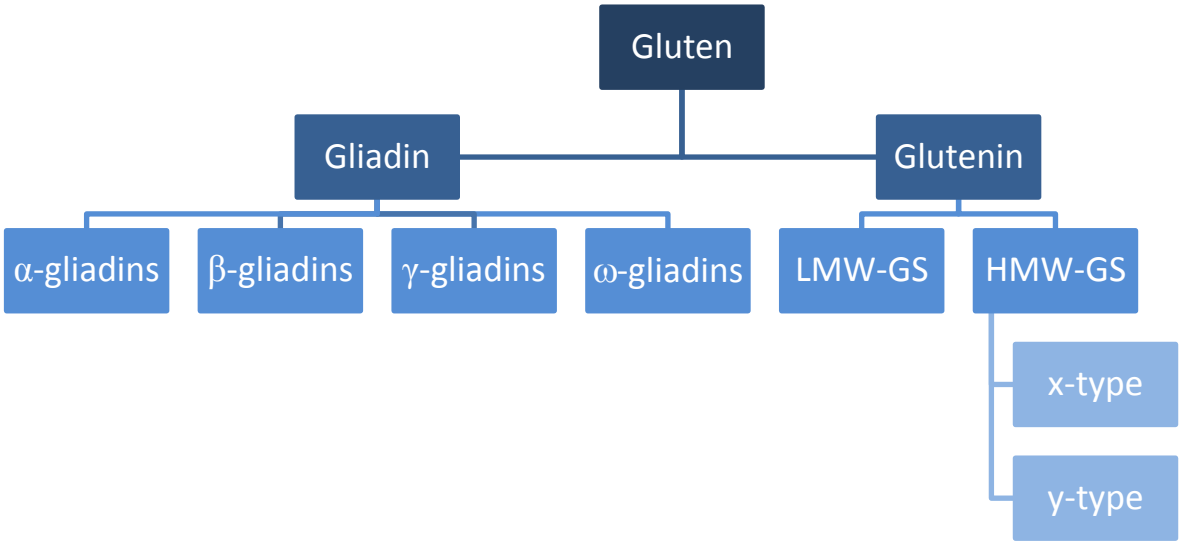


Figure 1.4. Breakdown of gliadin and glutenin subunits. LMW-GS = low molecular weight gluten subunits; HMW-GS = high molecular weight gluten subunits

1.3.3. Coeliac disease

Coeliac disease is an immune-mediated enteropathy in genetically-susceptible individuals[97, 104] precipitated by exposure to dietary gluten. Small intestinal damage can result in gastrointestinal symptoms including diarrhoea, constipation, excessive wind, abdominal pain

or discomfort, and bloating but also other non-gastrointestinal presentations (Table 1.5). The only available treatment of coeliac disease is lifelong strict avoidance of gluten. Once thought to be rare and only to occur in Western populations, coeliac disease is now considered a relatively common disease affecting about 0.6-1% of the world's population.[105] Despite this, most patients are not diagnosed or can present with atypical symptoms, due to the often asymptomatic nature of coeliac disease. Consequently, there are approximately 7-10 undiagnosed patients for each known coeliac disease patient.[106] Even with this increasing prevalence, the number of individuals embracing the gluten-free diet appears much higher than the projected number of coeliac disease patients.[97]

Table 1.5. Symptoms commonly experienced in patients with coeliac disease

Gastrointestinal symptoms	Non-gastrointestinal symptoms
• Diarrhoea • Constipation • Excessive wind • Abdominal pain or discomfort • Bloating • Vomiting	• Puberty and growth delay • Anaemia • Weight loss • Osteoporosis • Arthritis • Fractures • Dental abnormalities • Ataxia • Depression • Dermatitis herpetiformis • Miscarriage and infertility • Cognitive impairment

1.3.4. Non-coeliac gluten sensitivity

NCGS was original described in the 1970s, but only in recent years have an increasing number of publications called attention to an apparently novel yet escalating entity. Individuals with NCGS describe experiencing symptoms associated with eating gluten-containing foods and show improvement when following a gluten-free diet, in the absence of coeliac disease or wheat allergy.[97, 104] Commonly reported features of NCGS include gastrointestinal symptoms such as pain, bloating and altered bowel habits, and extraintestinal ailments similar to those of coeliac disease, including eczema and/or rash, anaemia, ataxia, fatigue, cognitive impairment, and depression.[97] NCGS has been endorsed by an expert group as a definite clinical entity, with defined criteria for its recognition.[107] This criteria follows a full diagnostic evaluation where the aim of the confirmation of the diagnosis is to assess the clinical response to the gluten-free diet and then to measure the effect of reintroducing gluten after a period of treatment with the gluten-free diet.[107] A detailed flow diagram of the diagnostic process is shown in Figure 1.5. Despite this defined diagnostic procedure the diagnosis of NCGS remains largely a diagnosis of exclusion, whereby an elimination diet and 'open challenge' is used to evaluate whether health improves with the elimination or reduction of gluten from the diet.[97, 104] As such, many aspects of the epidemiology, pathophysiology, clinical spectrum and treatment are still unclear.

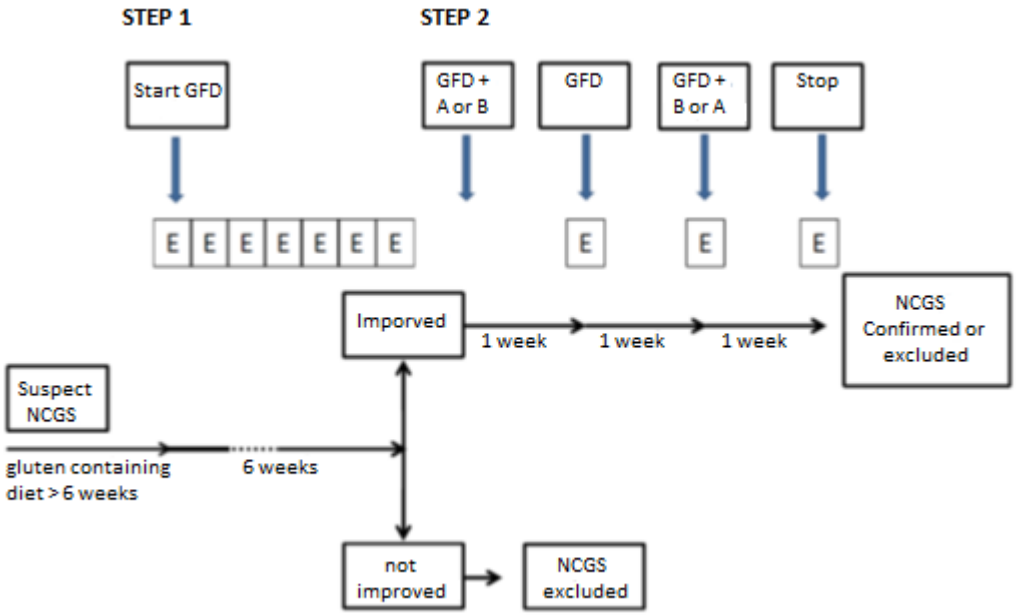


Figure 1.5. The flow diagram of the NCGS diagnostic process developed by Catassti et al.108 GFD=gluten-free diet; A = product A (gluten or placebo); B= product B (placebo or gluten); E=evaluation (questionnaire). The evaluation is performed weekly during Step 1 and daily during step 2

1.3.5. Gluten and mechanisms of action

In patients with coeliac disease, the pathological process of gluten ingestion is well understood where, immune responses to gliadin fractions promote an inflammatory response, characterised by infiltration of the lamina propria and the epithelium with chronic inflammatory cells and villous atrophy.[108] This response is mediated by the deamidation of gliadin peptides by tissue transglutaminase (tTG) which forms negatively charged amino acids that bind to the disease associated human leukocyte antigen (HLA)-DQ2 and -DQ8 receptors on the cell surface antigen-presenting cells (APCs).[109] Once bound, this complex is

presented with high affinity to the major histocompatibility complex (MHC) Class II T-cells. The CD4+ T-cell activation leads to the secretion of pro-inflammatory cytokines, particularly interferon-γ (IFN-γ),[110, 111] and metalloproteinases and other tissue-damaging mediators that induce crypt hyperplasia and villous injury.[108, 109, 112-114]

A variety of sequences from α-, γ-, and ω-gliadins, as well as from the glutenins have been identified to activate T-cells in patients with coeliac disease, although several hundred gluten peptides are predicted to be immunogenic.[98, 112, 115-117] Indeed, T-cell cross-reactivity against other prolamins including secalin and hordein has been confirmed.

In patients with NCGS, the mechanistic action of gluten remains unknown, despite an array of confusing data. Several initial studies proposed an important role of the intestinal innate immune system triggered by an adaptive immune response.[118, 119] However, more recent work has suggested the possibility of NCGS being characterised by an activation of both innate (non-specific defence mechanism) and adaptive (antigen-specific immune response) immunity (Table 1.6.).[120-122] There have even been retrospective reports of higher proportions of patients with NCGS developing autoimmune disorders, with antinuclear antibodies and showing DQ2/DQ8 haplotypes compared with patients with IBS.[123] Newer work has also focused on the role of gliadin on gut permeability in *ex vivo* conditions where it was shown to activate zonulin signalling resulting in increased intestinal permeability to macromolecules.[124] The possible effect of gliadin on zonulin release and signalling requires further elucidation. Regardless, over the years, there has been an increase in the number of factors have been postulated to play a role in the pathogenesis of NCGS but many of these studies have had disparate designs and their reported findings appear inconsistent. Furthermore, few studies have examined effects of potential NCGS biomarkers in healthy subjects. It may be that these changes are shown in both NCGS and healthy populations and thus are not disease specific. Table 1.6 summarises the elimination and challenge studies that have been performed to date

(adapted from Gibson et al[78]) but further work is needed. In the absence of diagnostic biomarkers, elimination and challenge studies are feasibly the most accurate way of determining the existence of the entity.

Table 1.6. Mechanistic challenge studies in non-coeliac gluten sensitive populations

Study	Population	Challenge substance	End point(s)	Patient response	Histology in those with NCGS or wheat induced abnormalities
Sapone 2011[119]	NCGS, coeliac disease, healthy controls	No challenge performed		Lower intestinal permeability; increased duodenal expression of Toll-like receptor-2; reduced expression of FOXP3 compared to controls and patients with coeliac disease	92% of patients had >30 intraepithelial lymphocytes/100 epithelial cells
Biesiekierski 2011[125]	NCGS	FODMAP depleted wheat protein	Intestinal permeability; inflammatory markers	No wheat protein specific induction of end points	No increase in intraepithelial lymphocytes in patients HLA-DQ2/8 positive, not reported for patients HLA-DQ2/8 negative
Carroccio 2013[126]	IBS with improvement of symptoms after gluten-free diet	Wheat flour; other food antigens	Tomato soup supplements with sachets of gluten-free flour	Wheat protein basophil activation in >80%	90% increase in intraepithelial lymphocytes; 90% increase in eosinophils

Bucci 2013[121]	NCGS, coeliac disease, healthy controls	Gliadin peptides; wheat protein	Markers of inflammation after incubation in vitro; basophil activation	No gliadin peptide specific effects; no evidence of basophil activation to gliadin peptides or wheat protein	44% increase in intraepithelial lymphocytes; No increase in eosinophils
Vazquez-Roque 2013[127]	IBS	Wheat protein	Intestinal permeability	Wheat protein containing diet associated with some evidence of increased intestinal permeability	No differences between dietary groups
Brottveit 2013[120]	NCGS, coeliac disease	Bread	Mucosal cytokine response; Intraepithelial lymphocyte response	Increased interferon~y response; No intraepithelial lymphocyte response	33% of patients had >25 intraepithelial lymphocytes/100 epithelial cells
Biesiekierski 2013[86]		FODMAP depleted wheat protein	Intestinal permeability; inflammatory markers	No wheat protein specific induction of end points	1 participants elicited a positive T-cell response, no other differences between dietary groups
Hollon 2015[128]	Coeliac patients with active disease; Coeliac patients in remission; NCGS; Non-coeliac controls	Gliadin	Barrier function; cytokine secretion after incubation ex-vivo	No gliadin specific increase in intestinal permeability in NCGS compared to non-coeliac controls; Higher concentrations of IL-10 measured in non-coeliac controls compared to NCGS	No difference in barrier function between NCGS and non-coeliac controls;

1.3.6. Elimination and challenge studies

That wheat consumption is associated with the induction or worsening of gastrointestinal symptoms in patients without coeliac disease and that its withdrawal from the diet resulted in symptomatic improvement was first reported by Jones et al.[129] Since then, several well-controlled elimination and challenge studies have been reported with somewhat conflicting results. A comprehensive overview of these studies is provided in Table 1.7. In short, five of the eight reported studies included participants defined as having NCGS and fed them wheat gluten, which was shown to significantly worsen gastrointestinal symptoms.[130-134] However, in two of these reported studies, convincing gluten-specific effects were only observed in the minority of patients.[132, 134] For example, a placebo-controlled re-challenge of 35 patients revealed that just one third of patients were able to correctly identify the flour that contained gluten from that which did not and thus had a gluten-specific response.[134] Additionally, 49% reported a specific response to the gluten-free flour.[134] Authors argued that patients may have been sensitive to the FODMAP, namely fructan, component of the flour and that this may have influenced results. Given the small quantity of fructans present, this seems unlikely.[135] Hence, the number of patients who truly had NCGS is uncertain. Secondly, studies have included patients with intraepithelial lymphocytosis thereby comprising those with potential coeliac disease.[130, 134] In such cases, a specific symptomatic response to gluten is more likely.

The remaining three trials were performed by the same group with similarly conflicting results. The first, a parallel group study found that patients were significantly worse with gluten ingestion for overall symptoms, pain, bloating, wind, satisfaction with stool consistency and tiredness.[125] The second (comprising two back-to-back challenges) used a cross-over design on a low FODMAP dietary background and could find no evidence of a gluten-specific triggering of symptoms in such patients.[86] However, in all participants,

gastrointestinal symptoms consistently and significantly improved during reduced FODMAP intake, but significantly worsened to a similar degree when their diets included gluten or whey protein.[86] Either patients did not have NCGS as self-reported or the study design precluded its recognition. Interestingly, despite the lack of symptom exacerbation following gluten consumption, participants continued to restrict gluten at study completion as they subjectively described feeling better.

Table 1.7. Summary of double-blind, placebo-controlled trials in non-coeliac patients with irritable bowel syndrome (IBS) symptoms and suspected non-coeliac gluten sensitivity (NCGS)

Study	Population	N	Type of trial	Mode of administration of gluten/wheat (g/day)		Duration	Results
Cooper 1980[131]	NCGS	8	Double-blind, cross-over RCT	Tomato soup supplemented with sachets of gluten containing flour (20 g/day)	Tomato soup supplements with sachets of gluten-free flour	4 weeks with randomisation of sachets randomly through each day for the first 3 days of weeks 2 and 4	Significant worsening of overall symptoms for each patients in the week of gluten-containing flour administration compared to the control
Biesiekierski 2011[125]	IBS with improvement of symptoms after gluten-free diet	34	Double-blind, RCT	Gluten-free bread/muffin supplemented with carbohydrate-depleted wheat protein (16	Gluten-free bread/muffin	6 weeks with daily administration of bread/muffin with or without wheat protein randomly	Significant worsening of overall symptoms in patients with wheat protein ingestions compared to those without wheat protein ingestion

				g/day)			
Carroccio 2012[130]	IBS with improvement of symptoms after gluten-free diet	276	Double-blind, cross-over	Wheat flour capsules (13 g/day)	Xylose containing capsules		Significant worsening of overall symptoms in the weeks of wheat administration compared to the weeks without wheat ingestion
Biesiekierski 2013[86]	IBS with improvement of symptoms after gluten-free diet	37	Double-blind, cross-over, RCT	Food with high (16 g/day) or low (2 g/day) of carbohydrate-depleted wheat protein	Gluten-free food with whey protein (16 g/day)	2 week low FODMAP run-in period, then 1 week with high or low gluten diet or placebo, followed by a 2-week washout before crossing over to the next diet	Significant improvement of overall symptoms during reduced FODMAP diet and significant but similar worsening of symptoms on a diet with wheat protein or placebo
Biesiekierski 2013[86]	IBS with improvement of symptoms	22	Double-blind, cross-over,	Food with carbohydrate-depleted wheat	Gluten-free food with whey protein (16	3 days with gluten, whey protein or	Significant but similar worsening of symptoms in all dietary arms – no

	after gluten-free diet – subset of population as Biesiekierski et al		RCT	protein (16 g/day)	g/day) or placebo	placebo diet with ≥3-day washout before crossing over to the next diet	patient with specific wheat protein-mediated response
Di Sabatino 2015[132]	NCGS	61	Double-blind, cross-over RCT	Capsules containing purified gluten (4.375 g/day)	Capsules containing rice starch	1 week with one type of capsule, 1 week washout before crossing over to another week with the other type of capsule	Significant worsening of overall symptoms after gluten ingestion compared to placebo
Shahbazkhani 2015[133]	IBS with improvement of symptoms after gluten-free diet	72	Double-blind RCT	Gluten powder (50 g) mixed with 150 ml water	Gluten-free powder (50 g) mixed with 150 ml water	6 weeks with twice daily administration of gluten containing or gluten-free powder	Significant worsening of symptoms in the gluten-containing group compared to placebo

Zanini 2015[134]	NCGS	35	Double-blind, cross-over RCT	Gluten containing flour (10 g/day)	Gluten-free flour (10 g/day)	10 days with one type of flour, 2 week washout before crossing over to another 10 days with the other type of flour	Significant but similar worsening of symptoms in both dietary arms; Gluten containing flour only correctly identified in 34% of participants

1.3.7. Issues in the definition and evaluation of NCGS

The reason for this apparent heterogeneity of results requires close examination. First, major interpretative problems relate to the nature of the populations being studied and reported. It has, up until now, been well accepted that NCGS patients do not have villous atrophy but might have an increased number of intraepithelial duodenal lymphocytes. Lymphocytic enteritis is a non-specific lesion that might be associated not only to coeliac disease but also to *Helicobacter pylori* infection, SIBO or use of anti-inflammatory drugs.[122] However, the most frequent cause of lymphocytic enteritis in patients with positive HLA-DQ2/DQ8 after exhaustive diagnostic work-up has been coeliac disease, ranging from 16-43%.[122] As such, several studies have included patients with intraepithelial lymphocytosis in the duodenum and evidence of immunological activation that potentially might be part of the spectrum of coeliac disease. In this respect, the exclusion of coeliac disease by combined histological and serological assessment while consuming adequate gluten is imperative[136] not only with respect of misdiagnosing NCGS in coeliac disease patients but also on overestimating the response to a gluten-free diet in NCGS patients.[122]

Second, nocebo responses can be an issue in re-challenge arms as evident in several of the reported studies.[86, 134] For example, just one third of patients fulfilling the clinical diagnostic criteria for NCGS experienced symptom reoccurrence following recent double-blind gluten challenges.[134] Despite randomised, double-blinded, placebo-controlled, cross-over studies being the gold standard for dietary trials it may be that alternate designs, such as parallel designs, are required in NCGS populations. Alternatively, it may be imperative that cross-over designs are used in this group to identify the minority who do have true gluten sensitivity. Parallel-group studies assess differences in population-based symptom reporting.

Third, the active product used in the challenges could also have potentially influenced the results. Depending on the study, carbohydrate-deplete wheat protein, whole wheat flour or purified gluten were used in varying quantities (Table 1.7). These substrates were also delivered via variable mediums including in whole foods or capsules (Table 1.7). In order for reasonable comparisons to be made between trials, a consensus on the active product type and quantity used in future challenges should be sought. This is particularly important given the large variability in gluten exposure when for example; whole wheat flour is compared to purified gluten (Figure 1.6). Interestingly, within coeliac populations, it is well documented that the inflammatory response is caused by the gliadin fraction of the gluten protein.[108] It is, therefore, possible that those with NCGS are responding in a similar manner. If so, controlled exposure to the individual wheat gluten proteins in challenge trials involving individuals with NCGS may prove beneficial.

Fourth, besides gluten it is also possible that other components in wheat, such as FODMAPs, may contribute to symptoms (at least in those related to IBS) experienced by NCGS patients. This was observed convincingly during the run-in period in the Biesiekierski et al[86] study and more recently in work evaluating the efficacy of both the gluten-free and low FODMAP diets in patients with NCGS where the proportion of NCGS patients responsive to carbohydrate restriction outnumbered that of patients responding to a gluten-free diet.[137] Although FODMAPs may be the offending agent, other potential culprits present within the wheat protein such as ATIs and glycoproteins have been postulated to play a role.

Finally, gluten restriction may be associated with changes in mental state and may not be directly related to a reduction in gastrointestinal symptoms. As previously described, recent work by Biesiekierski et al[86] revealed that despite continued gastrointestinal symptoms on a gluten-free diet patients with NCGS continued to restrict gluten as they reported feeling better.

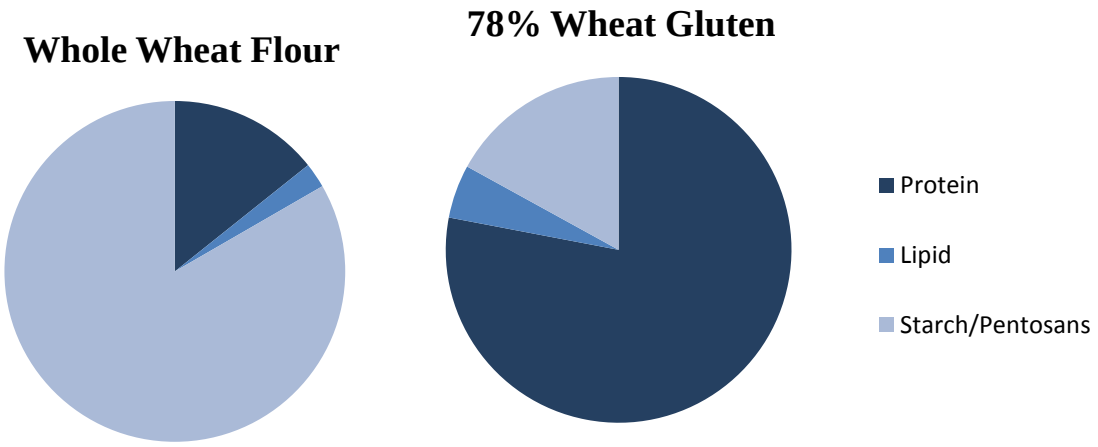


Figure 1.6. Wheat protein breakdown according to the percentage of wheat gluten present whole wheat flour and 78% wheat gluten concentrate

1.3.8. Gaps in the evidence

NCGS is an emerging novel entity that still requires elucidation. Considering the lack of available evidence with regard to epidemiology, diagnostic criteria and pathogenesis, knowing how to treat NCGS patients remains controversial. With several well-designed elimination and re-challenge trials failing to observe gluten-specific effects in the majority, treatment of NCGS may not be as simple as employing a gluten-free diet. As such, several points are worth considering in the current thesis.

The first consideration is that the improvement reported in NCGS patients on a gluten-free diet is related to changes in mental state not necessarily gastrointestinal symptoms *per se*. Mood disorders and cognitive impairment has been extensively explored within the coeliac population where psychological aliments are common in untreated patients with reversal of this effect observed on a gluten-free diet. The relationship between psychological manifestations and NCGS has seldom been studied. One recent publication explored trait

anxiety and depression in patients with NCGS following gluten exposure and found patients had higher baseline psychological scores compared to healthy controls but that these scores did not differ significantly following the consumption of gluten.[138] Mental state was not specifically explored. It may be that the reversal of mental state amongst this entity, not personality trait, which contributes to why such patients feel better when following a gluten-free diet despite the continuation of gastrointestinal symptoms. These findings coupled with those reported by Biesiekierski et al[86] provide the rational for investigating this concept within the current thesis where two successive challenge studies were conducted. The first of these was an exploratory investigation and the second was a longer more detailed RCT. Results from these two investigations are reported in Chapters 4 and 5 respectively.

The second consideration is that gluten restriction does improve gastrointestinal symptoms in patients with NCGS and that previous re-challenge trails have failed to observe a gluten-specific effect due to the highly selected cohort and the subsequent varying methodologies employed. It is possible, therefore, that previous studies have precluded any consistent gluten-specific effects. Gluten itself is a complex mixture of hundreds of related but distinct proteins found not only in wheat but also in rye and barley. The major grain protein of wheat gluten includes the gliadin and glutenin prolamins which together make up 85-90% of the total grain proteins. In coeliac disease, it has been shown that immune responses to the specific gliadin fraction of gluten are responsible for promoting an inflammatory response which results in various degrees of intestinal injury. Despite the clear understanding of the physiological mechanisms involved in coeliac disease, the mechanistic action of wheat gluten protein in patients with NCGS remains unknown.

It is possible that specific T-cell responses to gliadin may also be occurring in patients with NCGS and may or may not be related to the toxic peptide sequence associated with coeliac disease. Work on mechanisms with individual fractions of wheat gluten protein is only

possible with isolation studies. The structural complex of wheat gluten protein, the isolation of the various gluten fractions and the importance of doing work with isolated fractions as opposed to whole wheat gluten is explained and reported in Chapter 6, where the first large-scale gliadin and glutenin isolation methodology is discussed.

The final consideration is that there is in fact no gluten-specific effect in patients with NCGS and that other food components may be responsible for the exacerbation of gastrointestinal symptoms amongst this cohort. As discussed, other than the obvious presence of gluten, there is good evidence that wheat and other grains contain significant quantities of FODMAPs, particularly fructans. Interestingly, analysis of commonly consumed grain and cereal products has shown that wheat-derived products contain the highest fructan content.[139] The products with the lowest fructan content are mostly gluten-free, being based on rice, oat, quinoa and corn ingredients. It is possible, therefore, that 'gluten restriction' automatically reduces a patients' dietary fructan intake and may be the reason that patients report improved gastrointestinal symptoms on a gluten-free diet as previously reported within this entity.[86]

1.4. Gut-Directed Hypnotherapy

1.4.1. Understanding gut-directed hypnotherapy

Gut-directed hypnotherapy is one of the most widely studied psychological treatments within IBS populations and was first described by Whorwell and colleagues at the University Hospital of South Manchester.[140] Typically, a session of gut-directed hypnosis involves an introduction where suggestions for imaginative experiences are presented. A hypnotic induction will then follow, with the aim of allowing the subject to enter an altered

consciousness or trance state. Once in the trance state, suggestions are made for the control and normalisation of gastrointestinal function (normally on a repetitive basis) and metaphors are used for bringing about improvement (Table 1.8). This differs from other forms of psychological treatment where therapy is provided to the patient in a conscious state.

1.4.2. Brain-gut axis

The brain-gut axis refers to the bi-directional flow of information that takes place between the brain and the gastrointestinal tract. The organisation of homeostatic reflexes within the brain-gut axis allows afferent signals arising from the lumen of the gut to be transmitted via various visceral afferent pathways to the CNS.[141] Reflexes that generate appropriate gut responses to physiological as well as pathological afferent gut signals occur at the level of the ENS, the spinal cord and the pontomedullary nuclei and limbic regions.[141] Through such reflexes, vagal visceral afferent inputs play an important role in such diverse functions as modulation of emotion, pain, satiety and immune responses.[141]

While reflex circuits within the ENS, in principle, can regulate and synchronize all basic gastrointestinal functions (motility, secretion, blood flow), coordination of gut functions with the overall homeostatic state of the organism requires continuous and close communication between the CNS and the gastrointestinal tract.[141] Descending corticolimbic influences can set the gain and responsiveness of these reflexes, impose distinct patterns of motor responses on lower circuits, and modulate visceral pain transmission.[141] Such descending modulation can be triggered by cognitive or emotional influences, or in response to environmental demands, and can override local reflex function during sleep, in the context of environmental stressors, or during strong emotions such as fear and anger.[141]

The brain-gut axis, therefore, plays an important role in the regulation of many vital functions including the regulation of digestive processes, in the modulation of the gut-associated

immune system, and in the coordination of the overall physical and emotional state of the organism with activity in the gastrointestinal tract.[141] As such, peripheral and central alterations in brain-gut interactions are likely to underlie the pathogenesis of symptoms in all patients with chronic intestinal disorders.

No single pathophysiological mechanism can explain all symptoms across all patients with chronic intestinal disorders. It is likely that varying patterns of dysregulation in the interactions between the CNS and the respective abdominal end organ are involved in different subsets of patients. While dysregulation at first onset of symptoms may be purely functional and driven primarily by abnormal autonomic system activity, chronicity of symptoms may be associated with neuroplastic and structural changes in the brain, spinal cord and gastrointestinal tract.[141]

Despite the lack of scientific data, one may speculate that there are different ways chronic intestinal disorders can develop from dysregulation within the brain-gut axis. Longstanding transient dysregulation of homeostatic reflexes (in the periphery and/or centrally) may gradually result in neuroplastic peripheral and/or central changes, leading to permanent dysregulation.[141] Alternatively, formation of maladaptive interoceptive memories may create central mechanisms by which pain and discomfort can be experienced in contexts of emotional distress, without any abnormal peripheral responses.[141] Recent work by Koloski and colleagues has provided support for both the brain-gut and gut-brain hypothesis in IBS patients specifically.[142] In a cohort of subjects without IBS at baseline, higher levels of anxiety at baseline, were a significant predictor of developing IBS 12 years later.[142] Further, IBS at baseline, without elevated levels of anxiety and depression at baseline, had significantly higher levels of subsequent anxiety and depression at follow-up.[142] These finding were interpreted as showing that the CNS and gastrointestinal tract are likely to interact bi-directionally in IBS.[142]

Several general modulators within the brain-gut axis have been proposed to alter brain-gut interactions in chronic intestinal disorders. These include centrally-targeted pharmacological and non-pharmacological therapies. Multiple sites can be targeted within the brain-gut axis, and these include matostatin, opioid, 5-hydroxy tryptamine-3 ($5\text{-}HT_3$) and neurokinin receptors and corticotrophin releasing factor-1 (CRF_1), and can influence symptoms involving gastrointestinal function and emotion.[141] Regarding non-pharmacological therapies, several psychological treatments have been shown to be effective in improving gastrointestinal symptoms and psychological state amongst various chronic intestinal disorders in numerous high-quality clinical trials.

Table 1.8. Common gut-directed suggestions and metaphors used during hypnosis

Suggestions	
Improvement in pain & bloating	"There will be no more pain, no more bloating and no more discomfort"
Improvement in bowel habits	"Your bowel habits will continue to improve day by day, week by week and month by month"
Improvement over time	"You will continue to get better and better and better"
Metaphors	
River	The flow of the river is a representation of the flow of the gastrointestinal tract. Patients control the flow of their river according to their needs.
Hand warmth	The warmth of the hands represents calmness and control. Patients visualise feelings of calmness and control over their gastrointestinal tract while placing their hands on their abdomen.
Medicine	Taking medicine improves gastrointestinal function. Patients envisage the medicine providing protection against pain, bloating, discomfort and abnormal bowel habits.

1.4.3. Efficacy of gut-directed hypnotherapy in irritable bowel syndrome

A summary of RCTs evaluating the efficacy of gut-directed hypnotherapy compared to usual treatment, supportive therapy or wait-list controls amongst the IBS population are presented in Table 1.9. Detailed analysis of the gastrointestinal outcomes from those RCTs is shown in Table 1.10 and psychological outcomes in Table 1.11.

1.4.3.1. Randomised control trials

The first RCT conducted to assess the change in gastrointestinal symptoms, following a course of individualised gut-directed hypnotherapy in unselected patients with IBS refractory to standard medical treatment, was reported by Whorwell et al,[143] where greater improvements in individual symptoms of abdominal pain, distension and bowel habit (all $p<.0001$) were observed in the gut-directed hypnotherapy group (n=15) compared to the psychotherapy control (n=15). The magnitude of the effect was large. For example, abdominal pain changed from a mean weekly score 14 (maximum score 21) to 12 in the placebo group and 2 in the hypnotherapy group. Similar observations of improvement have been further substantiated by more recent work within the literature.[144, 145b, 146a, 147] All described overall gastrointestinal symptom improvement, ranging between 24-73%, following gut-directed hypnotherapy compared to control interventions (Table 1.9). Improvement was observed regardless of whether participant populations were unresponsive to standard medical treatment at enrolment [146a, 147] or not.[144, 145b] Individual symptomatic responses differed between studies and can be seen in Table 1.9.

A positive effect has also been shown in group, as opposed to individualised, gut-directed hypnotherapy, where overall greater improvement in IBS-IS scores were observed in 28 (61%) out of 46 gut-directed hypnotherapy patients compared to 18 (41%) out of 44 of those allocated to the active control (absolute difference 20%, 95% CI: 0-40%, $p = .05$).[148] Long-term maintenance of symptomatic improvement was observed in four of the five above-mentioned individualised studies[144, 145b, 146a, 147, 148] and ranged from 2-months to 1-year. Superior improvement in one or more psychological domain (e.g. anxiety, depression, and well-being) was also seen following gut-directed hypnotherapy compared to control conditions in several studies.[143, 144, 146a, b, 148]

Not all studies have reported significant improvements with gut-directed hypnotherapy compared to control interventions. In a paper that reported the results of two separate RCTs, with similar design features, a greater reduction in gastrointestinal symptoms following hypnotherapy (compared to wait-listed control) was only observed in one of the two included studies.[146] In this paper, 138 patients were randomised to study 1 (n=90) or study 2. Those in study 1 were randomised to receive the treatment or control in private psychology practices whereas those in study 2 were randomised to receive the treatment or control in a small country hospital. In both studies, IBS-related symptoms were improved post-treatment in the gut-directed hypnotherapy groups ($p<.05$), but not in the control groups. As described, in study 1, a significantly greater improvement could be detected in the gut-directed hypnotherapy group compared to the control group (mean difference 3.7, 95% CI: 0.3-7.2, $p=.03$), but this was not observed in study 2 (mean difference 0.3, 95% CI: -0.2-0.9, $p=.22$). This may have partly related to the lack of power in the latter study where only 48 participants were enrolled.

One RCT targeted physiological mechanistic changes in patients with IBS as its primary outcome measure.[149] Gut-directed hypnotherapy reduced the sensory and motor components of the gastro-colonic response compared with supportive therapy only when evaluated by colonic distension before and after a one-hour duodenal lipid infusion. The results paralleled the reported clinical improvement in 10 of 14 patients following hypnotherapy compared to 5 of 14 in the control group, although the clinical differences did not reach statistical significance ($p=.06$). Despite this, the greatest understanding of mechanistic changes following a course of gut-directed hypnotherapy can be obtained from observational studies, and are explained in detail below.

Thus, the majority of the published trials on gut-directed hypnotherapy as a treatment for IBS provide evidence to suggest that gut-directed hypnotherapy is efficacious, with the main

measure effect being a reduction in gastrointestinal symptom scores. Six out of seven (86%) IBS studies indicated a significant reduction in global gastrointestinal symptoms following gut-directed hypnotherapy compared to those in the control groups. The observed improvement occurred irrespective of patient responsiveness to standard medical treatment, the bowel habit of the patients and regardless of whether the therapy was provided individually or in group settings. This improvement was maintained long-term in four of five studies.

1.4.3.2. Comparative studies

Two comparative RCT studies with active psychological treatments, including biofeedback[150] and education intervention[151], revealed that gut-directed hypnosis did not provide superior therapeutic change compared to the active controls. Comparative studies using other treatment modalities have not been conducted.

1.4.3.3. Uncontrolled studies

While several other studies have further explored the use of hypnosis in IBS, these were non-randomised observational studies,[152-165] single case reports[166-168] or a comparison of two types of hypnotherapy for IBS.[145, 169-171] They have uniformly suggested that gut-directed hypnotherapy may be useful in controlling gut symptoms. The most impressive was an audit of 1,000 consecutive patients in which 76% had a 50-point reduction in the IBS Symptom Severity Score after 12 sessions of hypnotherapy over three months.[165] Other studies have not included gastrointestinal symptoms as a primary or secondary outcome but have focused more specifically on possible mechanistic changes following a course of gut-directed hypnotherapy[172, 173] or likely predictors of response to hypnosis.[174, 175]

Long-term follow-up studies have mostly been observational, other than the study recently published by Moser et al[148], and have too reported sustained beneficial effects of gut-directed

hypnotherapy for the treatment of IBS over time[156, 176] and in group settings.[177] Gonsalkorale et al explored the long-term follow up of 204 patients and demonstrated that approximately 4 out of every 5 patients who responded to treatment fully retained their therapeutic benefits for a minimum of 1 year (outcome assessed for up to 5 years after treatment) and that most continued to see further improvements in bowel symptoms after the end of the treatment course.[156]

Table 1.9. Characteristics of the study population, methodology for hypnotherapy and control population used in randomised controlled trials of gut-directed hypnotherapy in patients with irritable bowel syndrome

Study	Studied population		Interventions				
	Number (male)	Disease	Gut-directed hypnotherapy				Control
			Number of sessions	Duration of sessions	Duration of therapy	Audiotape provided	
Whorwell 1984[143]	30 (4)	IBS	7	30 min	12 weeks	Yes	Psychotherapy
Galovski 1998[144]	13 (2)	IBS	12	30-60 min	12 weeks	Yes	Symptom monitoring (wait-list)
Palsson 2002b[145]	24 (9)	IBS	7	45 min	12 weeks	Yes	Delayed therapy (wait-list)
Simren 2004[149]	28 (9)	IBS	12	60 min	12 weeks	No	Supportive therapy

Roberts 2006[147]	81 (12)	IBS	5	30 min	5 weeks	Yes	Usual medical care
Lindfors 2011a[146]	90 (19)	IBS	12	60 min	12 weeks	No	Supportive therapy
Lindfors 2011b[146]	48 (9)	IBS	12	60 min	12 weeks	Yes	Wait-list control
Moser 2013[148]	90 (19)	IBS	10	45 min	12 weeks	Yes	Supportive talks with medical treatment

Table 1.10. Gastrointestinal symptom outcomes of randomised controlled trials using gut-directed hypnotherapy (GDH) in patients with irritable bowel syndrome. NNT = Number needed to treat

Study	Outcomes	Outcome scoring method	% of responders	Results		NNT
				At completion of therapy (GHD compared to control)	At follow-up	
Whorwell 1984[143]	Pain; distension; bowel habit	Likert scale	N/A	Greater individual improvement in abdominal pain, distension and bowel habit (all $p<.0001$)	Not assessed	N/A
Galovski 1998[144]	Diarrhoea; constipation; pain; bloating; flatulence; belching; nausea	Composite Primary Reduction Score (CPRS); Symptom diary	GDH 73% Control 0%	Greater overall improvement (mean difference .84, $p=.016$).Greater individual improvement in constipation ($p=.015$), abdominal pain ($p=.012$) and flatulence ($p=.006$) but not diarrhoea, bloating, belching or nausea	Improvement maintained in 44% 2-months post-treatment	N/A
Palsson 2002b[145]	Overall gastrointestinal (GI) symptom improvement; pain;	Symptom diary	N/A	Greater overall improvement ($p=.002$). Greater individual improvement in pain (mean difference -3.9, $p=.049$) and	Improvement maintained 10-months post-treatment. 68% mean estimated	N/A

	bloating; proportion of hard/loose bowel movements; frequency bowel movements			proportion of hard/loose stools (mean difference -.16, *p*=.003) but not bloating or frequency of bowel movements	degree of change	
Simren 2004[149]	Colonic sensory thresholds; tonic and phasic motor activity	Barostat procedure	GDH 71% Control 36%	Colonic sensitivity following duodenal lipids reduced post-GDH for pain (33±2.7 mm Hg vs. 26±3.3 mm Hg, *p*<.01) and following the control intervention for gas (22±1.7 mm Hg vs. 16±1.6 mm Hg, *p*<.01), discomfort (29±2.9 mm Hg vs. 22 ±2.6 mm Hg, *p*<.01) and pain (33±2.7 mm Hg vs. 26±3.3 mm Hg, *p*<.01). Reduced balloon volumes during lipid infusion were seen in the control intervention (141±15 ml vs. 111±19 ml, p<.05) but not after GDH (83±14 ml vs. 80±16 ml, *p*>.20)	Not assessed	3

Roberts 2006[147]	Overall GI symptom improvement; pain; constipation; diarrhoea	Symptom score based on Rome II criteria	N/A	Greater overall improvement (mean difference 8.5, 95% CI: 2.3-14.7, *p*=.008). Greater individual improvement in pain (mean difference 12.5, 95% CI: 2.4-22.6), *p*=.02) and diarrhoea (mean difference 7.6, 95% CI: 0.2-15.1, *p*=.046) but not constipation	Improvement maintained 1-year post-treatment but mean change in GDH was not significantly superior	N/A
Lindfors 2011a[146]	Bloating; gas; pain; loose stools; urgency; hard stools; incomplete evacuation	Scores of individual symptoms were combined into two domains (1) sensory symptom score (pain, bloating, gas) (2) bowel habit score (loose stools, urgency, hard stools, incomplete evacuation)	GDH 38% Control 11%	Greater overall improvement (mean difference 3.7, 95% CI: 0.3-7.2, *p*=.03) and sensory symptom score (mean difference 2.2, 95% CI: .5-3.1, *p*=.01) but not bowel habit score	Improvement maintained in 42% 1-year post-treatment	4
Lindfors 2011b[146]	Pain, bloating, constipation,	Gastrointestinal Symptom Rating	GDH 24% Control	No overall greater improvement. Greater individual improvement in	Improvement maintained in 28% 1-	9

	diarrhoea, satiety	Scale (GSRS) IBS version	11%	bloating (mean difference .82, 95% CI: .30-1.3, *p*=.003). Independent analyses revealed overall greater improvement following GDH (*p*<.05) but no improvement was seen in the control group	year post-treatment	
Moser 2013[148]	Overall GI symptom improvement	IBS Impact Scale (IBS-IS)	GDH 61% Control 41%	Overall greater improvement (absolute difference 20%, 95% Confidence Interval (CI): 0-40.2%, *p*=.046)	Improvement maintained in 54% of GDH patients and 25% of the controls 1-year post-treatment	5

Table 1.11. Psychological symptom outcomes of randomised controlled trials using gut-directed hypnotherapy in patients with irritable bowel syndrome

Study	Outcomes	Outcome scoring method	Results	
			Initial (post-treatment)	**Delayed (follow-up)**
Whorwell 1984[143]	Well-being	Likert scale	Overall greater improvement in well-being (p<.0001) in the gut-directed hypnotherapy (GDH) compared to control intervention	Not assessed
Galovski 1998[144]	Depression; anxiety	Beck Depression Inventory; State-Trait Anxiety Inventory	Overall greater reduction in state (p=.04) and trait (p=.014) anxiety but not depression pre vs post-treatment. Comparisons not made between interventions	Reduced state and trait anxiety maintained 2-months post-treatment
Palsson 2002b[145]	Psychopathology; somatisation; autonomic functioning	Symptom Checklist–90 Revised (SCL-90R); The Stress-related Physical Symptoms Inventory (SPSI); Autonomic functioning test	Overall greater reduction in SCL-90R (p=.002) and SPSI (p=.0001) scores but not autonomic functioning pre vs post treatment. Comparisons not made between interventions	Not assessed
Simren 2004[149]	Not assessed			

Roberts 2006[147]	Quality of Life (QOL)	IBS-QOL	Overall improvement in QOL (p<.001) pre vs post treatment. No difference in improvement observed between interventions	Improvement maintained 12-months post-treatment
Lindfors 2011a[146]	QOL; anxiety and depression	IBS-QOL; Hospital Anxiety and Depression Scale	Improvement in QOL (mental health, sleep & social role subscales, all p<.05) pre vs post treatment. A greater reduction in anxiety (p<.05) but not depression was observed in the GDH compared to control intervention	Improvement maintained 1-year post-treatment
Lindfors 2011b[146]	QOL; anxiety and depression	Short Form-36 (SF-36); Hospital Anxiety and Depression Scale	Improvement in physical (p<.05) but not mental QOL pre vs post treatment. A greater reduction in anxiety (p<.05) but not depression was observed in the GDH compared to control intervention	Improvement not maintained 1-year post-treatment
Moser 2013[148]	QOL; anxiety; depression	SF-36; Hospital Anxiety and Depression Scale	Overall improvement in QOL (p=.006) and reduction in anxiety and depression in the GDH compared to control intervention	Improvement maintained 1-year post-treatment

1.4.4. Mechanism of action

The precise mechanisms by which gut-directed hypnotherapy exerts an efficacious effect are poorly understood. Regardless, there is strong evidence that gut-directed hypnotherapy can influence psychological and physiological outcomes, including motility, visceral sensitivity, immune function and central processing.

1.4.4.1. Physiological factors

Gut-directed hypnotherapy has an effect on various physiological indices:

- *Gut motility:* In patients with IBS, gut-directed hypnotherapy reduces fasting distal colonic motility[173]
- *Visceral sensitivity:* The effect of gut-directed hypnotherapy on rectal sensitivity has been addressed with differing results. Initial work revealed improved rectal sensitivity amongst patients with diarrhoea-predominant IBS,[161] an effect that was later confirmed within all IBS subtypes.[159, 178] In contrast, other work has failed to observe this effect.[145a, 172] The apparent inconsistency in results may be due to differences in methodology or whether adult[145a, 159, 161, 178] or paediatric[172] populations were studied
- *Immunological effects:* Self-hypnotherapy has been shown to antagonise decreases in natural killer (NK) T-cell counts induced by the stress of examinations. Furthermore, changes in CD3+, CD4+ and CD8+ lymphocyte counts before exams were also reduced by self-hypnosis.[179, 180] More recently, one session of gut-directed hypnotherapy in 17 patients with active ulcerative colitis reduced systemic and rectal mucosal inflammatory responses. Specifically, it reduced serum interleukin (IL) -6 concentration by 53%, circulating NK cell numbers by 18%, and rectal mucosal release of substance P by 81%, histamine by 35% and IL-13 by 53%[181]

- *Effects on central processing:* Functional magnetic resonance imaging (fMRI) provides insights into cortical activation patterns to painful rectal stimuli, where patterns differed in patients with IBS compared to controls.[182] Specifically, pain has been shown to result in greater activation of the anterior cingulate cortex than non-painful stimuli in IBS subjects.[8] Using this technique, gut-directed hypnotherapy has a normalising effect on the aberrant central processing of visceral signals in patients with IBS[151]

1.4.5. The challenge in designing high quality trials

Conducting well-designed clinical trials using hypnotherapy is difficult. In order for researchers to be able to draw causal conclusions about the efficacy of an intervention, they must compare the treatment condition with control group that accounts for improvements caused by factors other than the treatment.[183] In pharmacological studies, the control group can receive treatment identical to that of the experimental group (through a placebo pill) meaning that participants cannot tell whether they are in the experimental or the control treatment.[183] Therefore, any difference between the groups on the outcome measure can be attributed to the effect of the treatment.[183] However, pharmacological-standard study designs are difficult in the realm of psychology, where it is almost impossible to match expectations between treatment and control groups.[183] Participants in psychological interventions typically know what treatment they received. Measuring the effectiveness of a therapy to a no-treatment control condition is, therefore, compromising and possibly inadequate. However, this has been the predominant control used in studies to date. An alternative is to compare the therapy with an active control group, but this too can lead to expectations about the effectiveness of the therapy.

1.4.6. Gaps in the evidence

Obtaining high-quality evidence for efficacy of gut-directed hypnotherapy in patients with IBS is constrained by difficulties in designing a blinded placebo. An alternative is to compare gut-directed hypnotherapy to a therapy with proven efficacy, like the low FODMAP diet. The low FODMAP diet is a good comparator for several reasons. We have a sound understanding of the mechanistic action of FODMAPs, the low FODMAP diet has been shown to reduce gastrointestinal symptoms in the majority of patients and is equally efficacious regardless of IBS bowel habit subtype. Therefore, the technique of comparing gut-directed hypnotherapy to the low FODMAP diet was explored in the current thesis and is reported in Chapter 7.

1.5. Directions of the Current Thesis

The link between gastrointestinal disease, diet and psychological status is seldom studied. While there is a large body of evidence that examines either dietary or psychological interventions for the treatment of various gastrointestinal conditions including NCGS and IBS, very few encapsulate the two. The overall aims of the current thesis are therefore, twofold. The first aim is to explore the existence of NCGS in relation to dietary exacerbation of both psychological and gastrointestinal symptoms. The role of wheat gluten and its specific protein classes will also be examined where an isolation study will enable future specialist work with the isolated gliadin and glutenin protein subclasses. The second aim is to gain a greater understanding of the role of gut-directed hypnotherapy, as a psychological therapy, in the reduction of gastrointestinal and psychological symptoms in patients with IBS when directly compared to the low FODMAP diet, the first-line dietary therapy now applied within this entity.

Chapter 2 - Aims and Hypotheses

An increasing number of patients worldwide are reportedly sensitivity to gluten without evidence of coeliac disease, so called non-coeliac gluten sensitivity (NCGS). As discussed in Chapter 1, little is known about the NCGS entity despite the numerous mechanistic, elimination and re-challenge trials. Nonetheless it has been endorsed by an expert group as a definite clinical entity[107] characterised by symptoms that usually occur soon after the ingestion of gluten-containing food, disappear with gluten withdrawal and relapse following gluten challenge.

What remains unknown is what exactly patients are reacting to within specific gluten-containing products. It may be gluten itself, and if so, the precise protein responsible for this effect requires elucidation. Potential problematic wheat gluten protein classes include gliadins, glutenins, albumins and globulins. Alternatively, it may be other components in wheat, primarily fructans, which from a collective group known as FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols) that may be responsible for symptomatic induction. Another consideration, however, is the potential effect that gluten has with regard to psychological symptoms. For example, recent evidence suggests that many patients with NCGS retain gastrointestinal symptoms on a gluten-free diet but continue to restrict gluten as they report feeling better.[86] Therefore, it may be through the improvement of psychological rather than gastrointestinal symptoms that is responsible for this improvement.

The current thesis aims to begin to explore these potential responsible components. Chapters 4 and 5 describe the series of studies undertaken to understand the role of psychological symptoms in patients with NCGS where it was anticipated that a major effect of gluten in those with NCGS would be on mental state and not necessarily on gastrointestinal symptoms.

Insight into the component of the wheat gluten protein that is potentially responsible for this effect was also explored where an isolation study was conducted to enable future work with purified gliadin, the toxic fraction of gluten in patients with coeliac disease, and glutenin. Chapter 6 describes this isolation process and its potential application in future studies within the NCGS entity.

Psychological health and its effects on brain-gut interactions is an area of important consideration. This may be true of NCGS populations and has certainly been shown within IBS cohorts.[142] Thus, therapeutic ways of manipulating these interactions are warranted. An additional focus of this thesis, therefore, was to identify new and novel treatments for gastrointestinal symptoms and psychological disturbance in functional gastrointestinal disorders using psychological modalities. The modality of specific interest was gut-directed hypnotherapy where it was compared to a therapy with proven efficacy, the low FODMAP diet, in patients with irritable bowel syndrome. The results of this work are presented in Chapter 7. Specific aims and hypotheses of each the abovementioned studies are detailed below.

Chapter 4: The effect of gluten on the psychological state of subjects with non-coeliac gluten sensitivity: A pilot study

Aim

- To investigate the effect of gluten on psychological state and gastrointestinal symptoms in patients with NCGS

Hypothesis

- That gluten ingestion would worsen the psychological state of those with self-reported NCGS

Chapter 5: The effect of gluten on psychological indices, quality of life and fatigue in subjects with non-coeliac gluten sensitivity

Aim

- To conduct a larger and more detailed study that investigates the effect of gluten on psychological indices including anxiety, depression and cognitive function, as well as quality of life and fatigue, in patients with NCGS
- To confirm previous findings that a major effect of gluten in those with NCGS is on psychological state and not gastrointestinal symptoms

Hypotheses

- That gluten adversely affects psychological state in those with NCGS
- That the adverse effects of gluten will be restricted to current feelings of depression

Chapter 6: The development and characterisation of a method for the large-scale isolation of gliadin and glutenin suitable for human consumption

Aims

- To isolate the gliadin and glutenin fractions of the gluten protein on a large (~5-10kg) scale
- To produce gliadin and glutenin that can safely be consumed by humans and used in future clinical trials

Hypothesis

- That the gliadin and glutenin fractions of the gluten will be easily extracted from the other polypeptides based on their differing solubility properties

Chapter 7: A randomised comparison of the short and longer term efficacy of gut-directed hypnotherapy with that of the low FODMAP diet on gastrointestinal and psychological symptoms in subjects with irritable bowel syndrome

Aims

- To determine whether the efficacy of gut-directed is comparative to that of the low FODMAP diet
- To compare the collective benefit of combining these therapies

Hypothesis

- Participants would report similar gastrointestinal and psychological improvements regardless of whether they received gut-directed hypnotherapy or the low FODMAP diet but that those who received both therapies would experience an enhanced effect

Chapter 3 - General Methods

3.1. Participants

Participants were recruited through newspaper advertisements in metropolitan Melbourne, on social media including Facebook and Twitter, via the Monash University Department of Gastroenterology webpage, and through the Functional Gut Clinic at the Alfred Hospital. Participants were included if they were aged >18 years of age, met Rome III criteria for irritable bowel syndrome (IBS), and had coeliac disease excluded by either a normal duodenal biopsy (Marsh 0) performed at endoscopy while on a diet containing adequate gluten (i.e. at least 4 slices of wheat bread or its equivalent per day for six weeks) and/or the absence of the HLA-DQ2 and HLA-DQ8 haplotype. Participants in Studies 4 and 5 also needed to fit additional criteria for non-coeliac gluten sensitivity (NCGS) including gastrointestinal symptom improvement following the implementation of a gluten-free diet, current well-controlled gastrointestinal symptoms on a gluten-free diet, adherence to a gluten-free diet for a minimum of 6 weeks preceding enrolment and reported worsening of symptoms on a gluten-containing diet. Exclusion criteria included March 1 or 2 lesions on duodenal biopsy, other significant gastrointestinal disease (such as cirrhosis or inflammatory bowel disease), other clinically significant co-morbidity, intake of non-steroidal anti-inflammatory agents, use of systemic immunosuppressant medication, previously diagnosed or reported psychiatric disorder, excessive alcohol intake, pregnancy and inability to give written informed consent. Participants enrolled into study 7 were also required to be naive to both gut-directed hypnotherapy and the low FODMAP (Fermentable Oligosaccharides, Disaccharides,

Monosaccharides And Polyols) diet. Any participant who had previously undergone gut-directed hypnotherapy or been instructed on the low FODMAP diet was excluded.

3.2. Ethics

All studies described in this thesis were approved by either the Eastern Health Research and Ethics Committee or The Alfred Research and Ethics Unit. Trials were also registered with the Australian Clinical Trials Registry (Chapter 4: ACTRN12613000768796; Chapter 5: ACTRN12614000726651; Chapter 7: ACTRN12612000585820).

3.3. Randomisation and Blinding

Randomisation for all studies was conducted according to a computer generated list executed by http://www.randomization.com. In studies reported in Chapters 4 and 5, randomisation codes were held by an independent observer, in accordance with the CONSORT statement and guidelines of randomised controlled trials[184]. The study reported in Chapter 7 was not blinded.

3.4. Measurements

The following measurements were used consistently throughout the thesis.

3.4.1. Gastrointestinal symptoms

Gastrointestinal symptoms were assessed using a 100 mm visual analogue scale (VAS), where 0 indicated no symptoms and 100 represented the worst symptoms ever experienced. The VAS can be adapted for any symptom, overall and individual symptoms, and involved the participant placing a mark at a point relevant to their degree of symptom severity. A ruler was then used to calculate the marked score. The questions asked using the VAS were overall gastrointestinal symptoms, abdominal pain or discomfort, bloating or distention, passage of wind (i.e., flatulence), satisfaction with stool consistency, tiredness and lethargy and nausea. The VAS is part of the validated IBS-SSS questionnaire[185] and is shown in Appendix 1.

3.4.2. State Trait Personality Inventory

Psychological mental states and traits were assessed using the State Trait Personality Inventory (STPI). The STPI was selected based on simplicity, validity and reliability.[186] It is an 80-item self-report questionnaire, with eight 10-item scales for measuring state and trait anxiety, depression, anger and curiosity. State items are used to assess current emotional state and are rated on a four-point intensity scale, where 1= not at all; and 4= very much so. Trait items assess emotional disposition and are rated on a four-point intensity scale, where 1= almost never; and 4= almost always. The range of possible scores for each subscale can vary from a minimum of 10 to a maximum of 40. The STPI is shown in Appendix 2.

3.4.3. Depression Anxiety and Stress Scale

The Depression Anxiety Stress Scale (DASS) is a set of three self-report scales designed to measure depression, anxiety and stress.[187] Each of the three scales contains 14 items, divided in subscales of 2-5 items with similar content. The depression scale measures dysphoria, hopelessness, devaluation of life, self-depreciation, lack of interest/involvement, anhedonia

and inertia. The anxiety scale measures autonomic arousal, skeletal muscle effects, situational anxiety and subjective experience of anxious affect. The stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal and being easily upset/agitated, irritable/over-reactive and impatient. Responses are scored on a four-point severity scale where, 0 = did not apply to me at all and 3= applied to me very much, or most of the time. Scores for depression, anxiety and stress are calculated by summing the scores for the relevant items. DASS severity ratings can be seen in Table 3.1 and is shown in Appendix 3.

Table 3.1. DASS severity ratings[187]

	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Very severe	28+	20+	34+

3.4.4. Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) comprises statements that the patient rates based on their experience over the past week.[188] The 14 statements are relevant to either generalised anxiety (n=7) or depression (n=7), the latter being primarily composed of reflections of the state of anhedonia (inability to enjoy oneself or take pleasure in everyday things usually enjoyed). Responses are scored on a scale from 3 to 0. A score of 11 or higher indicates the probable presence of the mood disorder with a score of 8 to 10 being suggestive of the presence of the respective state. The two subscales, anxiety and depression, have been found to be independent measures. In its current form HADS scores can be divided into four

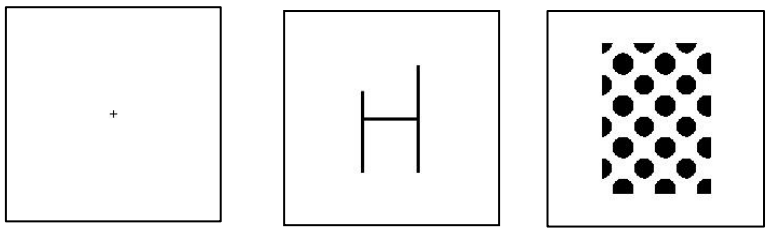
ranges; 0-7 = normal, 8-10 = mild, 11-15 = moderate, and 16-21 = severe. The HADS is widely accepted with good reliability and validity for the assessment of anxiety and depressive symptoms[189] and is shown in Appendix 4.

3.4.5. Subtle Cognitive Impairment Test

The SCIT is a brief (3-5 minute) computer-based perceptual judgement task[190] that is sensitive to minor changes in the speed and/or efficiency of cognitive processes.[191-194] Participants are asked to decide which of two parallel vertical lines (joined together to form an H) is shorter and then to indicate their decision by pressing the corresponding button (left or right) on a computer touchpad. On any given trial, the participant is asked to fixate on a crosshair in the middle of the screen and then the test stimulus is briefly presented on the screen, followed immediately by a pattern of filled black circles on a white background (the 'mask'). The mask reduces any further processing of the stimulus by blocking any afterimage of the stimulus. The participant receives repeated trials (crosshair, stimulus, mask) at each of eight stimulus durations between 16 ms and 128 ms. Trials are presented in a different pseudo-random sequence for each participant so that they cannot predict how long the stimulus will remain on the screen or on which side of the screen the stimulus is shortest. All stimuli were presented on a 15" flat screen of a DELL laptop computer.

Performance on the SCIT yields response times (ms) and error rates (%) for each of the eight stimulus exposure durations. Mean response time (ms) and mean error rate (%) are then calculated for each group as a function of exposure duration. Data for the four shortest exposures (16, 32, 48 and 64 ms) and the four longest exposures (80, 96, 112 and 128 ms) are pooled giving four dependent variables: *Response Time* for the Head (SCIT-RTH) and Tail (SCIT-RTT) of the SCIT distribution; and *Error Rate* for the Head (SCIT-EH) and Tail (SCIT-ET). Head response times and error rates reflect the speed and efficiency preconscious,

automatic cognitive processing respectively, while tail response times and error rates reflect the speed and efficiency, respectively, of more conscious and controlled cognitive processing. Collectively, they provide a measure of global cognitive processing that is sensitive to decrements in the signal processing that underpins the majority of cognitive domains.[194, 195] The SCIT has very high reliability and has been validated against numerous cognitive tests.[195]



Fixation Stimulus (500 ms) | Test Stimulus (16-128 ms) | Mask (500 ms)

Figure 3.1. Examples of subtle cognitive impairment test stimuli

3.4.6. IBS-QOL

Quality of life (QOL) was assessed using the IBS-QOL scale.[196, 197] It consists of 34 items and uses a 5-point Likert response scale to assess how much of each item describes the respondent's feelings to a particular symptom; not at all, slightly, moderately, quite a bit, and extremely or a great deal. All 34 items are scored through simple, summative scaling to derive an overall total score and eight subscales including dysphoria, interference with activity, body image, healthy worry, food avoidance, social reaction, sexual and relationships. To facilitate interpretation of scores, the summed total score is transferred to a 0-100 scale ranging from 0

(poor quality of life) to 100 (maximum quality of life). It is a highly reliable and valid self-administered questionnaire with an internal consistency of the overall IBS-QOL (0.95) exceeding the recommended cut-off of 0.70 for group comparisons and sufficient for individual comparison.[196] Scores of the IBS-QOL correlate strongly with other health status measures including the Short Form- 36 (SF-36), a generic measure of functional status and the Symptom Checklist 90 Revised (SCL-90), a measure of psychological distress. The IBS-QOL is show in Appendix 5.

3.4.7. Daily Fatigue scale

Fatigue was measured by the Daily Fatigue Impact Scale (D-FIS),[198] a questionnaire containing eight items investigating fatigue on cognition, physical functioning and daily activities. Answers were given on a 5-point Likert scale, where 0 equates to "no problem" and 4 to "extreme problem." A global score was derived from the sum of ordinal scores obtained for each item. Scores of more than 10 are consistent with the reports of subjects within the first 6 days of the onset of an acute flu-like illness. Scores of more than 20 are associated with a high likelihood of time lost from work. The D-FIS is shown in Appendix 6.

3.4.8. Salivary cortisol

Salivary cortisol is frequently used as a biomarker of psychological stress, as it is an accurate and practical alternative to blood determinations. Because several factors may influence concentrations (such as contaminating substances in saliva, diurnal rhythm of cortisol, sample storage), participants were provided with clear instructions (see Appendix 10) on collection, including sample collection taken at standardised times (in the evening at 2030 h). The Salimetrics Oral Swab (SOS; Salimetrics™, State College, USA) was used to collect saliva samples and stored inside a Swab Storage Tube (clear sterile plastic tube; Salimetrics™, State College, USA). All saliva samples were transported on ice and frozen at -20 °C until being

assayed externally (Stratech Scientific APAC Pty Ltd, Sydney, Australia) by competitive immunoassay using commercially available kits (Salimetrics™, State College, USA).

3.5. Statistical Analysis

Study-specific techniques are outlined in the respective chapters. Statistical programs used included; GraphPad Prism® (Version 5.02 for Windows, GraphPad Software, San Diego California USA), the R Statistical Software Package (R Development Core Team, R: A Language and Environment for Statistical Computing, Vienna, Austria) and SPSS® (SPSS for Windows, Chicago: SPSS Inc.; IBM).

Chapter 4 - The effect of gluten on the psychological state of subjects with non-coeliac gluten sensitivity: A pilot study

4.1 Background and Aims

Gluten, the major protein of wheat, has been established as the causative agent in the development of coeliac disease, characterised by small intestinal injury and immunological activation.[199] Gluten has also been implicated as a causal factor in the development of chronic functional gastrointestinal symptoms similar to those classified as irritable bowel syndrome (IBS).[200] In fact, non-coeliac gluten sensitivity (NCGS) has been proposed as a defined entity in which IBS-like symptoms markedly improve on a gluten-free diet, but coeliac disease has been excluded.[97, 104] However, understanding of this putative entity remains poor and controversial. Several descriptions of it have included patients with intraepithelial lymphocytosis in the duodenum and evidence of immunological activation that potentially might be part of the spectrum of coeliac disease.[119, 201-203] Furthermore, descriptions of the entity often do not take into account the potential for symptomatic improvement by reduction of other symptom inducing components of wheat, especially fructans, one of the short-chain poorly absorbed carbohydrates (FODMAPs [Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols]).[86]

A full description of the elimination and challenge trials conducted within NCGS cohorts are provided in Chapter 1 but two recent studies conducted by our own group (Department of Gastroenterology, Monash University) have challenged NCGS patients on a gluten-free diet, who had normal duodenal biopsies and/or were HLA-DQ2/8 negative, with carbohydrate-

deplete gluten in a blinded fashion.[86, 125] The first, a parallel group study found that patients were significantly worse with gluten for overall symptoms, pain, bloating, satisfaction with stool consistency and tiredness. No clues to the mechanisms were elucidated.[125] The second (comprising two back-to-back challenges) used a cross-over design on a low FODMAP dietary background and could find no evidence of gluten-specific triggering of symptoms in such patients.[86] Interestingly, participants opted to continue following a gluten-free diet upon study completion as they subjectively described feeling better.

Psychological health has been extensively explored within the coeliac population, where several neurological and psychiatric illnesses are common.[204, 205] Among them, a high prevalence of anxiety and depression has been reported in treated patients.[138, 206-209] In the majority of cases, this anxiety and depression is reported particularly as a personality trait whereby the behaviours and feelings are consistent and relatively enduring.[138, 206-209] However, a high prevalence of transitory mood state has been reported in untreated coeliac disease patients.[206] Interestingly, reversal of this effect was observed after one year on a gluten-free diet.[206] This observed change in temporary predisposition in coeliac patients following the removal of gluten may be similar in patients with NCGS.

The relationship between psychological health and NCGS has seldom been studied. One recent publication explored trait anxiety and depression in patients with NCGS where patients consumed four slices of gluten-containing white bread per day for three days.[138] Results revealed that patients had higher trait anxiety and depression scores at baseline compared to healthy controls but that these scores did not differ significantly following the consumption of gluten. Mood state was not explored, although mood change and other extra-intestinal symptoms including forgetfulness were common symptoms related to gluten intake reported by recently surveyed NCGS participants.[210] It may be that the reversal of psychological state

amongst this entity, not personality trait that contributes to why such patients feel better when following a gluten-free diet despite the continuation of gastrointestinal symptoms.

This concept was investigated in the current exploratory study of participants with IBS in whom coeliac disease had been excluded and a gluten-free diet had led to self-reported improvement in gastrointestinal symptoms. It was hypothesised that the ingestion of gluten by participants with NCGS would have a significant effect on psychological state and not necessarily on gastrointestinal symptoms.

4.2 Materials and Methods

4.2.1 Participants

Participants were recruited from a preceding study in which subjects with self-reported NCGS were challenged with diets containing varying amounts of gluten.[86] They were all invited to participate in the current exploratory study aimed in part to determine the effect of gluten on psychological state. The time between participation in the two studies varied from 8 to 17 months, so inclusion/exclusion criteria were re-confirmed (see Chapter 3, section 3.1).

4.2.2 Protocol

The exploratory study was a randomised, placebo-controlled, double-blind, cross-over dietary rechallenge study. Participants were assigned to a computer-generated randomisation sequence, held by an independent observer. Upon enrollment, participants were educated on a diet low in FODMAPs and it was asked that they continue a gluten-free diet low in FODMAPs for the duration of the study. After a three-day baseline period, participants then

received one of the three dietary challenges consecutively for three days, followed by a minimum three-day and maximum 14-day washout period between each diet (Figure 4.1). Participants were required to report symptom resolution before crossing over to the next diet. Challenge food was supplemented with gluten, whey or not supplemented (placebo). Whey protein isolate was used as a protein control and has a supposed rapid digestibility in the gut.[211, 212] All meals and snacks were supplied to participants (labeled "Diet A", "Diet B" and Diet C") during dietary challenges. Measurements included psychological state, cortisol secretion, and gastrointestinal symptoms. Psychological state and cortisol secretion were assessed prior to (baseline) and on day 3 of each dietary challenge. Gastrointestinal symptoms were assessed daily for the study duration. Participants unable to continue a treatment due to intolerable gastrointestinal symptoms were permitted to cease the study food of that particular arm, but continue to collect data as per day three and collect symptom and food diaries when not on the study diet. Participants then resumed any remaining treatment arms following the allocated washout period.

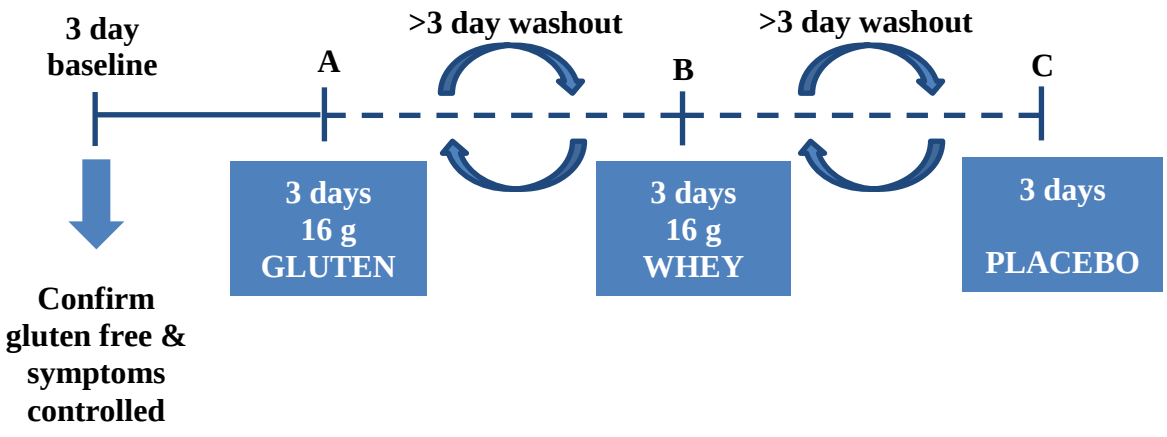


Figure 4.1. Study protocol outline

4.2.3 Study food preparation

Study food was prepared according to Australian and New Zealand Food Standards. All study food was supplied and other potential inducers of symptoms were minimised by the food being gluten-free, dairy-free, low FODMAP and low in food chemical content. Resources describing the content of natural food chemicals including salicylates, amines and glutamate were used in accordance with the approach described by the Allergy Unit, Royal Prince Alfred Hospital (RPAH; Sydney, Australia) Elimination Diet.[213] During dietary challenges food was supplemented with 16 g/day vital wheat gluten, 16 g/day whey protein isolate or no additional protein (placebo). All meals and snacks were provided but participants were asked to provide perishable items themselves. Guidance was given as to which perishable foodstuffs were appropriate. The meal plan was adequate in macronutrients, micronutrients and provided 8 MJ energy daily. Meals and snacks were similar in taste, texture and appearance across the three treatment conditions, confirmed with preliminary testing in five healthy people where the food containing the gluten could not be differentiated from those that did not. Participants with larger energy requirements were provided with additional low FODMAP, gluten-free, low-chemical, dairy-free meals and snacks. All food was prepared in the kitchens of Monash University. Meals were packed individually in food-grade foil containers or bags sealed with a cryovac Orved VM-12 vacuum sealer (Orved® Musile di Piave, Italy) to extend shelf life and quality of the food. Food was stored frozen at -20° C. All food was provided free of charge.

The gluten used was commercially available, carbohydrate-depleted wheat gluten (Vital Wheat Gluten; Manildra Group, Manildra, NSW, Australia). Protein characteristics were completed by Dr Ferenc Békés and determined using reversed-phased high-performance liquid chromatography (HPLC) and size-exclusion HPLC. Results of protein analysis are

shown in Table 4.1. The whey protein isolate (Resource® Beneprotein Instant Protein Powder; Nestle Healthcare Nutrition, Inc., Minneapolis, USA) was lactose-free and low FODMAP, as measured by methodologies described previously.[214, 215]

Table 4.1. Percentage distribution of the gluten used shown on reversed-phase high-performance liquid chromatography (HPLC) and percentage distribution of the protein content on the basis of size-exclusion HPLC

	Component	% content
Overall composition	Protein	75%
	Crude fibre	1.8%
	Lipid	7%
	Starch	15.6%
	Ash	0.6%
Protein Distribution	Gliadin	40%
	Glutenin	53.4%
	Non-gluten protein (albumin / globulin)	6.6%

4.2.4. Measurements

4.2.4.1. Adherence to the gluten-free diet

Adherence to the gluten-free diet was assessed by specific questioning and using a flow chart to give a numerical score.[216] The flow chart is based on four simple questions with a five-level score (0-IV), which from a clinical point of view can be grouped into three levels of classification (Table 4.2). This was crosschecked with the assessment of participants' baseline

three-day food diary. Adherence to dietary challenges was assessed by entries into a tick-box diary and unused food was counted at the end of each dietary challenge. Participants were also asked to document any additional foods consumed. These were evaluated by an experienced dietitian for gluten and FODMAP content using the Monash University FODMAP database.

Table 4.2. Classification for the evaluation of gluten-free diet compliance

Score	Classification
0 or I	Do not follow a strict gluten-free diet
II	Follow a gluten-free diet but with important errors that require correction
III or IV	Follow a strict gluten-free diet

4.2.4.2. Gastrointestinal symptoms

Gastrointestinal symptoms were assessed using a 100 mm visual analogue scale (VAS) as described in Chapter 3, Section 3.4.1. The VAS was completed daily for the duration of the study. Daily VAS scores were combined to obtain an average over the baseline period and each of the three dietary challenges (gluten, whey and placebo). Clinical significant change of symptoms was arbitrarily defined as a change of at least 20 mm.

4.2.4.3. Psychological indices

Psychological state was assessed using the State Trait Personality Inventory (STPI) and is described in Chapter 3, Section 3.4.2. Only anxiety and depression subscales of the STPI were calculated for the current study. The STPI was completed during the baseline run-in period and on day 3 of each dietary challenge.

4.2.4.4. Salivary cortisol

Salivary cortisol secretion was used as a biomarker of stress. Collection instructions were provided to participants to ensure influential factors were controlled, including sample collection taken at standardised times. Saliva samples were obtained during the baseline period and on day 3 of each dietary challenge. The results were expressed as micrograms per decilitre (μg/dl). More detail on salivary cortisol collection methodology is provided in Chapter 3, Section 3.4.8.

4.2.5. End-points

The primary end-point was the change in psychological state as measured on the STPI from the baseline run-in period to that at the end of the study treatment period. Secondary end-points included the change and comparison of salivary cortisol compared with the baseline run-in period, and the change in overall and individual gastrointestinal symptom scores.

4.2.6 Statistical analyses

A linear mixed model analysis for cross-over designs was undertaken for each of the mental health indices separately, with dietary condition and order of testing treated as fixed factors and participants as the random factor. A number of models and covariance structures were fitted to the data. The comparison of salivary cortisol across dietary challenges was assessed by repeated measures ANOVA. The gastrointestinal symptom data were not normally distributed across dietary challenges and so were analysed using the Friedman test. Where required, pairwise comparisons between each of the challenge conditions were undertaken and Type 1 error was controlled by the use of Hochberg False Discovery Rate (FDR) test.[217, 218] Two-tailed P-values at or below 0.05 were considered statistically significant.

4.3 Results

4.3.1 Participants

Twenty-two participants agreed to participate in the study. The subjects who participated in the preceding study, but were not able to return did so due to pregnancy/breast feeding (n = 3), travel (n = 3), time constraints (n = 8) or being unwilling to eat gluten (n = 4). Details of recruited participants are provided in Table 4.3. Briefly, they were aged 24-62 years and five were male. Predominant bowel habits were diarrhoea in eight, constipation in ten and alternating in four. Twelve participants were HLA-DQ2 and/or HLA-DQ8 positive. Twenty-one participants reported symptom resolution during the allocated washout period. One participant had an extended washout period (11.5 weeks) between her second and third diet treatment, but was included since her data did not influence the result of any analysis.

Table 4.3. Participant characteristics at baseline

Participant characteristics	
Number of participants	22
Gender	5 male
Median age (range)	48 (24-62) years
Predominant bowel habit	
Diarrhoea	36%
Constipation	46%
Mixed/Alternating	18%
HLA type	
DQ2 or DQ8 positive	55%

4.3.2 Dietary compliance

All participants undertook the three dietary challenges. One patient ceased the whey challenge (treatment first received) prematurely because of intolerable symptoms after lunch on day 2. Data continued to be collected as per day 3.

Nearly all meals (99, 96 and 99%) were consumed in the gluten, whey and placebo challenges, respectively. All patients adhered to the gluten-free, low FODMAP diet. Seven participants consumed snacks high in natural food chemicals (e.g., one banana), but this did not differ across the dietary challenges within participants.

4.3.3 Effect on psychological state

Two participants were considered outliers at baseline (>2 SD from the mean) and their responses were removed from analysis. A linear mixed model for cross-over designs was applied to the remaining 20 participants with fixed effects of condition (challenge) and order (sequence), as well as the interaction between these two factors, and participants entered as random effects. The model of best fit to the data for each of the STPI state and trait variables, determined by the lowest -2 restricted log likelihood value, was that with repeated measures on condition and unstructured covariance matrix.

The tests of fixed effects revealed that condition (F=5.994, p=0.011) had a significant effect on STPI state depression score, but order (F=3.036, p=0.06) did not. Further, no significant interaction between condition and order was observed (F=1.623, p=0.20). Exploration of the main effect of challenge condition revealed that state depression was significantly higher in the gluten condition than the placebo condition (p=0.010) (Table 4.4, Figure 4.2). Figure 4.3 shows paired participant STPI state depression scores across the three conditions. This

increase in STPI state depression score following gluten ingestion compared to placebo met criteria for statistical significance after controlling the FDR (p=0.017). Effect size between gluten and placebo (d=0.64) was moderate. Although state depression was higher in the gluten condition than in the whey condition this difference failed to reach significance (p=0.07, d=0.43) (Table 4.4, Figure 4.2). There was no difference between whey and placebo conditions (p=0.61, d=0.12) (Table 4.4, Figure 4.2). Eighteen participants (90%) had equal (n=4) or higher (n=14) STPI state depression scores on gluten compared to placebo. Condition had no effect on the other STPI state or trait indices (Table 4.4).

Table 4.4. Comparison of STPI state and trait indices for placebo, whey and gluten dietary challenges. Gluten was associated with a significantly higher state STPI depression score across the three groups. Data shown as mean, standard deviation (SD) and effect size (η_p^2).

STPI		Mean (SD)			*P*-value	η_p^2
		Placebo	Whey	Gluten		
State Indices	Depression	19.20 (3.82)	19.75 (4.98)	21.45 (4.86)	**0.011**	**0.38**
	Anxiety	17.55 (4.36)	17.05 (3.97)	18.05 (5.09)	0.65	0.05
	Curiosity	22.65 (7.86)	23.35 (6.48)	21.40 (6.31)	0.25	0.06
	Anger	10.80 (1.28)	10.70 (1.53)	11.25 (2.02)	0.56	0.04
Trait Indices	Depression	18.45 (4.65)	19.85 (5.40)	18.80 (6.18)	0.54	0.02
	Anxiety	18.30 (4.24)	18.80 (3.44)	18.45 (3.95)	0.21	0.04
	Curiosity	27.95 (6.71)	27.05 (7.22)	27.75 (7.45)	0.70	0.01
	Anger	15.90 (5.43)	14.80 (4.19)	15.35 (4.77)	0.11	0.06

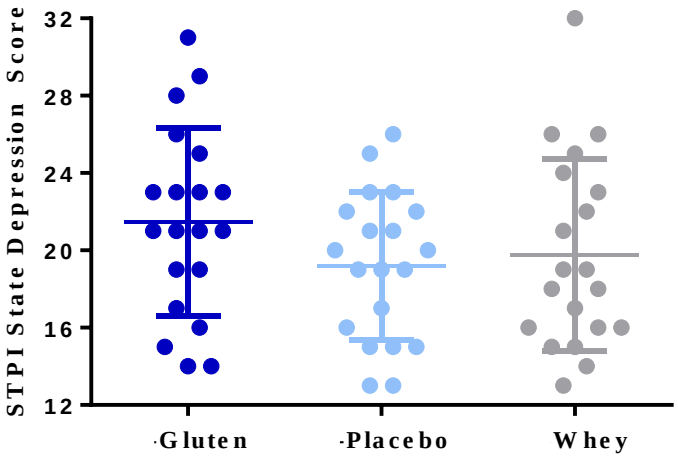


Figure 4.2. STPI state depression scores during the gluten, whey and placebo dietary challenges. A linear mixed model of fixed effects revealed that condition had a significant effect on STPI state depression score. Pairwise sub-analyses revealed that state depression was significantly higher in the gluten condition than placebo. No differences were found between gluten and placebo or placebo and whey. STPI: State Trait Personality Inventory

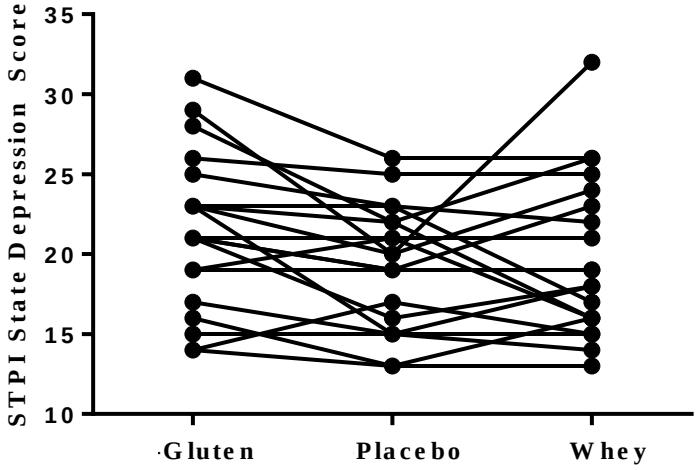


Figure 4.3. Paired STPI state depression scores across the gluten, whey and placebo dietary challenges. State depression scores were significantly higher in the gluten condition compared to placebo. No significant differences were found between gluten and whey or placebo and whey. STPI: State Trait Personality Inventory

4.3.4 Effect on salivary cortisol concentrations

One participant produced insufficient saliva for analysis and two participants failed to provide salivary samples on one or more dietary challenges. Their results were removed from analysis. No differences were found in salivary cortisol levels between or during the dietary challenges, $F(2,36)=1.17$, $p=0.31$ (Figure 4.4).

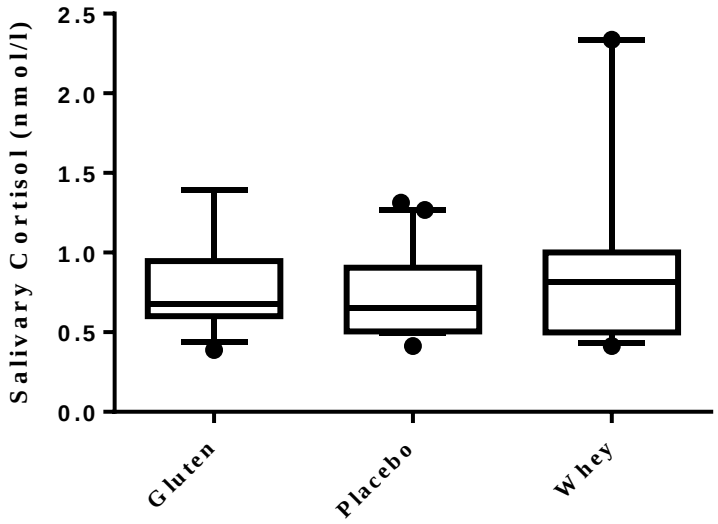


Figure 4.4. Salivary cortisol concentrations during the gluten, whey and placebo dietary challenges. The comparison of salivary cortisol across dietary challenges was assessed by repeated measures ANOVA. No differences were seen across the gluten, whey or placebo dietary challenges. Data shown as box and whisker plots (bar = median, box = interquartile range, whiskers = 10-90 percentile)

4.3.5 Effect on gastrointestinal symptoms

Comprehensive descriptions of gastrointestinal symptom results were published as part of a preceding thesis.[98] No differences were identified across the dietary challenges for overall gastrointestinal symptoms (Figure 4.5) or for individual symptoms. The order of the dietary challenges was associated with degree of symptomatic response, with the first intervention being associated with greater symptomatic changes than the second or third challenges, regardless of whether it contained gluten, whey or placebo (Figure 4.6).[86]

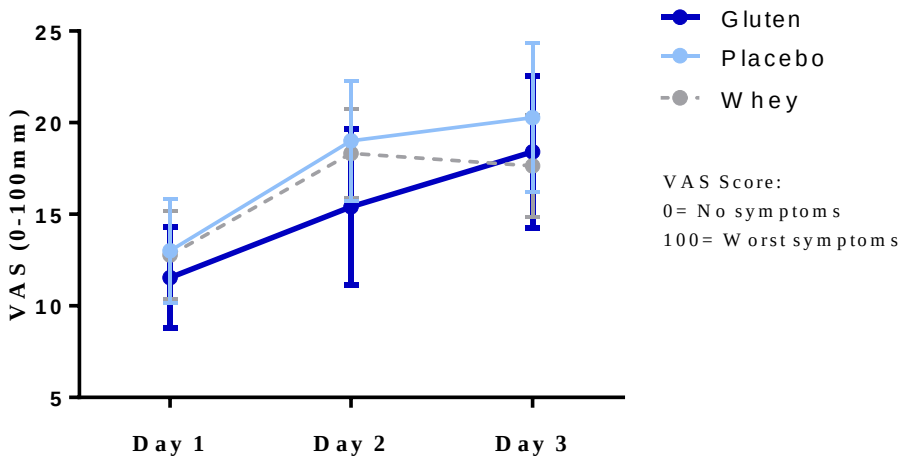


Figure 4.5. Overall gastrointestinal symptoms over the three-day study period during the gluten, whey and placebo dietary challenges. Gastrointestinal symptom data was analysed using the Friedman test. There were no significant differences for overall gastrointestinal symptoms during the gluten, whey or placebo dietary challenges. Data shown represent the mean ± SEM. VAS = Visual Analogue Scale

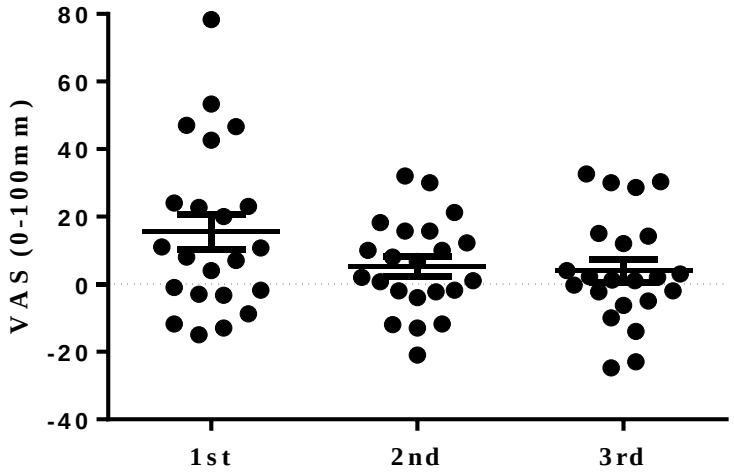


Figure 4.6. Change in overall symptom severity grouped in order of treatment arm received. The differences were compared by repeated measures ANOVA (p=.04). Differences were also compared between each group by a paired t-test (*p*=.07 between 1st and 2nd; *p*=.06 between 1st and 3rd; *p*=0.71 between 2nd and 3rd). VAS = Visual Analogue Scale

4.4 Discussion

The term NCGS has been defined as one or more of a variety of immunological, morphological or symptomatic manifestations that are precipitated by the ingestion of gluten in people in whom coeliac disease has been excluded.[219] Despite the supposedly sound definition, the NCGS entity is complex. While initial work was suggestive of a gluten-specific effect on gastrointestinal symptoms amongst this entity,[125] the subsequent double-blind, placebo-control, randomised, cross-over studies, including the present study, have failed to observe this interaction.[7] We have hypothesised that the reason why patients might feel better on the gluten-free diet is that gluten is having a detrimental effect on their psychological state and the cessation of gluten improves their well-being rather than the gastrointestinal

symptoms *per se*. Indeed, short-term exposure to gluten appeared to specifically induce current feelings of depression in the present study.

The observed change in current feelings of depression is appreciable and supported by a recent double-blind placebo-controlled, cross-over study in which depression was assessed using a 3 point Likert-scale.[132] According to Spielberger's norms for state depression scores,[186] the mean scores of participants in this study went from being largely "neutral depressive" in the placebo condition to being "mild depressive" following the consumption of gluten.[186] However, Spielberger's norms are based on clinical populations whereas patients in the current study had not been diagnosed as clinically depressed. That said, this observed change over a three-day intervention is plausible. Personality and mental states are widely accepted as being transitory and rapidly changing, often from moment to moment.[220] If such a change is indeed a gluten-specific effect, the mechanisms involved require elucidation.

One explanation might be alterations in cortisol secretion as circulating concentrations of cortisol are greater with negative affect (i.e., aversive moods such as anxiety, hostility and depression). However, the degree to which this association is due to stable individual differences (i.e., traits) or transient differences in affect (i.e., states) remains unclear.[221] There is currently no evidence that gluten ingestion can stimulate cortisol secretion, but this link has been seldom studied.[222] In the current study, cortisol concentrations during each dietary period were measured in saliva, a technique that has been shown to provide a feasible, accurate, and practical alternative to blood determinations.[223, 224] These were similar across all dietary treatments, indicating that state depression may not be as closely associated with cortisol secretion compared to trait depression, as previously described.[221]

A second potential mechanism is via alteration of brain serotonin (5-hydroxy-tryptophan, 5-HT). Decreased brain 5-HT concentration has been long suggested as a cause of

depression.[225] The synthesis of 5-HT in the brain is dependent on the availability of its amino acid precursor, tryptophan. Interestingly, recent work has identified a link between protein ingestion, tryptophan production and concentrations of 5-HT in the brain.[226] In this study, rats consuming food supplemented with food-grade wheat for two hours had modest reductions in concentration of tryptophan in the brain suggesting that 5-HT pathways are remarkably sensitive to various proteins present in food.[226] Whether carbohydrate-depleted gluten results in reductions of tryptophan concentration in the human brain requires further exploration. Nonetheless, serotonergic dysfunction due to impaired availability of tryptophan has been shown to play a role in various psychological conditions including depression.[227-230]

A third explanation involves the so-called gluten "exorphins". These opioid peptides derived from partially digested food proteins including gluten can modulate intestinal function,[222] and can cross the blood-brain barrier and interfere with pain-inhibitory systems, emotionality and memory processes by modulating other hormonal or neurotransmitter systems via the opioid receptors as well as endogenous opioid peptides in the central nervous system (CNS).[231] Such a possibility could be investigated by, for example, the concomitant use of naloxone to block opioid receptors.

A fourth possibility might involve gluten-mediated changes in gut microbiota. Several studies have reported intestinal dysbiosis in patients with coeliac disease.[232] Interestingly, some of the alterations in gut microbiota are restored after adherence to a gluten-free diet.[232] This suggests that these changes are secondary consequences of the disease and perhaps directly related to the consumption of gluten. Evidence supporting an important influence of gut microbiota on emotional behaviour and underlying brain mechanisms is well established in adult rodents[233-235] and is emerging in humans. A recent study has provided first evidence that probiotics can modulate the activity of brain regions involved in processing emotion and sensation in adult

women.[236] Whether three days is sufficient to induce changes in microbiota is uncertain, but this hypothesis requires further investigation in the NCGS population.

It is important to note that several key design issues may have adversely influenced the results. These limitations have been discussed fully with relation to the end-point of gastrointestinal symptoms.[86] With respect to psychological effects, there are four main limitations. First, it suffers from the issues associated with most pilot studies. The number of patients studied is relatively small and the psychological end-points used were restricted to one scale. Secondly, the duration of the dietary challenge might be considered too short to observe the maximum change in psychological states. However, a three-day gluten challenge has been shown to be long enough to capture the greatest magnitude of change in gastrointestinal symptoms amongst this entity[138] and psychological states are known to be transitory and rapidly changing.[220] Thirdly, the use of a cross-over design within the IBS population has been criticised mainly on the basis of the possibility of carry-over effects and on the undue influence that drop-outs might have on the analysis.[237] As suggested in Chapter 1, studies within NCGS populations may be better to employ parallel arm designs thus negating any carry over effects. While gastrointestinal symptoms had returned to baseline levels before proceeding with the next dietary challenge, an order effect was observed, with significantly more severe symptoms being induced with the first dietary challenge.[86] However, there was no evidence of an order effect on the psychological indices used and the indices were similar in patients after the whey and placebo arms. Furthermore, all participants completed the study. Finally, while adherence to the dietary intervention was ensured using the gold-standard of providing all food, such provision might differ substantially from a participants' usual dietary habits, with consequent increase in the participants' anxiety and negative responses to the intervention.[238] Importantly no difference was observed in anxiety or salivary cortisol levels

across the three dietary challenges and depression was only associated with the ingestion of gluten.

4.5. Conclusions and Future Directions

In conclusion, the findings of gluten-specific acute changes in current feelings of depression, with no effects on trait indicates, provide a clue that the improvement reported by participants may be in the perception of their general well-being rather than in gastrointestinal symptoms. Such an association requires a longer and more detailed examination where a greater number of psychological indices are used. A further evaluation was completed as part of this thesis and is reported in Chapter 5.

Chapter 5 - The effect of gluten on psychological indices, quality of life and fatigue in subjects with non-coeliac gluten sensitivity

5.1. Background and Aims

Non-coeliac gluten sensitivity (NCGS) is believed to be characterised by both gastrointestinal symptoms and extraintestinal manifestations whereby improvements are observed following the removal of gluten from the diet and worsening of symptoms occurs with the reintroduction of gluten.[97, 104] To date, the majority of elimination and challenge trials within NCGS populations have focused on gastrointestinal symptoms. In Chapter 4, however, short-term exposure to gluten was shown to specifically induce current feelings of depression with no effect on other indices or on emotional disposition.[239] The magnitude of the overall effect of gluten was appreciable in that participants' mean scores went from being largely 'neutral depressive' in the placebo condition to being 'mild depressive' following the consumption of gluten. That said, this observed change was over a short 3-day intervention and determined using only one psychological measure. Notably gastrointestinal symptoms were induced similarly across gluten and placebo dietary challenges. Gluten-induced worsening of gastrointestinal symptoms has been described in some[125, 130-133] but not all[86, 134] previous randomised control trial (RCT) investigations.

The lack of gluten-specific gastrointestinal symptom effect, presented in Chapter 4, confirms former suggestions that gluten may not be a specific trigger of functional gastrointestinal symptoms amongst the vast majority of the NCGS entity as was initially thought[86, 134] and highlights the importance of confirming the previous findings related to the induction of

depression before widespread circulation of these results is considered. This is particularly important given the potential impact of these findings with regard to not only patient treatment and but also food industry practices, given the steady increase in the gluten-free food market in recent years. As such, the importance of consistent results from well-designed studies is imperative to our understanding of this entity. With these findings, strong and reliable messages can be dispersed to both the community and food industry.

Therefore, the aim of the current chapter was to provide a longer and more detailed examination of the effects of gluten in patients with NCGS where endpoints including a greater number of psychological indices that assessed not only depression but also anxiety and cognitive function as well as quality of life (QOL) and fatigue were employed. Gastrointestinal symptoms were also considered. It was hypothesised that the ingestion of gluten by participants with NCGS would have a significant effect on psychological state and not necessarily on gastrointestinal symptoms, as preceding reports suggest.[86, 134, 239]

5.2. Materials and Methods

5.2.1. Participants

Participants were recruited through newspaper advertisements in metropolitan Melbourne, on social media, at the Functional Gastrointestinal Clinic at the Alfred Hospital, and through the Monash University Department of Gastroenterology webpage. All participants were included if they met inclusion/exclusion criteria as previously described (Chapter 3, Section 3.1) with the exception that patients did not need to fulfil Rome III criteria for IBS, since this is not part of the consensus definition for NCGS. NCGS is reportedly characterised by both

gastrointestinal and extraintestinal symptoms and given that gluten-specific changes were only previously observed for depression in Chapter 4, with no effects on gastrointestinal symptoms, it was decided that this criterion may be prohibitive to observing potential psychological effects. Therefore, there was no stipulation as to which symptoms (gastrointestinal or extraintestinal) had to reportedly improve on a gluten-free diet and worsen following the consumption of gluten.

5.2.2. Protocol

A double-blind, placebo-controlled, randomized cross-over study of 8 weeks duration was undertaken. Participants were assigned to a computer-generated randomisation sequence, held by an independent observer. It was a requirement of the study that all participants continue their usual gluten-free diet for the duration of the study. Upon enrollment, participants underwent a 2-week baseline run-in period before receiving one of two challenge bars daily for 14 days, followed by a 14-day washout period between each bar (Figure 5.1). Participants were required to report symptomatic resolution during the washout period before crossing over to the next challenge. Challenge bars were supplemented with gluten or not supplemented (placebo). Measurements included questionnaires that assessed psychological indices, QOL, fatigue and gastrointestinal symptoms and were assessed prior to (baseline) and on day 14 of each challenge. Participants unable to continue either challenge due to intolerable symptoms were permitted to cease that particular challenge, but continue to collect data as per day 14. Participants then completed the remaining challenge, if applicable.

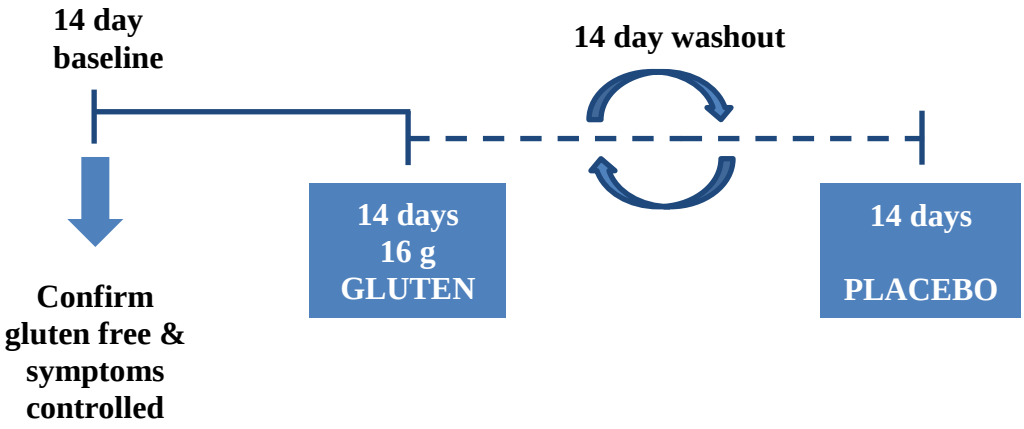


Figure 5.1. Study protocol outline

5.2.3. Challenge bar preparation

Challenge bars were prepared according to Australian and New Zealand food standards and the composition was based on a low FODMAP (fermentable, poorly absorbed short-chain carbohydrates) muesli bar formulation previously developed by Monash University. Bars were supplied at no cost to the participant and other potential inducers of symptoms were minimised by the bars being gluten-free, dairy-free and low FODMAP. During challenges, bars were supplemented with 16 g/day vital wheat gluten or no additional protein (placebo). Bars were similar in taste, texture and appearance, confirmed in preliminary testing in 8 healthy people where the bars containing the gluten could not be differentiated from those that did not.

The gluten used was commercially available, carbohydrate-depleted wheat gluten (Vital Wheat Gluten; Manildra Group, Manildra, NSW, Australia). Protein characteristics were completed by Dr Ferenc Békés and determined using reversed-phased high-performance

liquid chromatography (HPLC) and size-exclusion HPLC. Results of protein analysis are shown in Table 5.1.

5.2.4. Background diet

Participants were provided with a fourteen-day recording diary and instructions for its completion. Recorded information included the type (including brands and/or specific recipes) and the quantity of food consumed. Recorded information was then checked at the end of the baseline run-in period and assessed for intake of gluten-containing foods (dietary compliance). Once dietary compliance was established, participants were asked to consume this exact baseline diet during each of the challenges. This was done to ensure consistency across challenges.

Table 5.1. Contents of the gluten-enriched preparation (vital wheat gluten) used shown on reversed-phased high-performance liquid chromatography (HPLC) and percentage distribution of the protein content on the basis of size-exclusion HPLC

	Component	% content
Overall composition	Protein	75%
	Crude fibre	1.8%
	Lipid	7%
	Starch	15.6%
	Ash	0.6%
Protein Distribution	Gliadin	40%
	Glutenin	53.4%
	Non-gluten protein (albumin / globulin)	6.6%

5.2.5. Measurements

5.2.5.1. Adherence to the gluten-free diet

Adherence to the gluten-free diet was assessed by specific questioning and using a flow chart to give a numerical score.[216] Details of this flow chart are provided in Chapter 4, Section 4.2.4.1. This was cross-checked with the assessment of participants' 14-day food diary. Adherence to the gluten-free diet was arbitrarily defined as no more than three accidental exposures to gluten-containing foods throughout the duration of the study.

5.2.5.2. Consumption of challenge bars

Participants were asked to document the consumption of all challenge bars in the corresponding fourteen-day food diary and were instructed to return all uneaten bars at the end of each challenge period so adherence to the study protocol could be assessed.

5.2.5.3. Psychological indices

Psychological state was assessed using the State Trait Personality Inventory (STPI)[186] and the Depression Anxiety and Stress Scale (DASS)[187] as described in Chapter 3, Sections 3.4.2 and 3.4.3. Only anxiety and depression subscales of the STPI were calculated for the current study. The STPI and DASS were completed during the baseline period, on day 14 of the washout period and on day 14 of each challenge.

The Subtle Cognitive Impairment Test (SCIT) was used to assess cognitive function as described in Chapter 3, Section 3.4.5. The SCIT was completed during the baseline period, on day 14 of the washout period and on day 14 of each challenge. It was computer-based and required participants to attend the Department to complete that evaluation.

5.2.5.4. Quality of life

The IBS-QOL was used to determine disease-specific health-related QOL as described in Chapter 3, Section 3.4.6. The IBS-QOL was completed during the baseline period, on day 14 of the washout period and on day 14 of each challenge.

5.2.5.5. Fatigue

Severity of fatigue was evaluated by the Daily-Fatigue Impact Scale (D-FIS)[198] as described in Chapter 3, Section 3.4.7. The D-FIS was completed daily for the duration of the study. Daily D-FIS scores were combined to obtain an average over the baseline period, the washout period and each of the challenges.

5.2.5.6. Gastrointestinal symptoms

Gastrointestinal symptoms were assessed using a 100 mm visual analogue scale (VAS) as described in Chapter 3, Section 3.4.1. The VAS was completed daily for the duration of the study. Daily VAS scores were combined to obtain an average over the baseline period, the washout period and each of the challenges. Clinical significant change of symptoms was arbitrarily defined as a change of at least 20 mm.

5.2.6. End-points

The primary end-point was the difference in STPI state depression between gluten and placebo challenges. Secondary endpoints included the difference in all other STPI and DASS indices, cognitive function, QOL, fatigue, and gastrointestinal symptoms between gluten and placebo challenges and between gluten and baseline and baseline and placebo.

5.2.7. Statistical analysis

Power calculations were based on previous data from a 3-day assessment of gluten-specific effects[239] and allowed for drop-outs, missing data and error rate. Using the change in STPI state depression from baseline to the end of the intervention (day 14) as the primary end-point, 49 participants were required to detect a statistically significant difference between groups given an effect size of 0.2 with 80% power at a 2-sided 5% significance level. A linear mixed model analysis for cross-over design was undertaken for each of the psychological indices, QOL, fatigue and gastrointestinal symptom measures separately, with all four assessment times (baseline, washout, gluten & placebo) and the order of the gluten and placebo challenges treated as fixed factors and participants as the random factor. A number of models and unstructured covariance models were fitted to the data. Two-tailed *p*-values at or below 0.05 were considered statistically significant.

5.3. Results

5.3.1. Participants

Participant recruitment and flow is shown in Figure 5.2. Seventy-one individuals responded to advertisements but only 28 fulfilled the inclusion/exclusion criteria and were enrolled into the study. Nine participants withdrew prior to the baseline run-in period. One participant was on a waiting-list for an abdominoplasty and was called for treatment, one received a diagnosis of ovarian cysts, one revealed a previous diagnosis of diverticular disease, one had not been appropriately investigated for coeliac disease, two had family tragedies that resulted in their inability to participate and two claimed it was the wrong timing due to personal reasons. A

further two participants were lost to contact. The remaining two participants started but did not complete the study, were lost to contact and never returned the food diaries or questionnaires. Due to time constraints and other logistic issues, the study was terminated early when only 16 participants had completed the study with evaluable data. The characteristics these 16 participants are shown in Table 5.2. Of the participants who completed the study, 11 resided in Melbourne and 5 were from interstate (3 from New South Wales, 1 from Queensland and 1 from South Australia). As participants needed to come to the laptop computer that contained the SCIT software, only 6 participants undertook this aspect of the study.

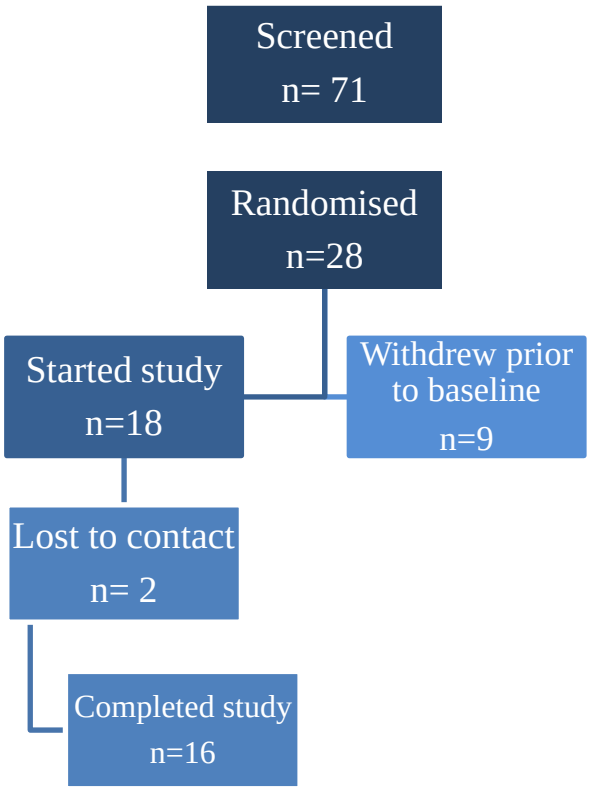


Figure 5.2. Participant recruitment and flow

Table 5.2. Participant characteristics at baseline

Participant characteristics	
Number of participants	16
Gender	6 male
Median age (range)	51 (37-71) years
HLA type	
DQ2 or DQ8 positive	44%

5.3.2. Dietary compliance

Sixteen participants undertook both challenge periods involving the gluten-free and gluten-containing bars. One patient ceased the gluten challenge prematurely (day 7) because of intolerable symptoms and only collected data up until this time-point. The data were carried forward. Nearly all challenge bars provided were consumed in the gluten and placebo bar challenges (98% and 98%, respectively). Fourteen participants adhered to a gluten-free diet for the duration of the study. The remaining two participants inadvertently consumed gluten during the baseline run-in period but maintained a strict gluten-free diet throughout both challenges. Thus, all were gluten-free during the challenge periods.

5.3.3. Effect on psychological indices

5.3.3.1. State Trait Personality Inventory (STPI) and Depression Anxiety Stress Scale (DASS)

Differences between gluten and placebo challenges were not observed for STPI state depression ($F(1, 15)=0.67$, $p=0.61$), state anxiety ($F(1,15)=0.01$, $p=0.93$), trait depression ($F(1, 14)=0.02$, $p=0.90$) or trait anxiety ($F(1, 14)=.54$, $p=0.47$) (Figure 5.3) nor was there a

significant change for any STPI outcome compared with baseline (Figure 5.4). Similarly, no differences between challenges were observed for DASS depression ($F(1, 15)=0.08$, $p=0.78$), anxiety ($F(1,15)=0.62$, $p=0.44$) or stress ($F(1, 15)=0.49$, $p=0.50$) or compared with baseline (Figure 5.5). Treatment order effects were not detected.

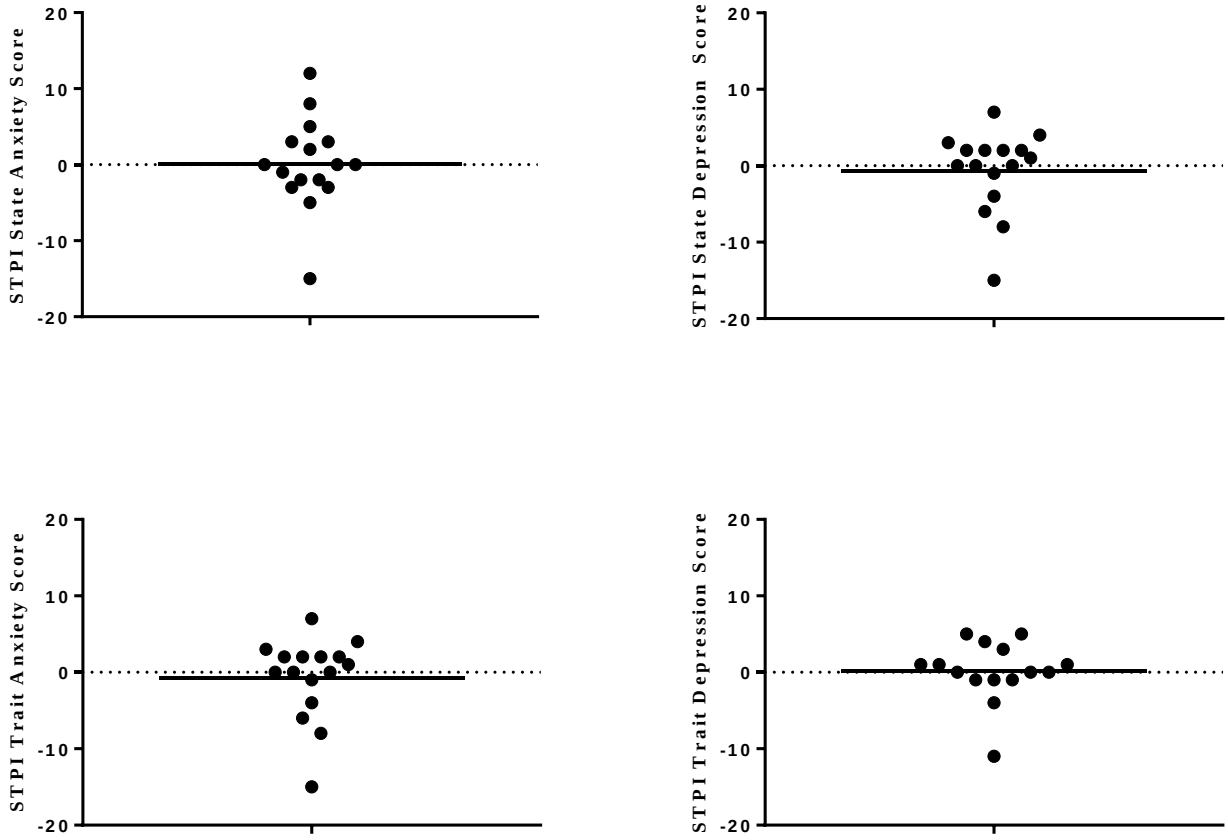


Figure 5.3. Difference in STPI indices from placebo to gluten. Data shown represent the mean. STPI = State Trait Personality Inventory

Figure 5.4. STPI state and trait depression and anxiety scores during the baseline period and gluten and placebo challenges. A linear mixed model of fixed effects revealed no effect of assessment time on STPI state or trait scores. STPI = State Trait Personality Inventory

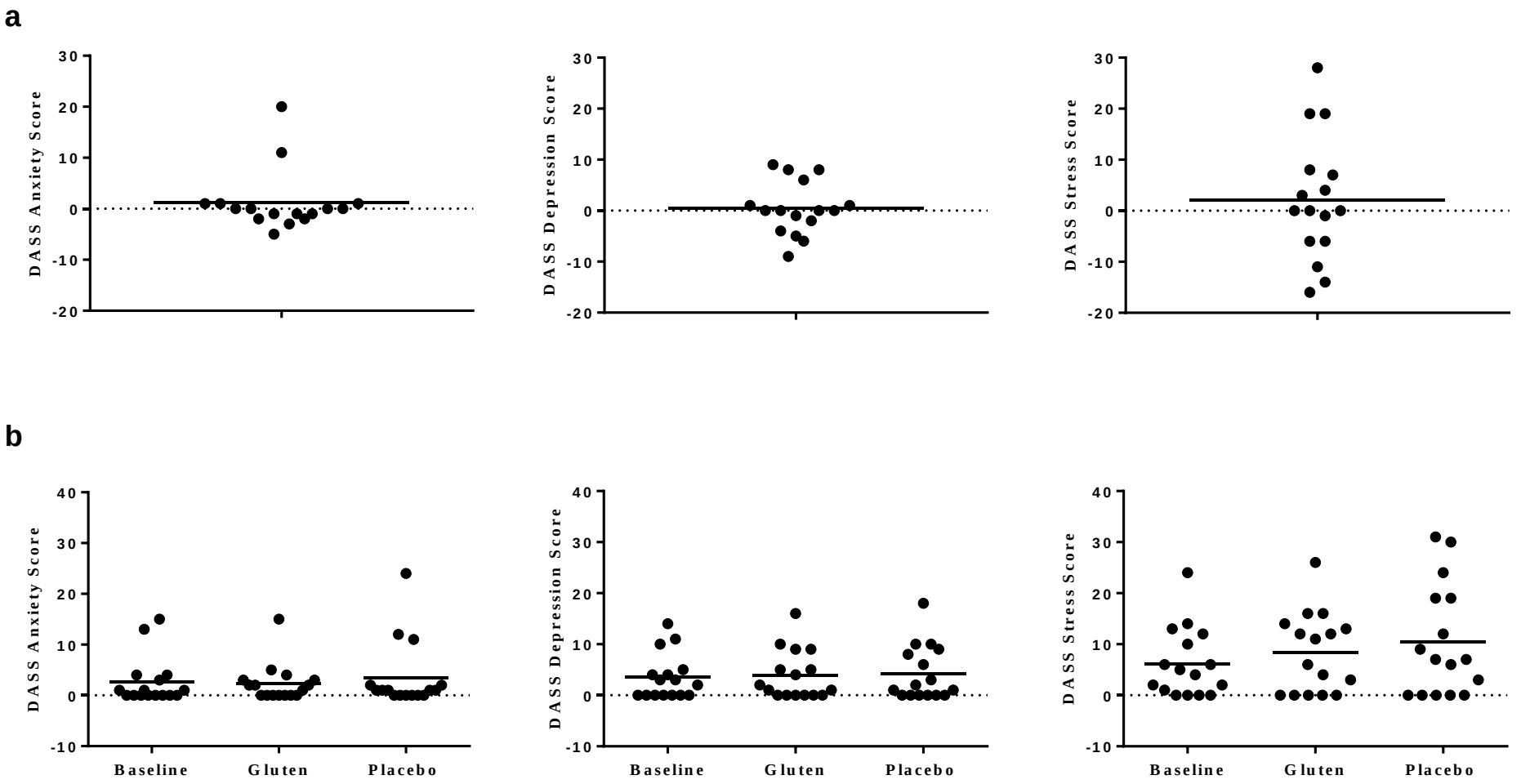


Figure 5.5. (a) Difference in DASS indices from placebo to gluten. Data shown represent the mean; (b) DASS indices during the baseline period and gluten and placebo challenges. A linear mixed model of fixed effects revealed no effect of assessment time on DASS scores. DASS = Depression Anxiety Stress Scale

5.3.3.2. Subtle Cognitive Impairment Test (SCIT)

Six participants completed the SCIT. The mean SCIT response times for the head of the SCIT distribution (SCIT-RTH; i.e., the collective mean response time for the four shortest exposure durations) for the gluten challenge were 80ms slower than those for placebo challenge (Figures 5.6 A and C). However, this difference failed to reach statistical significance ($F(1,5) = 2.99$, $p = 0.14$). SCIT-RTH following the gluten challenge was significantly slower than at baseline (42ms, $p = 0.05$) but there was no difference between baseline and the placebo challenge ($p = 0.29$). No significant effect of challenge order was observed ($F(1,4) = 0.01$, $p = 0.91$).

No significant difference between the gluten and placebo challenge conditions was found for mean SCIT response time for the tail of the SCIT distribution (SCIT-RTT: $F(1,5) = 2.62$, $p = 0.17$). No significant effect of challenge order was observed ($F(1,4) = 0.47$, $p = 0.53$), and no difference between baseline and either of the challenge conditions ($p < 0.05$).

A small but significant difference was observed in mean error rate for the head of the SCIT distribution (SCIT-EH) for the gluten challenge condition relative to the placebo challenge ($F(1,5) = 7.50$, $p = 0.04$) (Figure 5.6 B and D). However, no significant difference was found between gluten and placebo challenges for mean error rate in the tail of the SCIT distribution (SCIT-ET). For neither SCIT-EH nor SCIT-ET was any effect of the challenge order observed nor were there any differences found between the two challenges and baseline ($p < 0.05$).

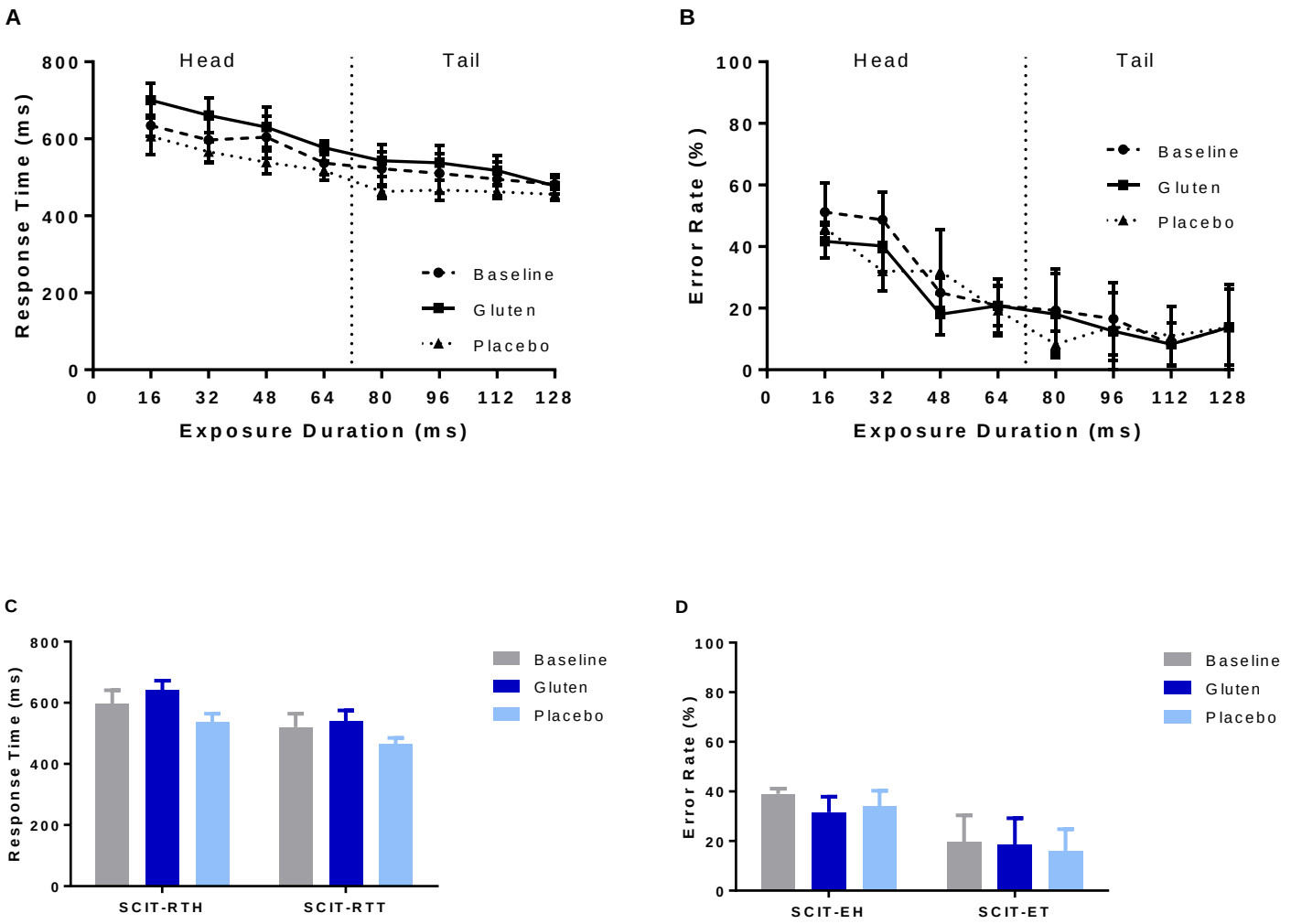


Figure 5.6. SCIT outcomes during baseline run-in period and gluten and placebo challenges. Mean SCIT response times (A) and mean percentage error (B) as a function of stimulus exposure duration. Mean head and tail response times (C) and mean head and tail errors (D) for baseline, gluten and placebo. SCIT = Subtle Cognitive Impairment Test

5.3.4. Effect on quality of life and fatigue

There were no differences in levels of QOL between challenges ($F(1,15)=0.02$, $p=0.90$) nor was there a significant change compared with baseline ($F(3,13)=0.47$, $p=0.71$) (Figure 5.7). Similarly, no differences in levels of fatigue were observed between the challenges ($F(1,15)=1.69$, $p=0.21$) or in comparison to baseline ($F(3,15)=2.32$, $p=0.07$) (Figure 5.8). No significant effect of challenge order was observed for QOL ($F(1,14) = 1.05$, $p = 0.32$) or fatigue ($F(1,14) = 0.001$, $p = 0.98$).

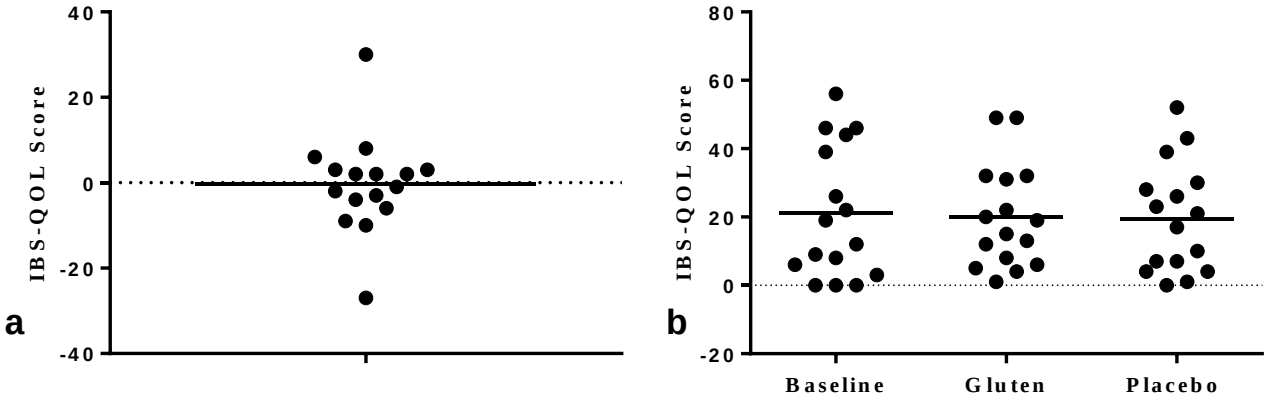


Figure 5.7. (a) Difference in IBS-QOL scores from placebo to gluten. Data shown represent the mean; (b) IBS-QOL scores during the baseline period and gluten and placebo challenges. A linear mixed model of fixed effects revealed no effect of assessment time on IBS-QOL scores. IBS-QOL = Irritable Bowel Syndrome Quality of Life

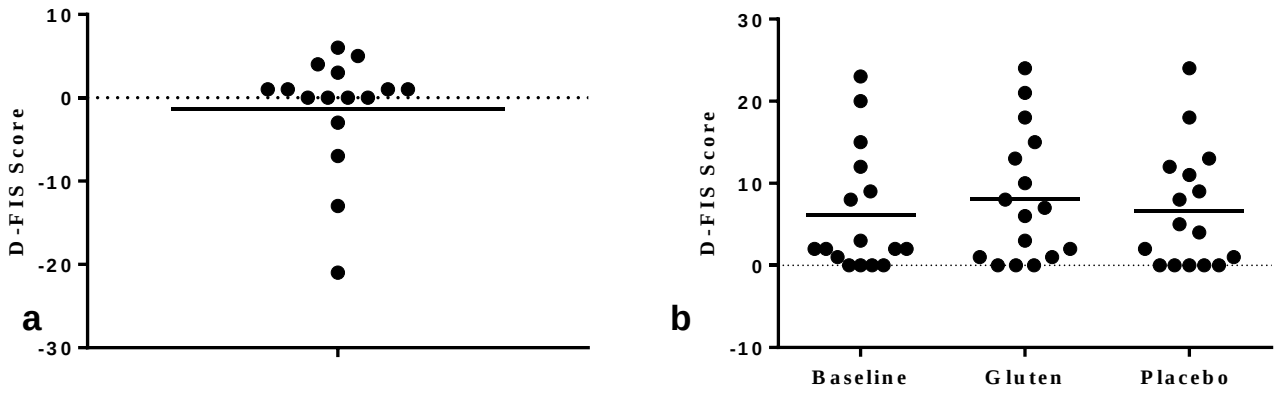


Figure 5.8. (a) D-FIS scores during the baseline period and gluten and placebo challenges. A linear mixed model of fixed effects revealed no effect of assessment time on D-FIS scores. (b) Paired D-FIS across gluten and placebo challenges. D-FIS = Daily Fatigue Impact Scale

5.3.5. Effect on gastrointestinal symptoms

Gastrointestinal symptoms at baseline were well controlled with a mean overall symptom score of 14 mm (range, 0-53 mm). Overall gastrointestinal symptoms worsened during each of the challenges with no difference in observed symptoms between gluten and placebo challenges ($F(1,15)=0.05$, $p=0.83$) (Figure 5.9) or from baseline ($F(3,14)=2.64$, $p=0.09$) (Figure 5.10). Similarly, no differences between challenges were seen for individual symptoms of abdominal pain ($F(1,15)=1.09$, $p=0.31$), bloating ($F(1,15)=0.98$, $p=0.34$), wind ($F(1,15)=0.02$, $p=0.90$), satisfaction with stool consistency ($F(1,15)=0.001$, $p=0.98$), or nausea ($F(1,15)=0.28$, $p=0.61$) (Figure 5.9). No differences between gluten and placebo challenges and baseline were observed for any individual gastrointestinal symptoms (Figure 5.10). Treatment order effects were not detected.

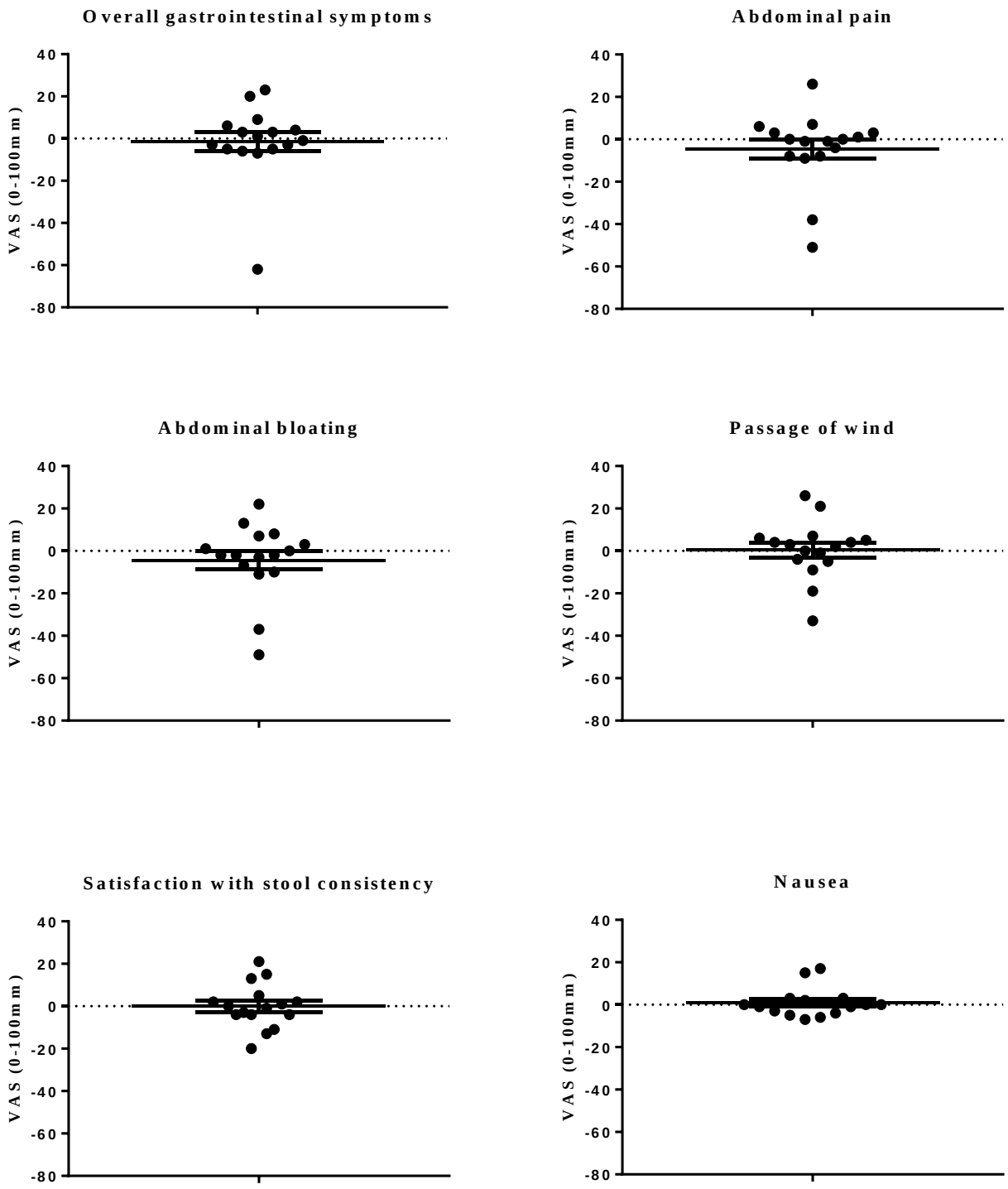


Figure 5.9. Difference in overall and individual gastrointestinal symptoms from placebo to gluten. Data shown represent the mean

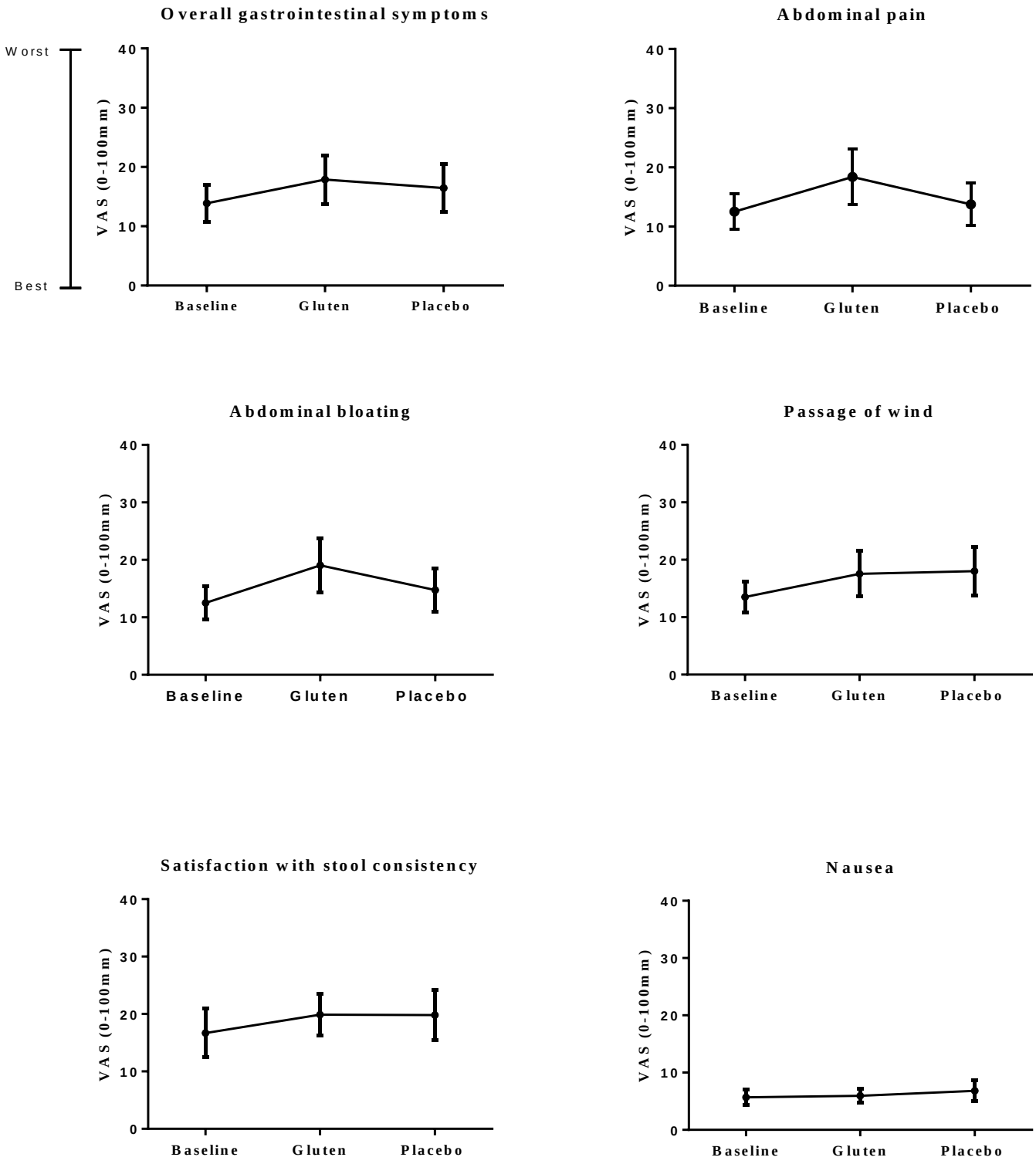


Figure 5.10. Overall and individual gastrointestinal symptoms during the baseline period and gluten and placebo challenges. A linear mixed model of fixed effects revealed no effect of assessment time on either overall or individual gastrointestinal symptom scores

5.4. Discussion

Studies amongst the NCGS entity have primarily focused on gastrointestinal symptoms with a lack of exploration towards the potential relationship between gluten ingestion and extraintestinal manifestations, with the notable exception of the results reported in Chapter 4. In that chapter brief (3-day) exposure to gluten specifically induced current feelings of depression where it was suggested that patients with NCGS might feel better on a gluten-free diet due to changes in mental state despite the continuation of gastrointestinal symptoms.[239] The results of the current study have examined longer exposure to gluten (14 days) and have not supported this observation. This double-blind, placebo-controlled, randomised, cross-over study has failed to reveal any evidence of specific changes to psychological indices associated with depression following gluten-ingestion. As found in Chapter 4 and by others[86, 134] gluten-specific effects on gastrointestinal symptoms were not observed.

Discrepancies in study design between Chapter 4 and the current study may have influenced results. First, all study food was provided to participants in Chapter 4 whereas participants followed their own usual gluten-free diet in the current study. This was done to minimise any negative associations of provided foods with symptom induction. While this was not an inherent problem in Chapter 4 where overall gastrointestinal symptoms were similar in both studies during the placebo challenges, is has been described as a limitation of previous investigations.[86] Regardless, symptoms were well controlled at baseline and no differences in extraintestinal or gastrointestinal symptoms were observed between the baseline period and the gluten and placebo challenges.

It is also possible that other potential inducers of symptoms including FODMAPs, dairy and naturally-occurring food chemicals, which were not controlled in the current study, may have contributed to symptomatic effects. Again, given that symptoms were well controlled at baseline (when participants were consuming diets that most likely contained FODMAPs, dairy and natural food chemicals), this seems unlikely.

As alluded to above, the challenge duration in the two studies differed from 3-days in the study reported in Chapter 4 to 14-days in the current trial. This increase in challenge duration was done to ensure that the maximal and stable in psychological state could be observed. Despite this, a plateau and weakening of the observed effect may have occurred over the 14-day challenge duration where an initial worsening of depression may have been evident on or around day 3 but by the time of reporting (i.e. day 14) participants had become accustomed to this heightened state where it was then considered 'normal'. Another consideration is that gluten-specific effects on depression appear relatively quickly and last for only a short period of time i.e. only a matter of days, in which case any acute changes would have been missed by the time of reporting. Such issues could be readily addressed by examining psychological changes at multiple time points in a subsequent study.

Several limitations of the current study may have also contributed to the lack of observed effects. First, with only 16 participants, the study may not have been adequately powered. Power calculations suggested 49 participants would be required to assess the primary endpoint of differences in STPI sate depression between gluten and placebo challenges. However, such power calculations were performed on different time-point after exposure to gluten and had assumed a constant effect of gluten on psychological symptoms. Recruiting participants was difficult and resulted in fewer numbers being included into the study than originally planned. Many potential participants were not prepared to eat gluten, several were only consuming a gluten-free diet some of the time and would lapse when it was not

convenient to adhere to such dietary limitations, and others were only avoiding gluten due to dietary FODMAP restriction and upon questioning did not believe that gluten itself was the inherent trigger of their symptoms. As such, these participants could not be included. However, the results of the study showed no hint of a specific effect and, if the trend continued, it would take hundreds of patients to demonstrate a change in state depression with gluten ingestion. Thus, power of the study is unlikely to be the reason for the negative results.

A second consideration was the heterogeneity of subjects sampled. Analyses that examined individual participants extraintestinal and gastrointestinal symptoms revealed that there seemed to be a minority who were notably worse on gluten compared to placebo and may be truly gluten-sensitive. There were, however, also a number of participants who experienced the worsening of symptoms on placebo. This high nocebo response has been similarly reported in other NCGS trials.[86, 132]

Despite these notable limitations, a progressive weakening of the assumption that gluten causes either gastrointestinal symptoms or extraintestinal manifestations in patients with NCGS is emerging where it should be considered that other components of food may be the culprit(s). Good evidence that wheat and other grains contain significant quantities of poorly-absorbed short-chain carbohydrates commonly known as FODMAPs now exists. For example, analysis of commonly consumed grain and cereal products has shown that wheat-derived products contain the highest fructan content.[139] The products with the lowest fructan content are mostly gluten-free.[139] As such, their concomitant reduction with the introduction of the gluten-free diet might lead to improved symptoms, wrongly perceived to be due to a reduction in gluten intake.[86] This could be true for both gastrointestinal symptoms and psychological manifestations where recent work within NCGS populations has revealed that by restricting FODMAP intake patients uniformly reduce gastrointestinal symptoms.[86] Furthermore, the restriction of fructose and sorbitol has been shown to improve symptoms of depression in

patients with fructose malabsorption where depression scores were reduced by 65% after four weeks on a fructose and sorbitol reduced diet.[240] The association between a low fructose and sorbitol diet, fructose malabsorption and improvement in mood and early signs of depression requires further exploration.

Preliminary examination of a test of subtle change in cognition, the SCIT, suggested a gluten-specific increase in response times on the SCIT-RTH. Of note, however, is the lack of significant difference between gluten and placebo challenges. This may have been purely a power effect. The magnitude of the response time differences between the gluten challenge and both baseline and placebo are equivalent to those observed in coeliac disease patients with so-called 'brain fog'.[191] In coeliac disease the slowing of the speed of cognitive processing has been linked to systemic inflammation following gluten induced inflammation in the gut. Regardless, only six participants were able to undertake this test, and despite large effect sizes, this outcome should be seen as indicative only, until a larger sample is investigated repeatedly over time.

5.5. Conclusions and Future Directions

Gluten exposure did not induce current feelings of depression or worsen most other measured indices in patients with self-reported NCGS. The only suggested gluten-specific effect was for subtle changes in cognition equivalent to what has been previously described in coeliac disease patients with brain fog. These results, however, are preliminary and require further investigation in both NCGS and healthy subjects. The data presented in the current study are best interpreted in light of those presented in the previous study, that gluten may have transient effects of feelings of depression, but this is not sustained. Thus, overall, gluten does

not induce depression, or change other psychological indices in patients with self-reported NCGS. Future work is needed to dissect the suggestion that subtle cognitive effects may be induced by gluten, and to more closely examine the time-related effects of gluten on psychological indices. The notion that gluten induces extraintestinal manifestations in patients with NCGS has not been supported.

Chapter 6 – The development and characterisation of a method for the large-scale isolation of gliadin and glutenin suitable for human consumption

6.1. Background and Aims

Evidence that gluten-ingestion contributes to extraintestinal manifestations and gastrointestinal symptoms in patients with non-coeliac gluten sensitivity (NCGS) is inconclusive. A potential cause of the variation in findings is due to the disparity in the challenge substrates employed where whole wheat flour, carbohydrate-deplete wheat protein or 'purified gluten' have been used. What remains unknown is whether the wheat protein class responsible for any observed effect is due to the gluten proteins (i.e. gliadin and/or glutenin) or the whether the albumin and globulin seed storage proteins may also play a role. Future work would benefit from challenging patients with pure substrates (i.e. free from contamination) so that any observable effects can be accurately attributed to the correct challenge medium as opposed to previous studies where challenge mediums have often contained several of these potential symptom inducers. Isolating the gluten fraction of wheat into gliadin and glutenin classes would create an opportunity for the specific effects of gluten, free from albumin and globulin and indigestible carbohydrate contaminants, to be observed independently of one another in patients with NCGS.

Isolating wheat gluten is a relatively simple process and has been carried out for centuries.[241] The process is so simple that it can be carried out in the kitchen or laboratory.[242] It is now also an efficient commercial process where the end-product is traded as "Vital Wheat Gluten" and is used by food industry where it is regenerated into functional gluten for a wide range of food (and non-food) products.[101]

Even though the methodology of isolating wheat gluten is well established, isolating protein classes including gliadins and glutenins free from albumins and globulins remains an extremely complex process. This is largely due to the type of interactions between protein and non-protein compounds through covalent and non-covalent interactions. Eliminating these interactions and separating the individual polypeptides is difficult, partly because of the huge number of relatively similar molecules but also because of their specific physical and chemical characteristics. Despite this, methods to isolate these prolamins have been developed where their extractability and solubility differs in various solvents. Gliadins, which are insoluble in most of the usual protein solvents, are soluble in 70% ethanol, glutenins are soluble in dilute acid or alkali, albumins are soluble in water and globulins in dilute sodium chloride concentrations.

Methods to individually extract gliadins, glutenins, albumins and globulins have been previously based on a four-step sequential extraction method. However, this process is only suitable to separate protein classes in milligram scale quantities for analytical purposes. With some alteration, methods can be scaled up to a gram level, but various factors inhibit producing larger quantities. The largest scale of "preparative isolation" of gliadins claims to produce about 500 grams of non-food-grade gliadin. However, this method used extremely expensive and time consuming technology.[243] No study has isolated large quantities of human-grade gliadin or glutenin. With only small and non-food grade gliadin being previously isolated, no direct dietary studies have been able to assess the effects of gliadin

specifically in gluten sensitive patients. The aim of the current study was to develop a method to isolate gliadin and glutenin from the wheat gluten protein on a large enough scale to undertake such assessment of the effects of these individual gluten proteins in human subjects in dietary studies.

6.2. Materials and Methods

The isolation methodology was created by Dr Ferenc Békés and carried out by Dr John Pearce in a purpose-built, fire-safe facility designed for processing materials with ethanol (Manildra Group, Bomaderry NSW, Australia). Fifty kilograms of commercially available wheat gluten containing 78% protein (Manildra Group, Manildra NSW, Australia) was added to 500 Litres (L) of 70% aqueous ethanol. The mixture was outgassed with nitrogen and stirred for one hour at an ambient temperature. After settling, the residual solid (glutenin) was separated from the liquid (gliadin) by decantation and freeze-dried. The ethanol content of the collected gliadin-rich filtrate was increased to about 85% by the additional of 450 L of 100% food-grade dry ethanol and filtered over a nylon mesh screen. To the collected gliadin aggregate, ice water was then added and stirred to agglomerate. The gliadin-rich fraction was isolated by winding the “stringy” material onto a paddle, thereby eliminating most of the ethanol, an essential step prior to the product being freeze-dried. Figure 6.1. outlines the step-by-step isolation procedure.

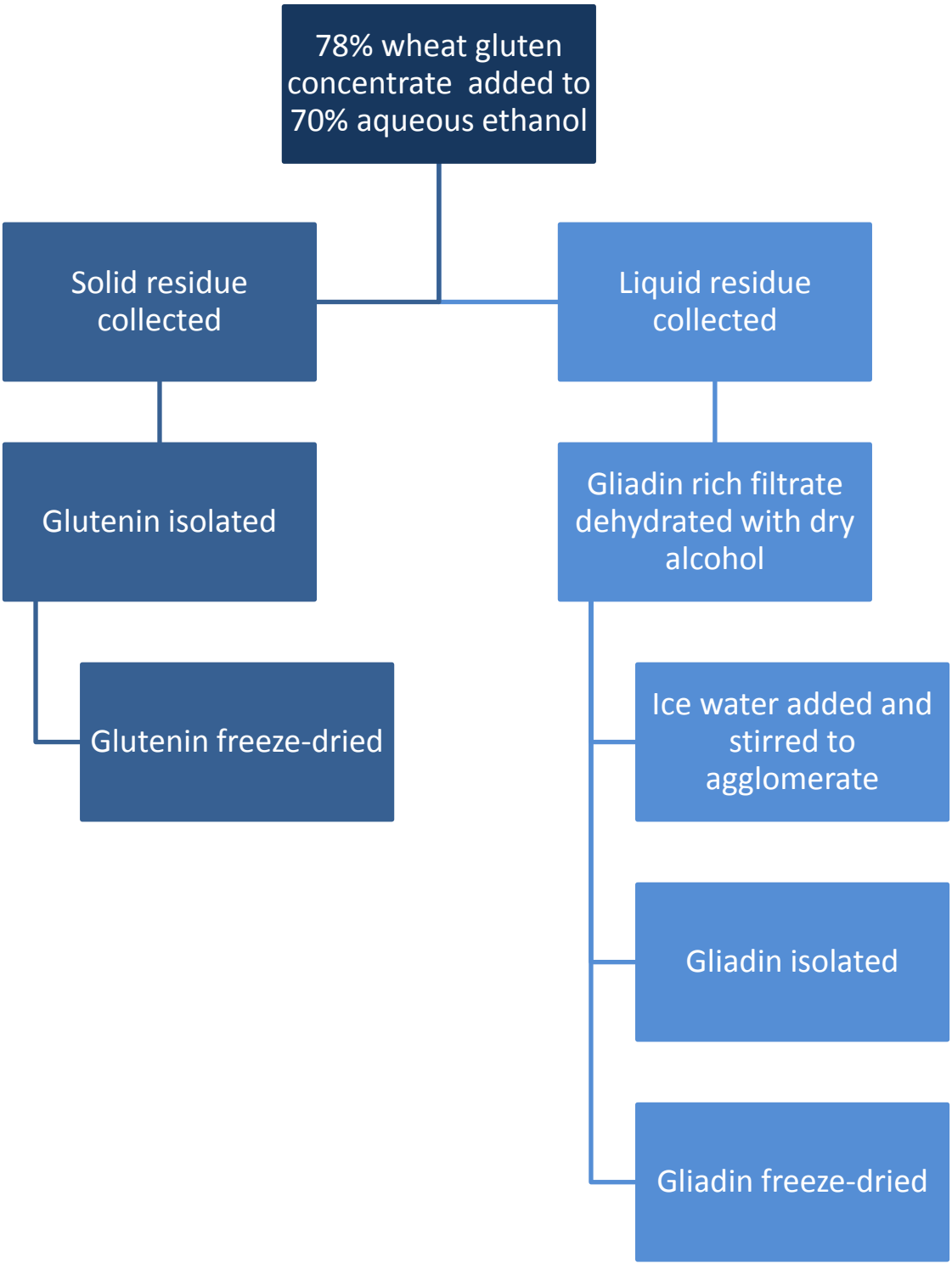


Figure 6.1. Gliadin isolation step-by-step procedure

The end-product was analysed for protein content using a standard Dumas methodology based on determining nitrogen content.[244] Clear separation was further confirmed using size-exclusion high performance liquid chromatography (HPLC) as previously applied for the investigation of wheat flour.[245, 246] Ten milligram (mg) isolates were mixed with 1 ml 0.5% sodium dodecyl sulfate (SDS) phosphate buffer and sonicated for 15 seconds, ensuring that the sample was completely dispersed within the first 5 seconds. The mix was then centrifuged for 10 minutes at 17,000 g and the supernatant was filtered through a 0.45 µm polyvinylidene fluoride (PVDF) filter. Twenty microliter (µl) extracts were injected into 0.5 ml/min aqueous acetonitrile buffer and 0.05% trifluoroactic acid, with a running time of 35 min. The proteins were detected at a wavelength of 214 nanometres (nm). SE-HPLC analysis was carried out on a Beckman Gold Nouveau chromatograph at the Department of Biochemistry and Food Technology of the Technical University of Budapest, Hungary on a SEC3000 column. Results have been expressed as a % of total protein content, relating the area under the peak of interest to the area of the total chromatogram.

6.3. Results

Gliadin was successfully isolated utilising the above methodology where ~5-10 kg was obtained. Starting concentrations of baker's flour, 78% wheat gluten and the total protein contents acquired in the gliadin and glutenin protein classes are presented in Table 6.1. Clear separation of these isolated proteins was further confirmed using size exclusion HPLC (Figure 6.2).

Table 6.1. Breakdown of Baker's flour, 78% wheat gluten and the isolated gliadin and glutenin protein fractions per g/100g

	Baker's Flour	78% Wheat Gluten	Gliadin (isolated fraction)	Glutenin (isolated fraction)
Protein	9.5-13%	78.2	95.1	80.5
Lipid	1.8-2.2%	4.7	0.6	6.4
Starch/Pentosans	85-90%	17.1	4.3	13.1
Total	100	100	100	100
Protein Composition				
Glutenin	45-55%	51.9	6.7	69.9
ω gliadin	5-12%	7.3	5.4	8.3
αβγ gliadin	30-40%	39.5	87.3	20.3
Soluble proteins	8-15%	1.3	0.6	1.5
Total	100	100	100	100

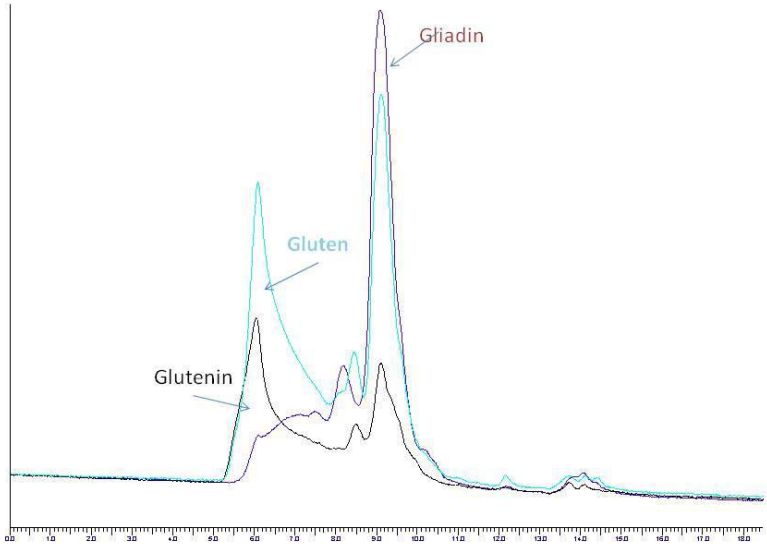


Figure 6.2. Separation of gliadin and glutenin using size-exclusion liquid chromatography

6.4. Discussion

Methodology to isolate wheat gluten protein is well established and requires a relatively simple process, but up until now, isolating gliadin and glutenin was more complex. The complexity stemmed from the inability to easily separate and harvest the individual prolamins. As such, only small scale, individual prolamin isolation methodologies had been previously adopted. As a result of new methodologies and access to flammable organic solvent (ethanol) safe pilot-scale processing facilities, the current study reports the first large pilot-scale isolation of human food-grade gliadin.

There were two novel aspects to the methodology applied in the present study. First, the isolation of gliadin was carried out from 78% purified wheat gluten concentrate not from

whole wheat flour as to reduce the potential for contamination where non-protein carbohydrate fractions are high (see Figure 1.6; Chapter 1). It also negated the need to extract the albumins and globulins having largely been depleted along with the starch and non-starch polysaccharides in the gluten manufacturing process. Similarly, this reduced the quantity of source material and the need for even larger processing facilities in order to eliminate the starch, fibre and water-soluble components. Secondly, the known differences in solubility of glutenin and gliadin in 70% ethanol/water mix and its sanitising effect, together with bacterioststic effect of ice water (~^{0}C) in the final recovery stage, to segregate the gliadins and glutenins were adopted in order to satisfy the criteria for "food grade quality" as set by the Australia New Zealand Food Authority thus enabling the end-product to be used in human clinical trials.

A total of about 10 kg each of the gliadin-rich fraction and of the glutenin-enriched fraction were obtained, compared with gram estimates from previous work.[243] Good purity of gliadin-enriched fraction was confirmed by size exclusion HPLC. The glutenin-enriched fraction was not exhaustively extracted with 70% ethanol so it still contains a significant proportion of gliadin. This could be processes further by additional extraction with 70% ethanol.

Reproducibility of the described methodology is likely to be good, assuming access to necessary facilities where large quantities of ethanol can be safely used and recovered for re-use. Access to large freeze-drying facilities is also necessary and time consuming given the time required to dry the materials. The ability to test the purity of the end product is also imperative, especially if the end product is going to be used in human clinical trials. Limitations to this methodology would be met if the abovementioned requirements, i.e., facility access where large quantities of ethanol can be safely used, access to large-scale freeze-dryers and the associated time constraints could not be met.

A further limitation of the produced end-product is the potential contamination of the sample from small quantities of other gluten and non-gluten proteins including albumins and globulins such as ATIs and glycoproteins, which are the natural protein pesticides found in wheat. While amounts of these potential contaminants in the end-product were low, only small concentrations may be needed to elicit an effect.[247] These remaining contaminants may be able to be similarly removed by subsequent washing with water, a process which could be considered before its use in future clinical trials. The potential contamination of the end-product with indigestible carbohydrates is not of concern given the purity of the sample obtained. For example, even if small quantities of FODMAPs are present, this would not be enough to elicit symptomatic effects.

Nevertheless, the effective large-scale isolation of gliadin and glutenin in the current study creates an opportunity for future research to be conducted using the obtained human-grade gliadin and glutenin in dietary trials. Previous elimination and re-challenge trials have produced inconclusive evidence of a gluten-specific effect on gastrointestinal symptoms within the NCGS entity. There is, however, emerging evidence that wheat gluten may be associated with psychological symptoms and that it may be that the improvement reported by participants is due to the perception of their general well-being rather than in gastrointestinal symptoms *per se*. If such a relationship between wheat gluten ingestion and psychological symptoms exists within the NCGS cohort it would be worthwhile assessing the direct effect of gliadin and glutenin on psychological state and thereby to determine whether either of these fractions is the specific gluten protein responsible for psychological change amongst this entity.

6.5. Conclusions and Future Directions

The relationship between psychological health and NCGS requires further elucidation. Should it be determined that the reported improvement in patients with NCGS on a gluten-free diet and the worsening of symptoms following gluten-ingestion, is due to changes in mental state and not gastrointestinal symptoms, it would be meaningful to understand to what wheat gluten protein these changes relate. Whether these changes are specific to the gliadin or glutenin classes of the wheat gluten protein requires elucidation. The effective isolation of gliadin and glutenin protein classes with reasonable purity in the current study creates an opportunity for future human dietary trials to assess the effects of these singular proteins on the psychological status of patients with NCGS.

Chapter 7 - A randomised comparison of the short and longer term efficacy of gut-directed hypnotherapy with that of the low FODMAP diet on gastrointestinal and psychological symptoms in subjects with irritable bowel syndrome

7.1 Background and Aims

Irritable bowel syndrome (IBS) is common and affects approximately 5-12% of the population in Western countries.[3, 4] There is no known cure for the condition and treatment often requires a multimodal approach where dietary, psychological and pharmacological approaches are commonly applied. Dietary therapies are appealing to most IBS suffers with the most recent strategy to have considerable impact being the restriction of indigestible and slowly absorbed short-chain carbohydrates, collectively known as FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols).

FODMAPs have been shown to induce gastrointestinal symptoms in IBS patients[80] mainly due to their poor and slow intestinal absorption with subsequent osmotically-driven increase in small intestinal water content and colonic fermentation producing gas.[1, 91, 92] The evidence-base for efficacy of the low FODMAP diet is strong [81, 88] with a recent well-powered blinded

placebo-controlled cross-over study confirming previous observations that approximately 70% of patients gain clinically significant benefit.[82] As such, the low FODMAP diet is increasingly applied by health professionals in patients with IBS as a first-line dietary therapy.[82, 87]

Another promising approach in reducing symptoms in patients with IBS is through the application of gut-directed hypnotherapy wherein suggestions for the control and normalisation of gastrointestinal function are made to the subconscious mind.[248] Several controlled trials and observational studies have reported reductions in overall and individual gastrointestinal symptoms in between 24-73% of participants with gut-directed hypnotherapy.[143, 146-148, 154] Its potential mechanisms of action on the brain-gut axis are multiple with evidence spanning psychological effects through to physiological gastrointestinal modifications. Regardless, obtaining similarly robust evidence of efficacy for gut-directed hypnotherapy to that of the low FODMAP diet is constrained by difficulties in designing a blinded placebo.

Participants in psychological studies typically know what intervention they are receiving.[183] Therefore, measuring the effectiveness of a therapy to a no-treatment control condition is inadequate. An alternative is to compare the therapy with an active control group with proven efficacy. This technique was employed in the current randomised clinical trial (RCT) which aimed to determine the efficacy of gut-directed hypnotherapy compared to that of the low FODMAP diet. An additional aim was to compare the collective benefit of combining these therapies. It was hypothesised that participants would report similar gastrointestinal and psychological improvements regardless of whether they received gut-directed hypnotherapy or the low FODMAP diet but that those who received both therapies would experience an enhanced effect.

7.2 Materials and Methods

7.2.1 Participants

Participants were recruited through newspaper advertisements in metropolitan Melbourne, on social media and through the Monash University Department of Gastroenterology webpage. Participants met inclusion and exclusion criteria as described in Chapter 3, Section 3.1.

7.2.2 Protocol

All patients were assessed by a Gastroenterologist with regards to inclusion and exclusion criteria. Participants were randomised, according to a computer-generated list, to receive gut-directed hypnotherapy, education in a low FODMAP diet or a combination of both. The study was not blinded. The effectiveness of the treatments was evaluated using questionnaires that assessed gastrointestinal symptoms and psychological indices concerning anxiety and depression and quality of life (QOL). All participants completed the questionnaires prior to treatment and directly after treatment (week 6). Long-term follow-up data was also collected 3 and 6 months after completion of the treatment. Follow-up data included gastrointestinal symptoms, psychological symptoms and dietary adherence (for those in the low FODMAP diet and combined treatments) and was posted to participants with a pre-paid envelope for easy return. Participants were asked to refrain from using any alternative treatment of their choosing until they had reached the 6-month follow-up time-point. All participants gave written, informed consent.

7.2.3 Interventions

Gut-directed hypnotherapy

Those randomised to receive gut-directed hypnotherapy underwent six, one-hour hypnosis sessions weekly for six weeks. The sessions were based on the well-established Manchester model.[143] They were scripted (i.e. the same for each participant) and were conducted with Simone L Peters. Participants were also provided with a pre-recorded compact disc (CD) that was identical to the first session's script and asked to listen to it daily during the 6 week intervention. After the gut-directed hypnotherapy was completed, the participants kept the CD and were able to listen to it at their choosing. Continued use of the CD in the follow-up period was not a requirement of the study. Adherence to gut-directed hypnotherapy was measured according to the attendance at scheduled sessions and to daily use of the CD during the intervention phase. Adherence was arbitrarily defined as no more than two missed days of listening to the CD per week over the 6-week study period, as recorded at each hypnosis session.

Low FODMAP diet

A gastrointestinal dietitian highly experienced with the delivery of the low FODMAP diet educated the participants in a one-hour session on the principles of the low FODMAP diet including the mechanistic action of FODMAPs at the beginning of week 1. Participants were asked to restrict foods containing high and moderate amounts of all types of FODMAPs and to consume only foods that contained low amounts of FODMAPs. They were given written information outlining the principles of the diet, lists of high, moderate and low FODMAP containing foods, instructions on how to read food labels for FODMAPs, and several recipe ideas. Participants were instructed to follow the diet strictly from the beginning of week 1 to the end of week 6. Weekly telephone contact was made to encourage compliance. Participants were not permitted to discuss additional matters during this contact. At week 6, participants underwent a review as per current best practice. Those who reported symptomatic improvement at review were educated on the reintroduction phase (detailed below) and those

who failed to show improvement were instructed to return previously excluded foods back into the diet (i.e., without following the reintroduction phase). Adherence to the low FODMAP diet was assessed during the weekly telephone contact where direct questioning was used to determine the level of adherence which was arbitrarily defined as no more than three accidental exposures to high FODMAP containing foods over the 6-week study period.

The reintroduction phase: Aimed to liberalise the diet whilst maintaining good symptomatic control as per current best practice. Tolerance levels for each participant and to each FODMAP were determined by reintroducing one FODMAP subgroup per week (except for oligosaccharides) and then monitoring any symptomatic response. Reintroduction of oligosaccharides occurred more gradually where one fructan containing food (wheat vs garlic) was introduced per week. If symptoms were experienced participants stopped the reintroduction and waited until they were symptom free before reducing the serving size to half and trying again. Alternatively, participants could assume that the FODMAP was a problem for them and continue onto the next FODMAP reintroduction. If symptoms were not experienced participants could either gradually increase the number of foods that contained the particular FODMAP they were challenging and continue to assess their response (i.e. determining their threshold) until the amount they previously consumed was reached or maintain that amount and type of FODMAP in their diet and continue onto the next FODMAP reintroduction. This process was continued until each individual FODMAP was tested. Information on what individuals did during the reintroduction phase was collected at the 6-month follow-up.

Combined treatment

Those in the combined condition received both the gut-directed hypnotherapy and the low FODMAP diet treatments on the same day as described above.

7.2.4 Measurements

7.2.4.1. Gastrointestinal symptoms

Gastrointestinal symptoms were assessed using a 100mm visual analogue scale (VAS) as described in Chapter 3, Section 3.4.1. Differences of 20 mm or more over time in an individual were arbitrarily considered clinically significant, as previously applied.[86]

7.2.4.2. Psychological indices

Psychological indices were assessed using the State Trait Personality Inventory (STPI),[186] and the Hospital Anxiety and Depression Scale (HADS)[188] as described in Chapter 3, Sections 3.4.2 and 3.4.4. Only anxiety and depression subscales of the STPI were calculated and reported.

7.2.4.3. Quality of life

The IBS-QOL was used to determine disease specific health-related QOL[197, 249] as described in Chapter 3, Section 3.4.6.

7.2.5 Long-term follow-up

Long-term follow-up data were collected 3 and 6-months post-treatment. Gastrointestinal symptoms, psychological indices concerning anxiety, depression and QOL were assessed as outlined above. In addition, participants in the low FODMAP diet and combined treatments completed a questionnaire that identified their current dietary status in terms of whether they continued to follow the low FODMAP diet strictly or following reduction of foods as instructed (referred to as 'attenuated' low FODMAP diet) or had stopped following the diet altogether. Information on whether any alternative treatments had subsequently been undertaken was also obtained. Long-term CD use data was not obtained.

7.2.6 End-points

The primary end-point was the change in overall gastrointestinal symptoms across the gut-directed hypnotherapy, low FODMAP diet and combined treatment groups from baseline to week 6 as measured by the VAS. Secondary end-points included the change in overall gastrointestinal symptoms across the three groups from baseline to 3 and 6-months post-treatment; the change in individual symptoms of abdominal pain, bloating, wind, satisfaction with stool consistency, and nausea from baseline to week 6 and 3 and 6-months post-treatment; the change in psychological indices concerning anxiety, depression and in QOL across the gut-directed hypnotherapy, low FODMAP diet and combined treatment groups baseline to week 6 and 3 and 6-months post treatment as measured by the STPI, HADS and IBS-QOL.

7.2.7 Statistical analysis

Power calculations were based on previous data[147] and allowed for drop-out, missing data and error rate. Using the change in overall gastrointestinal symptoms from baseline to the end of the intervention (week 6) as the primary end-point, 78 participants were required to detect a statistically significant difference between groups given an effect size of 0.2 with 80% power at a two-sided 5% significance level. Intention-to-treat analyses were performed on all data from baseline to week 6 but per-protocol analyses were applied for data from baseline to 6-months as there was no satisfactory way of dealing with the participants who failed to return their long-term follow-up questionnaires. Participants who were enrolled and randomised but who withdrew prior to any intervention were excluded from the analysis. Symptom data of participants who started treatment (during week 1) but withdrew prior to the end of week 6 were included and adjusted by carrying forward the last observation. Mixed between-within subjects analysis of variance was conducted to assess the impact of treatment condition (gut-

directed hypnotherapy, the low FODMAP diet and the combined condition) across four time points (week 1, week 6, and 3 and 6-month follow-up). One-way analysis of variance and t-tests were used to assess time and condition interactions. The relationship between overall gastrointestinal symptoms and psychological indices for each treatment was determined using Pearson product-moment correlation coefficient. Type 1 error was controlled by use of the False Discovery Rate adjustment technique.

7.3 Results

7.3.1 Participants

Participant recruitment and flow is shown in Figure 7.1. One hundred and forty six individuals who responded to advertisements but only 78 fulfilled the inclusion/exclusion criteria and were enrolled into the study. Four participants withdrew prior to the initiation of the randomised intervention. One participant became injured and was not able to fulfil attendance requirements, one was required to travel overseas due to unforeseen circumstances, one revealed a previous diagnosis of diverticular disease and one simply failed to attend the first treatment session. A further two participants started treatment but withdrew prior to week 6 (during weeks 3 and 5; both in the combined treatment). No demographic (Table 7.1), gastrointestinal or psychological (Table 7.2) differences were identified at baseline between treatment groups. Sixty two participants (84%) completed and returned the 6-month follow-up questionnaire.

7.3.2 Adherence during the interventions

Adherence to the low FODMAP diet was achieved in 21 participants (88%) in the low FODMAP diet and 19 (76%) in the combined treatment group with no difference observed between groups. Non-adherence was largely the result of >3 accidental exposures to high FODMAP foods but no participant abandoned the diet completely. Adherence to daily listening of the CD in the gut-directed hypnotherapy treatment group was achieved in 18 participants (72%) and 19 participants (80%) in the combined condition. Only three participants reportedly stopped listening to the CD completely.

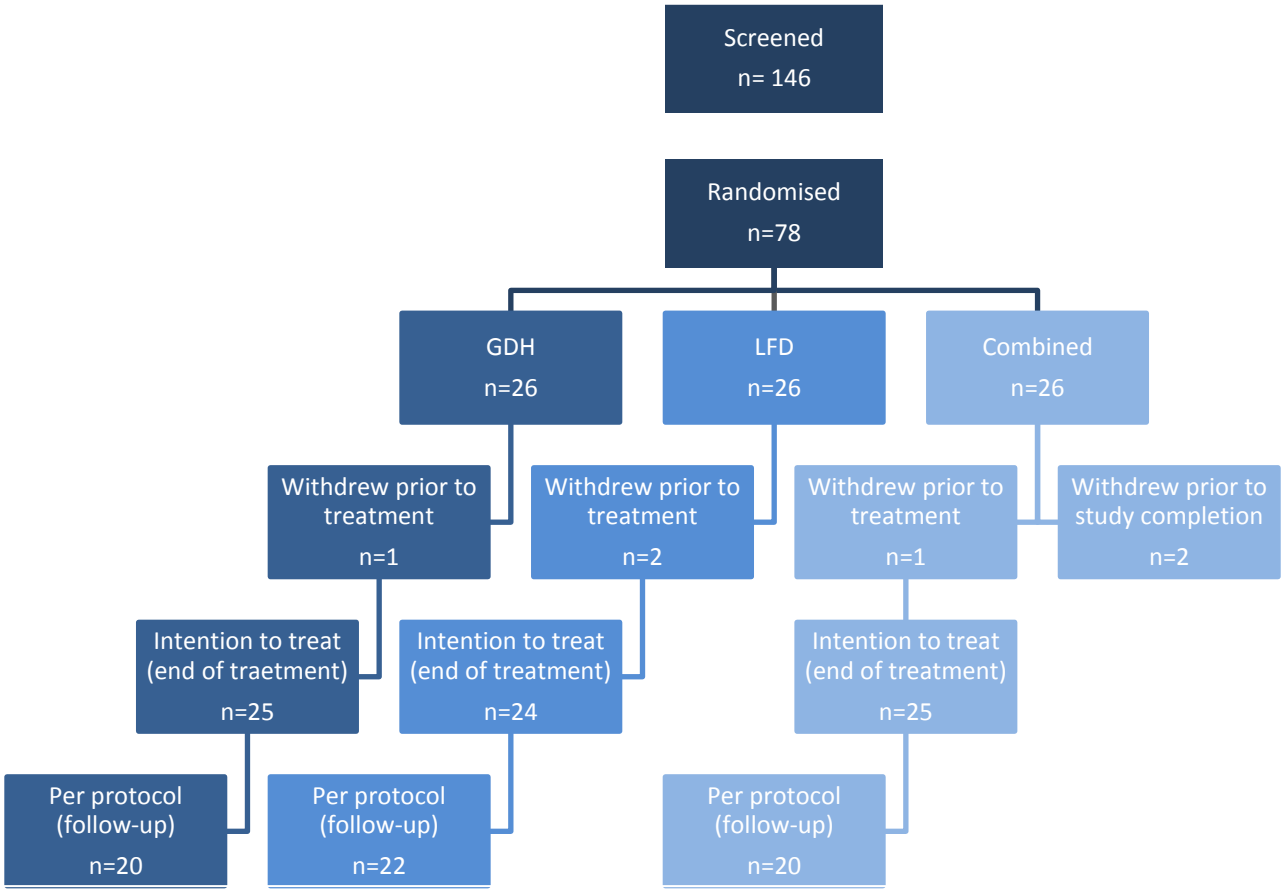


Figure 7.1. Recruitment pathway and reasons for withdrawals. GDH= gut-directed hypnotherapy; LFD= low FODMAP diet

Table 7.1. Participant demographics at baseline. GDH= gut-directed hypnotherapy; LFD= low FODMAP diet

	GDH	LFD	Combined treatment	P-value
No. of participants	25	24	25	*ns*
Gender, male	3 (21%)	5 (36%)	6 (43%)	*ns*
Median age	40	34	39	*ns*
(range)	(20-72) years	(23-66) years	(23-63) years	
IBS subtype				*ns*
Diarrhoea	7 (28%)	10 (42%)	13 (52%)	
Constipation	11 (44%)	5 (21%)	7 (28%)	
Mixed/alternating	7 (28%)	9 (37%)	5 (20%)	

Table 7.2. Participant gastrointestinal, psychological and quality of life characteristics at baseline between treatment groups. Data shown represents the mean (95% CI). GDH= gut-directed hypnotherapy; LFD= low FODMAP diet

	GDH	LFD	Combined	P-value
Gastrointestinal symptoms				
Overall	65 (60, 70)	61 (54, 68)	62 (56, 69)	*ns*
Pain	53 (44, 63)	53 (44, 63)	54 (44, 64)	*ns*
Bloating	68 (61, 75)	59 (50, 67)	58 (47, 70)	*ns*
Wind	69 (60, 77)	63 (55, 71)	61 (52, 70)	*ns*
Stool consistency	62 (51, 73)	70 (60, 81)	58 (46, 69)	*ns*
Nausea	25 (14, 37)	22 (13, 32)	24 (10, 37)	*ns*
Psychological measures				
STPI State anxiety	17 (15, 19)	18 (16, 20)	18 (15, 20)	*ns*
STPI State depression	19 (17, 21)	19 (17, 21)	19 (17, 21)	*ns*
SPI Trait anxiety	21 (19, 23)	21 (19, 23)	21 (18, 23)	*ns*
STPI Trait depression	18 (16, 21)	17 (15, 19)	19 (16, 22)	*ns*
HADS anxiety	8 (6, 10)	8 (7, 10)	9 (7, 11)	*ns*
HADS depression	4 (3, 5)	3 (2, 4)	4 (3, 6)	*ns*
IBS-QOL	56 (46, 65)	57 (47, 68)	60 (53, 67)	*ns*

7.3.3 Effect on gastrointestinal symptoms

There were no differences across the three treatment groups for overall gastrointestinal symptoms from baseline to week 6 (primary end-point) or from baseline to 6-months post-treatment (p=.67 and p=.14, respectively; one-way-between-groups ANOVA) (Table 7.3, Figure 7.2 and 7.3). Participants in each treatment reported significant improvements from baseline at each time-point. Individual symptoms of abdominal pain, bloating, wind, and stool consistency improved in each treatment group with no differences across groups from baseline to week 6 and 6-months post-treatment (Table 7.3, Figure 7.2). Improvement in nausea was observed across all treatment groups at week 6 but only those who received gut-directed hypnotherapy maintained improvement at 6-months (Table 7.3, Figure 7.2). For subjects taught the low FODMAP diet, the degree of symptomatic improvement was similar for each instructing dietitian (data not shown).

Eighteen of 25 participants receiving gut-directed hypnotherapy (72%), 17/24 of those receiving the low FODMAP diet (71%) and 18/25 (72%) receiving the combined treatment, improved at week 6. This improvement was maintained 6-months post-treatment in 74% receiving gut-directed hypnotherapy, 82% the low FODMAP diet and 54% of participants receiving the combined treatment. Worsening of symptoms, as defined as an increase of ≥ 20mm on the VAS from baseline to 6 months post-treatment, was reported in one participant in the gut-directed hypnotherapy group (4%), four in the low FODMAP diet group (18%) and seven (32%) in the combined group (Figure 7.2.).

7.3.4 Effect on psychological indices

No significant change in state anxiety or depression was observed across or within the gut-directed hypnotherapy, low FODMAP diet or combined treatment from baseline to week 6 or 6-months post-treatment (Table 7.4). No change in trait anxiety or depression was observed at either time-point for those in the low FODMAP diet or combined treatments. However, as illustrated in Figure 7.4, trait anxiety and depression significantly reduced in participants who received gut-directed hypnotherapy from baseline to 6 months post-treatment.

As shown in Table 7.4 and Figure 7.4, HADS anxiety significantly reduced in all three treatment groups from baseline to week 6 but was only maintained 6 months post-treatment for those in the gut-directed hypnotherapy and low FODMAP diet treatments. No difference in the degree of improvement at week 6 was observed across treatment groups (p=.90; one-way repeated-measures ANOVA). HADS depression significantly improved from baseline to week 6 in those who received the low FODMAP diet or combined treatments but this was not maintained 6 months post-treatment. Only a trend for a reduction in HADS depression was observed from baseline to week 6 for those patients who received gut-directed hypnotherapy, but this was the only treatment to reach statistical significance 6 months post-treatment.

7.3.5 Quality of life

IBS-QOL significantly improved in all three treatment groups by a mean of 14-20 points from baseline to week 6 and by 12-21 points from baseline to 6 months post-treatment (Table 7.4, Figure 7.5). There was no difference in the change across the groups.

Table 7.3. Change in overall and individual gastrointestinal symptoms from baseline to week 6 and 6-months post-treatment. Comparisons made by paired-samples t-tests. Data shown represents the mean difference (95% CI). GDH= gut-directed hypnotherapy; LFD= low FODMAP diet

	Baseline to week 6						Baseline to 6-months					
	GDH	***p* value**	**LFD**	***p* value**	**Combined**	***p* value**	**GDH**	***p* value**	**LFD**	***p* value**	**Combined**	***p* value**
Overall	-33 (-41, -25)	<.0001	-30 (-42, -19)	<.0001	-36 (-45, -27)	<.0001	-38 (-50, -27)	<.0001	-30 (-43, -16)	<.0001	-27 (-40, -14)	<.0001
Pain	-27 (-37, -16)	<.0001	-26 (-39, -14)	<.0001	-31 (-42, -20)	<.0001	-33 (-46, -20)	<.0001	-30 (-41, -20)	<.0001	-29 (-41, -16)	<.0001
Bloating	-35 (-46, -24)	<.0001	-37 (-51, -24)	<.0001	-36 (-48, -24)	<.0001	-40 (-53, -28)	<.0001	-29 (-41, -17)	<.0001	-30 (-45, -15)	<.0001
Wind	-37 (-50, -25)	<.0001	-41 (-53, -30)	<.0001	-34 (-43, -24)	<.0001	-32 (-43, -19)	<.0001	-33 (-46, -20)	<.0001	-29 (-43, -15)	<.0001

Stool consistency	-33 (-43, -23)	<.0001	-42 (-54, -29)	<.0001	-32 (-45, -19)	<.0001	-35 (-47, -22)	<.0001	-34 (-47, -21)	<.0001	-23 (-38, -8)	.009
Nausea	-14 (-22, -5)	.003	-11 (-20, -1)	.03	-16 (-27, -5)	.008	-17 (-28, -6)	.005	-10 (-23, 4)	*ns*	-12 (-26, 1)	*ns*

Table 7.4. Change in psychological status and quality of life from baseline to week 6 and 6-months post-treatment. Comparisons made by paired-samples t-tests. Data shown represents the mean difference (95% CI). GDH= gut-directed hypnotherapy; LFD= low FODMAP diet

	Baseline to week 6						Baseline to 6-months					
	GDH	***p* value**	**LFD**	***p* value**	**Combined**	***p* value**	**GDH**	***p* value**	**LFD**	***p* value**	**Combined**	***p* value**
STPI State												
Anxiety	-.04	*ns*	-2	*ns*	-2	*ns*	-2	*ns*	-1	*ns*	.5	*ns*
	(-3, 2)		(-5, -.1)		(-4, .6)		(-4, .7)		(-4, 2)		(-3, 4)	
Depression	-.6	*ns*	-1	*ns*	-1	*ns*	-2	*ns*	-1	*ns*	.2	*ns*
	(-2, 1)		(-3, 1)		(-2, .1)		(-4, -.1)		(-3, 1)		(-2, 2)	
STPI Trait												
Anxiety	-2	*ns*	-2	*ns*	-.4	*ns*	-4	<.0001	-1	*ns*	.3	*ns*
	(-3, -.3)		(-3, -.2)		(-2, 1)		(-6, -2)		(-3, .3)		(-2, 2)	
Depression	-1	*ns*	-.9	*ns*	-1	*ns*	-3	.011	-.8	*ns*	.6	*ns*
	(-2, -.01)		(-2, .4)		(-3, .5)		(-5, -.7)		(-2, .2)		(-2, 3)	
HADS												
Anxiety	-2	.023	-2	.003	-2	<.0001	-3	.001	-2	.037	-1	*ns*
	(-3, -.2		(-3, -.6)		(-3, -1)		(-4, -1)		(-4, -.1)		(-3, .2)	

Depression	-.8	*ns*	-1	.032	-1	.038	-2	.001	-1	*ns*	-.4	*ns*
	(-2, .05)		(-2, -.1)		(-2, -.06)		(-3, -1)		(-2, .3)		(-2, .7)	
IBS-QOL	20	<.0001	14	<.0001	14	<.0001	20	<.0001	21	<.0001	12	.001
	(14, 26)		(7, 20)		(9, 19)		(13, 28)		(12, 30)		(5, 19)	

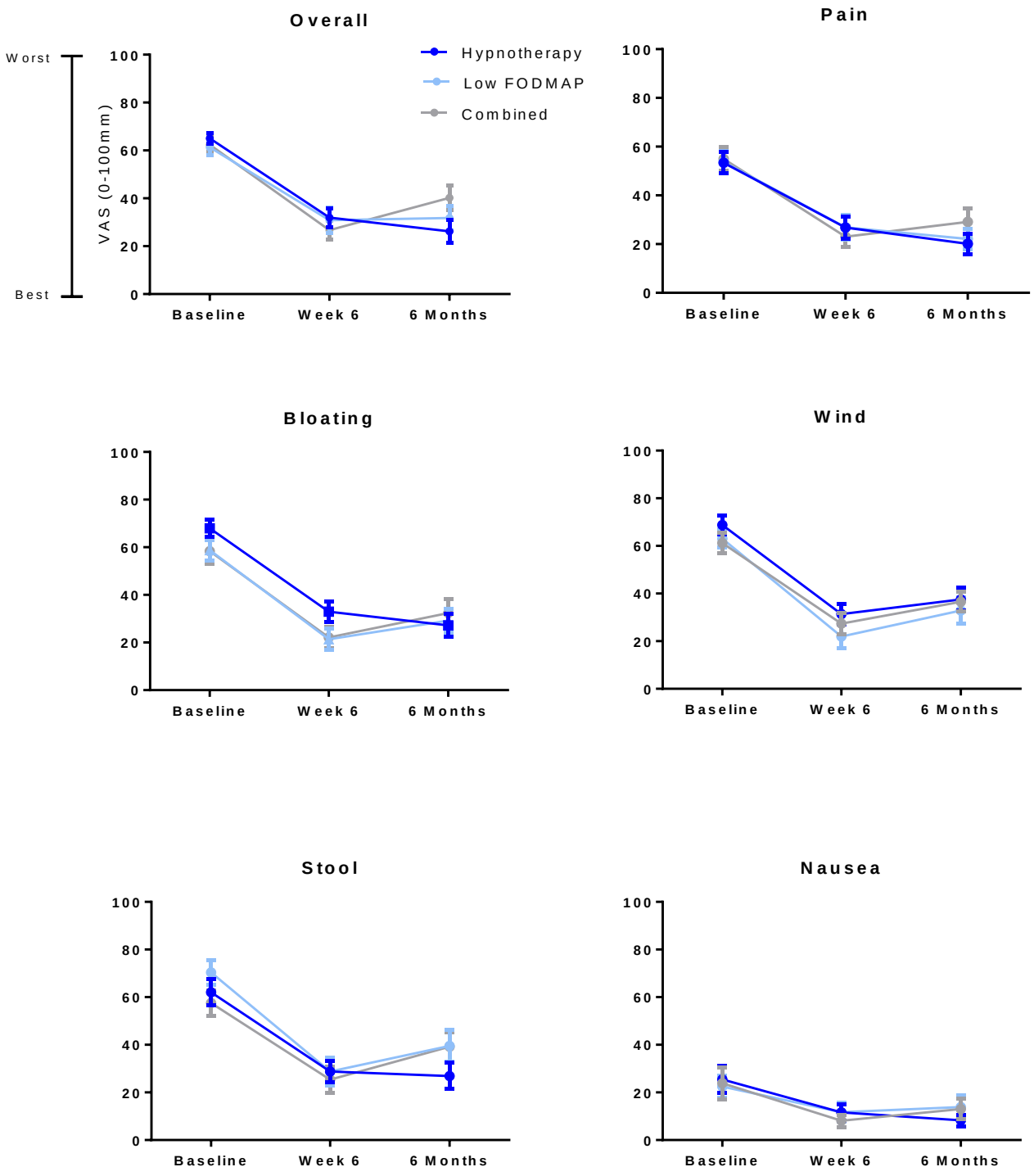


Figure 7.2. Overall and individual gastrointestinal symptom improvement over time and between treatment groups. Data was analysed using a mixed between-within subjects analysis of variance. There were no significant differences in overall or individual gastrointestinal symptoms between treatment conditions at each of the individual time points. Data shown represent the mean ± SEM

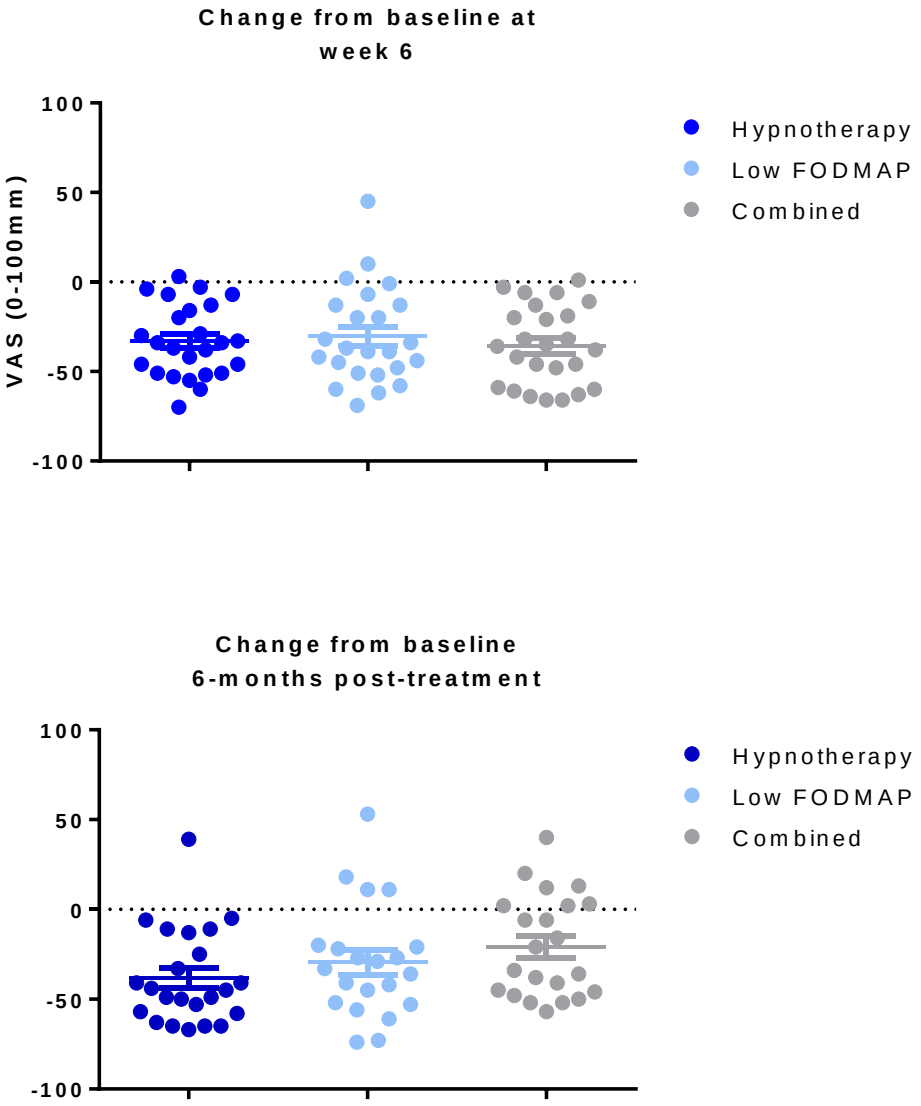


Figure 7.3. Change in overall gastrointestinal symptoms from baseline to week 6 and 6-months post-treatment. No difference in improvement was seen between treatment groups. Data shown represent mean ± SEM

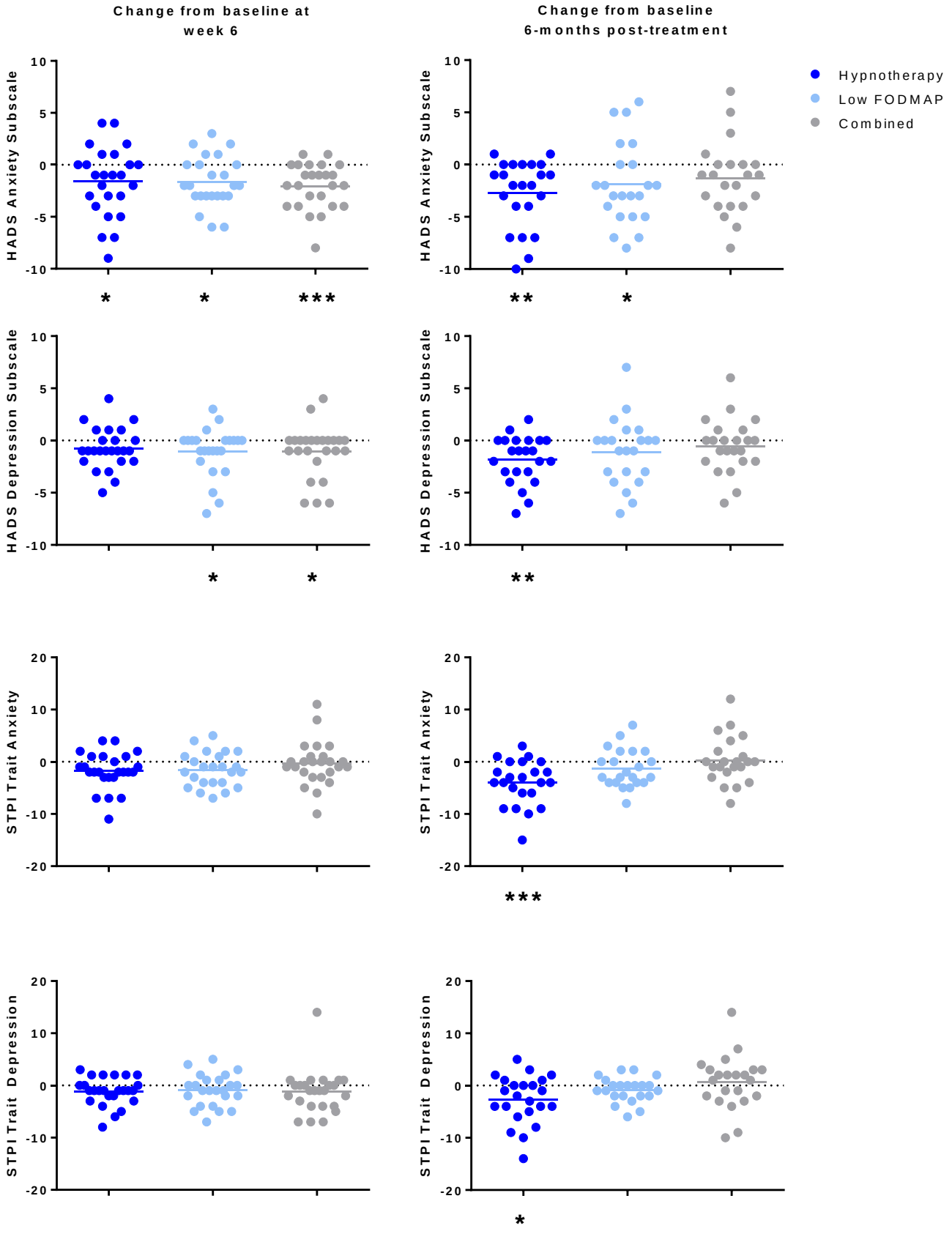


Figure 7.4. Change in HADS anxiety and depression and STPI anxiety and depression from baseline to week 6 and 6-months post-treatment. Data shown represent the mean. *p<.05; **p<.001; ***p<.0001

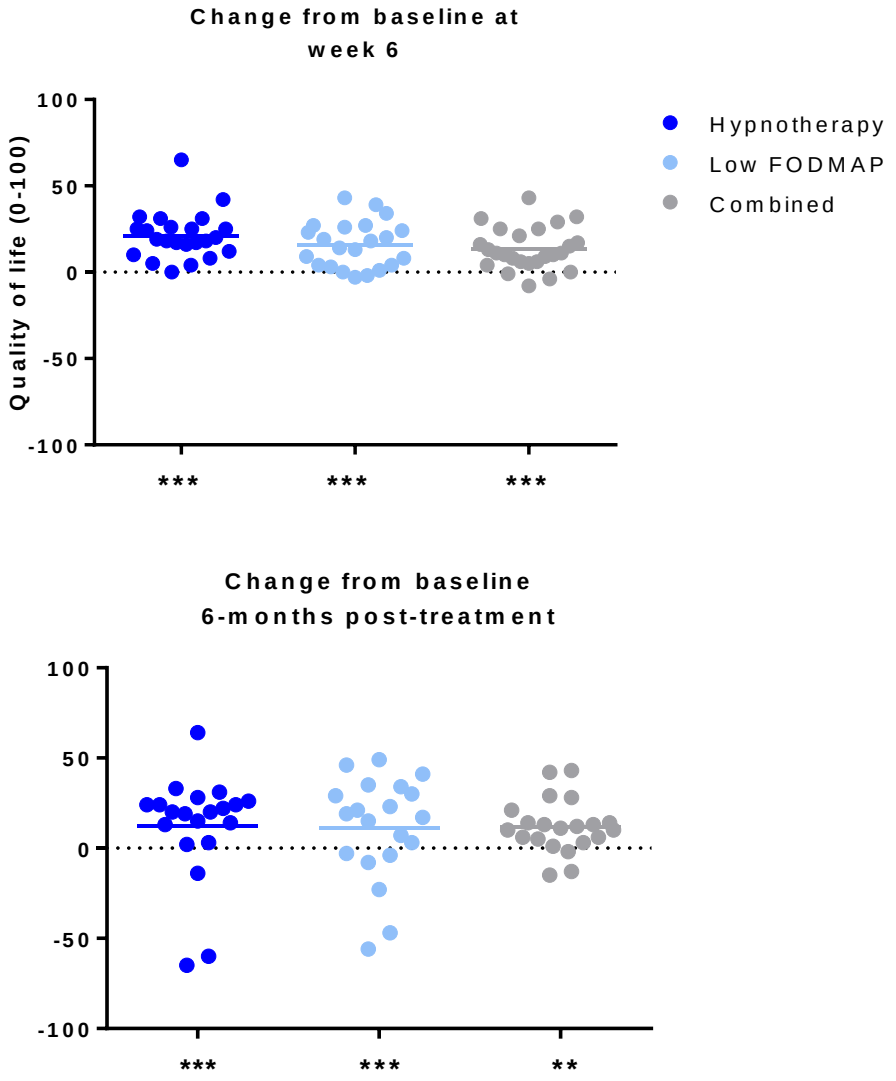


Figure 7.5. Change in quality of life from baseline to week 6 and 6-months post-treatment. Data shown represent the mean. *p<.05; **p<.001; ***p<.0001

7.3.6 Correlations

In order to determine whether changes in overall gastrointestinal symptoms were associated with changes in psychological indices for each treatment at the 6-week and 6-month time points, Pearson product-moment correlation coefficients were calculated. No correlations between overall gastrointestinal symptoms and psychological indices concerning anxiety, depression or QOL were identified for any treatment group from baseline to week 6. From baseline to 6 months post-treatment, overall gastrointestinal symptoms were directly associated with state depression (r =.49), trait anxiety (r =.42) and HADS anxiety (r= .72) and depression scores (r =.49; all $p<.05$) in the low FODMAP diet group. Overall gastrointestinal symptoms were directly associated with state (r =.46) and HADS anxiety (r =.54) in the gut-directed hypnotherapy group and with trait (r =.46) and HADS depression (r =.47; all $p<.05$) in the combined treatment. No correlations were identified between overall gastrointestinal symptoms and quality of life following any treatment.

7.3.7 Long-term follow–up

Three month follow-up data is not presented due to the maintenance of improvement at the 6-month follow-up time-point. Sixty two participants (84%) completed and returned the six-month follow-up questionnaire. Of the 22 who were in the low FODMAP diet group, two (9%) remained on a strict low FODMAP diet, 41% were following an attenuated low FODMAP diet and 50% abandoned the low FODMAP diet compared to 0%, 42% and 58% in the combined treatment, respectively. In the diet-only group, those remaining on the low FODMAP diet (strict or attenuated) at 6 months had a significantly greater symptomatic improvement at week 6 than those abandoning the diet (Figure 7.6a). However, the same was not the case for those receiving both diet and gut-directed hypnotherapy (Figure 7.6b). Eleven participants (15%) reportedly broke protocol and tried an alternative treatment/s in the six

months following study completion but no difference in adherence was observed between treatment groups (p=.73). Common alternative treatments included, acupuncture, Chinese medicine and other dietary changes. No participants in the gut-directed hypnotherapy treatment group reported trialling the low FODMAP diet or vice-versa.

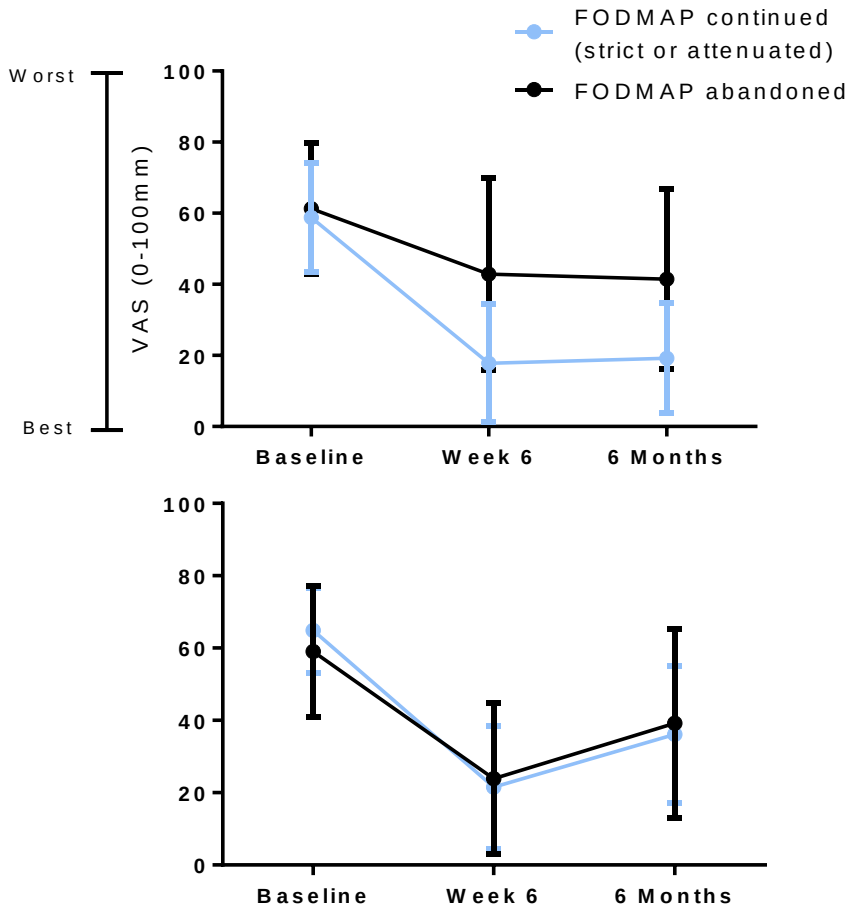


Figure 7.6. Overall gastrointestinal symptom improvement in the 41 participants who returned their 6-month follow-up questionnaires, according to whether they received (a) the LFD alone or (b) combined treatments. Those who reported greater symptomatic improvement at week 6 in the LFD treatment were most likely to continue on with the low FODMAP diet at 6-months (p=0.02, unpaired t-test). Symptoms at week 6 did not affect dietary status at 6-months for those who received the combined condition

7.4 Discussion

The therapeutic approach in patients with IBS involves dietary, psychological and pharmacological strategies. Obtaining high quality evidence for efficacy of psychological therapies has been challenging due to the difficulties designing appropriate placebo interventions. For gut-directed hypnotherapy, large observational cohorts and small RCTs with suboptimal placebo arms have suggested global reductions of symptoms in the majority of IBS patients. An alternative approach to obtaining more robust evidence is to compare efficacy against that of an intervention with a high level of evidence. The recent demonstration that the low FODMAP diet benefits all symptoms in 70% of IBS patients regardless of bowel habit subtype[82] permitted gut-directed hypnotherapy to be legitimately compared to the low FODMAP diet in patient's naïve to both therapies both on the effects on gastrointestinal symptoms (primary end-point), psychological indices concerning anxiety and depression, and QOL. Since the approaches are quite different in mechanisms of action, it was anticipated that they would have additive effects. The results of the current RCT clearly show that both therapies are efficacious to a similar degree and have durable benefits, but no signal of additive effects was evident. With the exception of an improvement in anxiety at 6-months in the low FODMAP diet treated arm, gut-directed hypnotherapy appeared to have a superior effect of changing psychological indices in the long-term.

The comparator therapy, the low FODMAP diet, showed efficacy in a similar proportion (about 70%) as similarly reported in previous observational and randomised studies.[81, 82, 87, 88] The durability of this response previously reported in a prospective observational cohort has been confirmed.[79] However, the actual dietary behaviour of patients taught the low FODMAP diet by a dietitian had not been previously reported at the time of study completion. Using a simple yet somewhat crude tool of self-assessment of adherence, few patients remained on the

strict FODMAP restriction that was recommended as initial therapy. This was likely to be in response to the reintroduction program directed by the dietitian so that the patients could liberalise their diets yet still continue to have symptomatic benefits. Interestingly, about one half of the patients reported stopping the low FODMAP diet over the follow-up period, many of whom had minimal or no benefit from the diet in the initial 6 weeks, although whether some restriction of particularly troublesome FODMAP rich foods, such as onions, was not specifically addressed. For those in whom the low FODMAP diet was the only therapeutic regimen introduced, response at 6 months was directly related to continued adherence to the low FODMAP diet, strict or attenuated. Such a difference was not the case in the combined treatment group presumably because of the efficacious effects of gut-directed hypnotherapy. These results are similar to new work which shows maintenance of therapeutic benefit 1 year post-treatment in those who responded to the low FODMAP diet regardless of the reintroduction of previously excluded FODMAPs.[85]

Gut-directed hypnotherapy achieved almost identical rates of response and mean magnitude of improvement at the end of therapy and at the 6-month follow-up. Likewise, QOL improved similarly. Hence, gut-directed hypnotherapy is comparative to low FODMAP diet in efficacy. Despite the very different portals of entry of the interventions (central nervous system versus luminal), the combination of gut-directed hypnotherapy and low FODMAP diet achieved response rates similar to either therapy alone and had numerically (though not statistically significantly) worse outcome after 6 months. Several reasons might be entertained for this. First, when considering VAS scores, the detection of an enhanced benefit of combing the two therapies may have been hindered by a ceiling effect. For example, healthy populations have reported similar gastrointestinal symptom scores to those reported at week 6 in all three treatment groups in the current study.[82] Secondly, despite the different portals of entry, the same disordered physiological processes may be the targets. Perhaps targeting the same

pathophysiology resulted in reduced rather than greater symptomatic improvement. Thirdly, the two therapies may have adversely affected each other. Patients may have not adhered to the diet or practised with the CD at home as seriously or well because they felt they were getting a 'double dose'. However, this seems unlikely since no evidence of this was detected in the assessment of adherence to dietary therapy. Fourthly, the results might reflect the nature of the patients. Up to one-third might not be readily amenable to any therapy and be regarded as have 'recalcitrant' IBS. Conversely, those readily amenable to modulation will respond to either effective therapeutic approach. The final possibility is that both were placebo effects. Against this contention is the strong published evidence for efficacy against placebo for the low FODMAP diet and the durability of improvement, a feature not observed in pharmacological studies.

Current understanding of the precise mechanism by which gut-directed hypnotherapy exerts an efficacious effect is limited. Regardless, there is strong evidence that gut-directed hypnotherapy can influence both psychological and physiological outcomes including motility,[173] visceral sensitivity,[159, 161, 178] immune function[179-181] and central processing.[151, 182] In the current study, only psychological aspects were addressed. Gut-directed hypnotherapy but not the low FODMAP diet or the combined treatment was associated with durable and increasing effect on anxiety and depression when two independent indices were used. Such effects were not apparent early, but emerged at the 6-months' assessment. However, symptomatic benefit did not correlate with improvement in psychological indices suggesting that this might not be the predominant mechanism of action for gut-directed hypnotherapy. More work is needed to further elucidate its independent role in relation to other factors involved in the treatment response.

The effect of restricting FODMAPs on psychological status is of interest, particularly in association with reports of improved depression in women with fructose malabsorption

following restriction of fructose intake.[240, 250, 251] When the patients were strictly low FODMAP there were some improvements in psychological status, but such changes correlated with symptomatic improvement, suggesting a causal relationship. However, these improvements were not sustained. If FODMAP intake is indeed associated with anxiety or depression, it would not be anticipated that improvements would be sustained since strict adherence to the low FODMAP diet was not an aim of the study or a feature of the participant's dietary behaviour. The divergence of psychological effects of the low FODMAP diet and gut-directed hypnotherapy does suggest that gut-directed hypnotherapy has specific psychological benefits, not just improvements associated with lower severity of gastrointestinal symptoms.

If expertise to deliver the low FODMAP diet and gut-directed hypnotherapy were available to manage a patient with IBS, it is uncertain which should be applied first. Certainly, the use of combined therapy is not supported. Predictors of response were not identified and the study was not of sufficient size to do this effectively. Gut-directed hypnotherapy carries some advantages. Adverse side effects of hypnotherapy are rare and when performed by an appropriately qualified and experienced practitioner, gut-directed hypnotherapy is considered exceptionally safe.[252] It is highly effective regardless of patients' individual hypnotic capacities.[144, 165] Disadvantages of gut-directed hypnotherapy include a lack of hypnotherapists skilled in gut-directed techniques, the financial burden of a therapeutic course and the time commitment needed (six weekly one-hour sessions as outlined in the current study).

The low FODMAP diet has the advantage that it utilises the interest in food-choice for better health thereby empowering the patients to influence their condition. However, several potential shortcomings of the low FODMAP diet are worth considering. The first relates to nutritional adequacy, which has only been specifically investigated in one study, where a

deficiency of calcium intake was noted.[81] Fibre intake is also at risk since wheat products, legumes and fruit and vegetables are an important part of fibre intake. Secondly, recent studies of the effect of altering FODMAP intake on the faecal microbiota have suggested a potential issue with regard to the loss of prebiotic effect of FODMAPs (particularly oligosaccharides) when adherence to the low FODMAP diet is strict,[253] although, in the present study, ongoing strict adherence was discouraged by the instructing dietitians and indeed practiced by only a minority of the participants. The third relates to the risk of precipitating an eating disorder such as orthorexia nervosa (the unhealthy obsession with eating healthy food).[254] While not recognised in the current Diagnostic and Statistical Manual of Mental Disorders (DSM-V), the increasing fixation of righteous eating within the community is undeniable. As such, a non-dietary therapy, such as gut-directed hypnotherapy, would be useful in preventing the escalation of such growing obsession.

The current study had some limitations. No direct questioning about the diets of individuals who only received gut-directed hypnotherapy was formally undertaken. Despite this, all participants were FODMAP-naïve and were unlikely to be over-restricting particular foods groups from their diet. Secondly, a small proportion of participants reported applying alternative treatment(s) between receiving the intervention and the study completion. This number was, however, evenly distributed across the groups and was thus unlikely to affect the overall primary end-point of gastrointestinal symptom change. Finally, the study was not adequately powered to assess predictors of response to the gut-directed hypnotherapy. Without a greater understanding of possible predictors, it makes it difficult to know in whom hypnotherapy should be applied.

7.5. Conclusions and Future Directions

In conclusion, the efficacy gut-directed hypnotherapy is comparative to that of the low FODMAP diet for relief of gastrointestinal symptoms and improving QOL in IBS patients, but these modalities do not show additive effects. Gut-directed hypnotherapy provides an additional benefit of improvement in psychological indices concerning anxiety and depression. Gut-directed hypnotherapy is an effective alternative to the low FODMAP diet and should be considered a viable modality as primary therapy for patients with IBS.

Chapter 8 - General Discussion

The preceding chapters of the current thesis have sought to explore the link between mental health and functional gastrointestinal disorders (FGID) where considerable contributions to our understanding of the role of mental health in patients with irritable bowel syndrome (IBS) and non-coeliac gluten sensitivity (NCGS) have been made. The following chapter highlights these contributions and considers the current state of the literature before drawing attention to some unanswered questions and future research directions.

8.1. Linking Mental Health and Non-coeliac Gluten Sensitivity

Issues around the observation that a greater number of individuals around the world are adopting a gluten-free diet have been examined in this thesis. In fact, this observed increase in gluten avoidance has now gone beyond levels that may be explained by the percentage of the population with the well-defined medical condition, coeliac disease. The reasons behind this increase are often associated with the belief that gluten consumption causes an increase in extraintestinal manifestations including changes to mental health, and gastrointestinal symptoms, where removal of gluten from the diet results in the reversal of these aliments. These patients, in the absence of coeliac disease or wheat allergy, are defined as having NCGS.[97, 104] Despite a considerable number of studies having had previously focused on the link between gluten ingestion and gastrointestinal symptoms[86, 125, 130-134] there appeared to be a sizeable lack of consideration that gluten ingestion may also contribute to extraintestinal

manifestations amongst this entity. Work presented in Chapters 4 and 5 has attempted to address this issue where gluten ingestion was explored in relation to psychological indices including depression, anxiety, stress and cognitive function as well as quality of life and fatigue.

(i) Chapter 4: A randomised, double-blind, placebo-controlled trial where 22 participants received one of three dietary challenges for 3 days was undertaken. Challenge food was supplemented with gluten (16 g/day), whey (16 g/day) or not supplemented (placebo). Results showed that gluten ingestion was associated with higher overall state depression scores compared to placebo where it was suggested that patients with NCGS may feel better on a gluten-free diet due to changes in mental state.

(ii) Chapter 5: The study presented in Chapter 5 was designed to be a longer and more detailed examination of the effects of gluten on extraintestinal manifestations in patients with NCGS than that reported in Chapter 4. It was randomised, double-blind, placebo-controlled trial where 16 participants received one of two different muesli bar challenges for 14 days. The muesli bars were supplemented with gluten (16 g/day) or not supplemented (placebo). Participants consumed their normal gluten-free diet throughout the study trial. Results showed no gluten-specific worsening of depression, anxiety, stress, quality of life or fatigue. The only significant outcome was for a hint that gluten-specific subtle changes in cognition might be present (n=6).

8.2. Gastrointestinal Symptoms and Non-coeliac Gluten Sensitivity

Evidence that gluten ingestion is associated with the worsening of gastrointestinal symptoms in patients with NCGS is scant. There have been some reports that suggest gluten-specific gastrointestinal effects amongst this entity[125, 130-134] but issues with patient selection and study design make these findings difficult to interpret. These issues need be considered before future investigations are undertaken, and are discussed in detail below. Other reports, notably, have failed to observe gluten-specific gastrointestinal effects,[86, 239] including those presented in Chapters 4 and 5.

(i) Chapter 4: No difference was identified across the dietary challenges for either overall or individual gastrointestinal symptoms. However, an order effect was apparent, with the first intervention being associated with greater symptomatic changes than the second or third challenges. Strong anticipatory responses are common, and this may be especially so in NCGS patients, the so-called *nocebo* response.

(ii) Chapter 5: Gluten-specific induction of gastrointestinal symptoms was similarly not described during this investigation. No order effects in this latter investigation were apparent.

8.3. What Part of Wheat is Responsible?

Wheat is the most cultivated gluten containing crop that appears in our everyday food supply. Everything from breads and pastas to sauces used as marinades contain wheat gluten. Notably,

however, wheat contains not only protein but also carbohydrate fractions. The potential impact of these two independent fractions in patients with NCGS is worth consideration.

8.3.1. Protein

8.3.1.1. Albumins, globulin, gliadins and glutenins

The protein content of wheat is a complex mix of different but related proteins including albumin, globulin, gliadin and glutenin classes, all of which have the potential to elicit symptomatic responses. For instance, many proteins in each of those sub-groups have been implicated in wheat allergy.[255] As such, it remains pertinent to ascertain whether wheat protein as a whole or gluten specifically is primarily responsible for changes to extraintestinal manifestations, including state depression and cognition, in patients with NCGS. Should an association be confirmed then attention can then be given to identifying the component/s of wheat protein are responsible for this effect.

8.3.1.2. Amylase-trypsin inhibitors and wheat lectins

Two other candidates in the wheat protein that potentially might induce symptoms are wheat α-amylase-trypsin inhibitors (ATI) and wheat lectins. ATI, the natural pesticides found in wheat, activate toll-like receptor 4 (TLR4) in the intestine and elicit strong innate immune effects not only *in vitro* but also *in vivo* in animal experiments after oral or systemic challenge.[247] This reaction may have broad implications for patients with NCGS.[247] Likewise, wheat lectin agglutinin (WGA) has received some attention as studies have been able to demonstrate that WGA can increase intestinal permeability and activate the immune system at very low doses.[256] While evidence regarding ATI and WGA is preliminary and confined to animal studies or work *in vitro*, they are worthy of further investigation.

8.3.2. Carbohydrate

There is no certainty that it is withdrawal of gluten from the diet that specifically improves symptoms in patients with NCGS. Nor can it be confirmed from elimination and re-challenge studies that gluten specifically triggers extraintestinal manifestations or gastrointestinal symptoms in patients with NCGS, as can be clearly demonstrated in the cohorts studied in this thesis. It should, therefore, be considered that other components of food may be the culprit(s).

There is good evidence that wheat and other gluten-containing grains contain significant quantities of poorly absorbed short-chain carbohydrates, particularly fructans.[139] It is likely, therefore, that 'gluten restriction' automatically reduces a patient's dietary fructan intake and may contribute to why patients with NCGS report feeling better on a gluten-free diet. The restriction of fructans (as well as other known FODMAPs [Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols]) has been shown to uniformly reduce gastrointestinal symptoms in patients with NCGS who, when challenged with gluten showed no symptomatic effects.[86] This association was similarly observed in Chapter 4 where no significant worsening of gastrointestinal symptoms was observed once dietary FODMAP intake had been controlled.

8.4. Unanswered Questions and Future Research Directions

8.4.1. What is the significance of the mental health findings in NCGS?

That exposure to gluten specifically induced current feelings of depression was shown in Chapter 4 but was unable to be reproduced in Chapter 5. Reasons for these discrepant findings

were discussed in Chapter 5, but should be readdressed here since variable findings seem to be attracted to studies with self-reported NCGS. The first and obvious issue was that of inadequate power. The first study was exploratory and provided data to enable power calculations for a subsequent study to be calculated. Challenges with patient recruitment led to far fewer participants being entered into the study than anticipated. However, detailed examination of the data obtained from those patients revealed not even a hint of effect. If power calculations were performed on the data obtained, then a future study would have required vast numbers of patients to have a chance of showing a gluten-specific effect. Hence, it is unlikely that power was responsible for the lack of effect. Differences in design of the study protocol may have been responsible. Participants in study one were exposed to each dietary intervention for 3 days versus those in the subsequent study where each challenge lasted 14 days. There was also no recorded reporting of psychological endpoints on day 3 in the subsequent study where any gluten induced feelings of depression may have been missed by reporting on day 14. Future studies should overcome this by examining psychological changes at multiple time points, including at day 3. The STPI has good test-retest reliability and so can be administered regularly.[257] Preliminary suggestions that gluten may induce subtle changes in cognition were also described. These results, however, require further investigation since the effect was derived from only 6 participants. Heathy controls should also be included to confirm that any gluten-specific psychological effects are characteristic of NCGS and not within the limits of 'normal'. The effects of gluten on mental health in healthy controls has seldom been studied, although it would be anticipated that any effects would be minimal. If changes in mental health are found to be gluten-specific, the mechanisms involved require elucidation.

8.4.2. What part of the wheat protein is responsible?

If it can be ascertained that the wheat protein is definitely responsible for the induction of depressive symptoms or changes to cognition in patients with NCGS, attention should be given to identifying the component of the wheat protein that is responsible for these effects. The successful development and characterisation of a method for the large-scale isolation of gliadin and glutenin in Chapter 6 creates an opportunity for effects of these proteins to be observed independently of one another in patients with NCGS. Contamination of the challenge mediums has been a criticism of previous NCGS investigations. Assessing symptom responses in a randomised, placebo-controlled, cross-over study of placebo, gliadin (8 g/day) and glutenin (8 g/day) for 14 days each and examining psychological changes at multiple time points (i.e. day 3, 7 and 14) is one suggested protocol.

8.4.3. Study design in NCGS populations: Working towards a consensus

A large problem within the NCGS literature is the extensive variation in study design. For example, some studies have used parallel designs[125, 133] while others have employed the cross-over technique.[86, 130-132, 134] The challenge medium has also differed where some studies have used carbohydrate-deplete wheat gluten[86, 125] while other have used whole wheat flour[130, 131, 134] and even purified gluten[132, 133] has been used on occasion, all with varying durations of exposure and dose. Furthermore, the study populations themselves have differed were some have included participants whom report any symptomatic improvement following gluten withdrawal (regardless of whether this relates to extraintestinal and/or gastrointestinal symptoms)[131, 132, 134] but others have required that participants also meet the definition of IBS.[86, 125, 130, 133] Coeliac disease has also not always been adequately excluded.[130] As such, it is important that consistent study designs are employed across research groups so that

worthwhile comparisons can be made between studies. The following recommendations are made for consideration:

(i) Cross-over-design: The use of cross-over versus parallel designs within NCGS populations is contentious. Given that high nocebo or anticipatory responses and large cross over effects are frequently described amongst NCGS cohorts, it could be argued that the cross-over design is not the ultimate design choice. Nocebo responses are, however, common in IBS studies where food is considered the culprit and may not be specific to NCGS populations. Notably, consistent issues with either anticipatory responses or cross-over effects were not observed between chapters in the current thesis (i.e. an order effect was seen in Chapter 4 but not Chapter 5). This, taken with the fact that NCGS is likely to exist in only a very small percentage of the population, makes the cross-over design apt in that it provides information about gluten specific responses in each individual. Parallel group studies may not be as suitable where a minority of patients are likely to have the response that is being measured, as seems to be the case with NCGS.

(ii) Patient-selection: Patients who meet the current criteria for NCGS should be included. NCGS may not be necessarily characterised by gastrointestinal symptoms, as Chapters 4 and 5 suggest, and so inclusion criteria stipulating a diagnosis of IBS may inappropriately exclude some participants. It is imperative that coeliac disease has also been convincingly excluded. Reviewing the quality of the duodenal biopsies is essential as is making sure the participants were consuming adequate amounts of gluten at the time of collection. The Australian Therapeutic Guidelines describes an adequate gluten challenge as the equivalent of four to six slices of bread (16-20 g gluten) per day for at least six weeks prior to being tested for coeliac disease.[258] Several former investigations have included participants that might be part of the spectrum of coeliac disease, with increased with intraepithelial lymphocytosis and evidence of

immune activation, and so it must be ensured that these participants are not included in future investigations where their reports are likely to distort results.

(iii) Re-challenge substrates: Some studies have used carbohydrate-deplete gluten, similar to what was used in the studies contained within this thesis, but others have also used whole wheat flour and purified gluten. Interestingly, what defines 'purified gluten' is often not described and so little is known about the actual purity of this product. It may be that the 'purified gluten' used was the same as what was employed in the studies contained within the current thesis but this cannot be ascertained by the published reports. Issues with whole wheat flour lie within the potential contaminants and so it is suggested that carbohydrate-deplete gluten is the most viable option. Additionally, the way in which the substrate is delivered including in whole foods or capsules is worthy of thought. Providing all food to participants can be costly and difficult given individual food preferences. As such, a more convenient way is to have participants consume their normal gluten-free diets, in which they feel comfortable, but utilise the knowledge of an experienced dietitian to ensure all meals are gluten-free and that the same meals are consumed consistently between challenges. This is what was done in Chapter 5 with relative ease and good compliance was achieved by participants. Food capsules is another consideration but given the large and multiple capsules that would be needed to deliver the necessary quantities of gluten prescribed (for example 10 capsules per day were needed to deliver 4.375 g gluten in a recent study[132]) and that capsules is not how people are usually exposed to gluten makes this options less appealing. Capsules can also be easily opened by participants where differences between the substances can be determined (i.e. via taste) and blinding is subsequently lost.

8.5. Gluten and Mechanisms of Action

It remains to be proven if and how gluten has direct causal effects on extraintestinal manifestations, including changes to mental state and cognitive function. Should such as association exist the proposed mechanisms responsible for these effects may include (1) a potential link between protein ingestion, tryptophan production and serotonin concentration in the brain (2) gluten exorphins, and (3) gluten mediated changes in gut microbiota. The pathophysiology of gastrointestinal symptoms associated with NCGS is also not well understood. Some studies have suggested an important role of the innate immune system but others have suggested it may be a mixed disease, with an activation of both innate and adaptive immunity.[118-122] Work from within our own department (Department of Gastroenterology, Monash University) has shown no differences for a variety of markers assessing immune reactions (predominantly coeliac-related adaptive immune pathways), inflammatory responses or poor digestibility of the gluten protein.[86, 125] Newer work has also focused on the role of zonulin signalling with regard to potential increased permeability to macromolecules of gliadin.[124] This work, however, is very preliminary having only been conducted in *ex vivo* conditions and requires further elucidation. Identifying biomarkers to diagnose NCGS would certainly make this entity easier to assess and would help to distinguish between those who are truly gluten sensitive and those in whom other potential components of wheat are most likely to cause symptoms.

8.6. Clinical Implications

It may be that the current diagnostic criteria for NCGS are inadequate. These criteria are not supported by latest gold-standard clinical trials where adequate symptomatic improvement

was not found amongst many patients with currently-defined NCGS following gluten exclusion, despite patients reporting that their symptoms had remarkably improved and were well controlled,[86] nor were symptoms uniformly invoked following the re-introduction of gluten into the diet.[86, 134] Many interpretations of the current definition also permit patients with possible coeliac disease to be included. As discussed, it is possible that gluten itself is not a specific trigger of gastrointestinal symptoms in patients with NCGS and that it is actually the concurrent reduction of fructans, or other wheat-associated components that may be responsible for the observed symptomatic improvements. Not surprisingly, due to such diagnostic difficulties, and limited and conflicting study findings, how to treat patients with possible NCGS remains controversial. Until a clear consensus is reached, the following management approach is suggested, as illustrated in Figure 8.1.

First, the diagnosis should not be entered into lightly. Dietary inadequacies are known to be common amongst those following a gluten-free diet and may relate to inherent deficiencies within the gluten-free diet itself.[259] Therefore, it is important for patients to undergo detailed medical assessment and diagnosis. To diagnose NCGS, it is imperative that coeliac disease is adequately excluded by reviewing the quality of the duodenal biopsies previously performed and whether they were taken when the patient was consuming adequate amounts of gluten. If no formal assessment of coeliac disease has been made then this should be undertaken. HLA typing is useful in patients whom are reluctant to undergo the gluten challenge where more than 98% of people with coeliac disease share the major histocompatibility complex II class HLA-DQ2 or HLA-DQ8 haplotype.[260] People who do not have the HLA-DQ2 or HLA-DQ8 haplotypes are unlikely to have coeliac disease and thus are not required to undergo the gluten challenge. The next step is a trial of the low FODMAP diet, which includes the reduction (but not exclusion) of gluten-containing grains by virtue of their usual co-existence. Only when the patient shows no or minimal symptomatic response to the low FODMAP diet, should gluten

be considered as the trigger. Following gluten exclusion, and provided there is marked improvement in symptoms, blinded challenges (that is, monitored reintroduction of gluten) can be subsequently undertaken. Although this approach is complex, a more practical approach will only be possible with the development of diagnostic biomarkers or other clinical predictors.

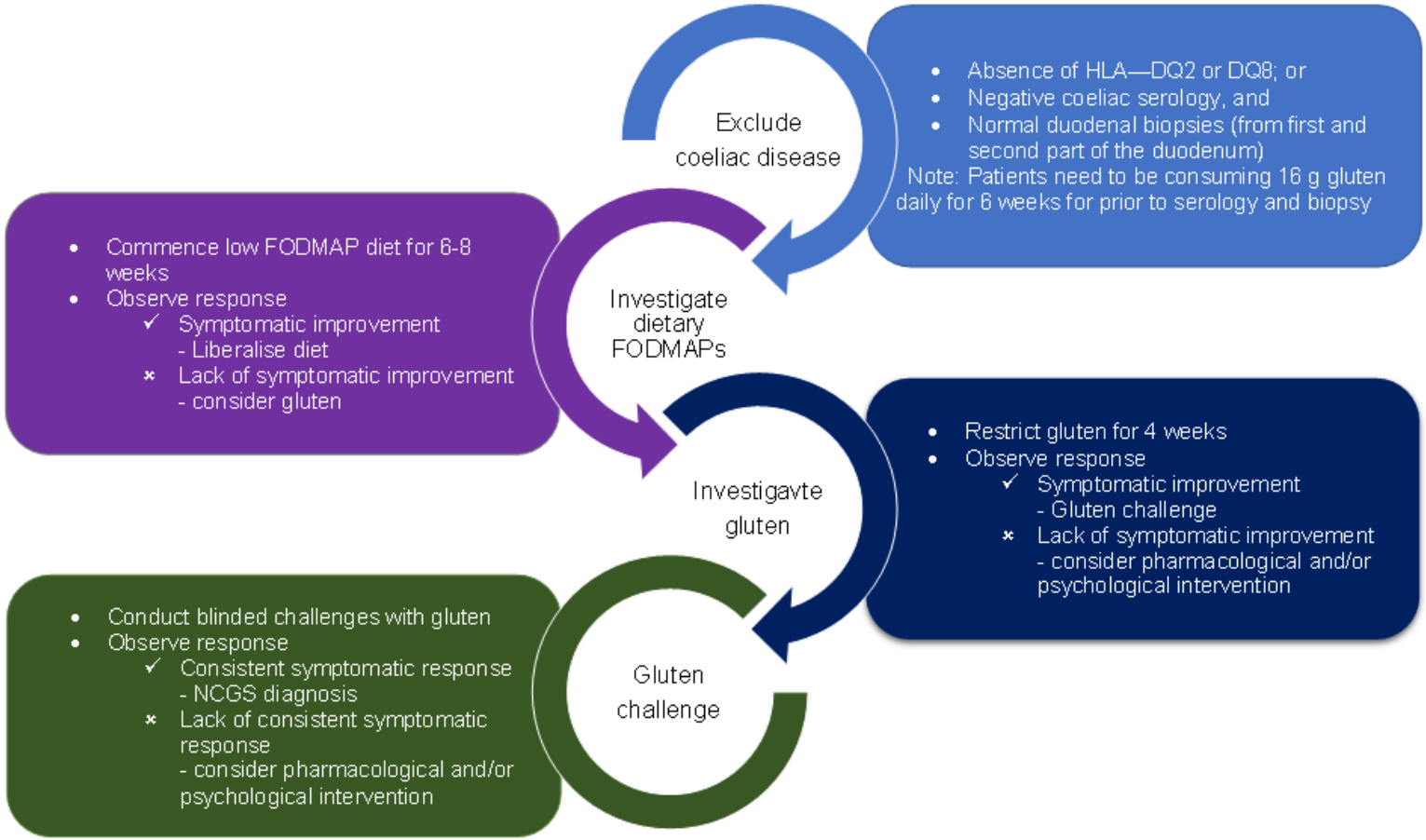


Figure 8.1. Suggested flow chart for diagnosing non-coeliac gluten sensitivity (NCGS)

8.7. Non-Coeliac Gluten Sensitivity or Irritable Bowel Syndrome?

The studies reported in Chapter 4 and 5, along with those in the literature, have failed to convincingly support a specific entity for NCGS in patients who would otherwise be classified as having IBS or other FGID. Notably, the low FODMAP diet is the dietary 'gold standard' for the treatment of IBS but this therapy does not help everyone where 30% of patients will continue to experience ongoing symptoms despite good dietary compliance. As such, additional therapeutic interventions are needed. It would be interesting to see a study of the application of a gluten-free diet in this setting.

8.8. Expanding the Irritable Bowel Syndrome Treatment Paradigm Using Gut-directed Hypnotherapy

Gut-directed hypnotherapy is becoming increasingly popular in the community as a treatment for IBS but its use in mainstream clinical settings continues to be limited its lack of high-quality evidence of efficacy. Largely this lack of evidence is due to difficulties in designing clinical trials with appropriately blinded placebos. An alternative to this would be to compare gut-directed hypnotherapy to a therapy with proven efficacy. This concept was explored in Chapter 7.

(i) Chapter 7: A randomised-controlled trial comparing the efficacy of the low FODMAP diet, gut-directed hypnotherapy and a combination of both was undertaken. Results revealed improvements in overall and individual gastrointestinal symptoms from baseline to the end of

treatment with no difference in the degree of symptomatic improvement between treatment groups. 71% of participants improved at week 6 with the low FODMAP diet, 72% with the gut-directed hypnotherapy, and in 72% who received the combination of both. These improvements were maintained long-term (6-months). Gut-directed hypnotherapy resulted in superior improvements in psychological indices, but all groups improved similarly for quality of life.

8.9. Gut-directed Hypnotherapy as a Viable Treatment in Patients with Irritable Bowel Syndrome

That the efficacy of gut-directed hypnotherapy is comparable to that of the low FODMAP diet for the relief of gastrointestinal symptoms makes gut-directed hypnotherapy a viable modality as a primary therapy for patients with IBS. While it is largely applied in patient's refractory to other treatment, given the observed improvements relatively untouched patients in Chapter 7, and considering the additional psychological benefits of gut-directed hypnotherapy over the low FODMAP diet, an argument that gut-directed hypnotherapy should be applied before the low FODMAP can be made. There are, however, several issues that remain either unanswered or noteworthy in progressing the more widespread use of gut-directed hypnotherapy in patients with IBS.

8.9.1. Mechanisms of action

The mechanistic action of gut-directed hypnotherapy is unknown. There is strong evidence that it can influence both psychological and physiological outcomes but only psychological aspects were addressed in Chapter 7. Here it was found that gut-directed hypnotherapy, but

not the low FODMAP diet or the combined treatment, was associated with the reduction of anxiety and depression as measured on the State Trait Depression Inventory (STPI) and the Hospital Anxiety and Depression Scale (HADS). These effects did not emerge immediately post-treatment but could be observed by the 6-month follow-up time-point. Notably, gastrointestinal symptom improvement did not correlate with improvement in psychological indices suggesting that this might not be the predominant mechanism of action for gut-directed hypnotherapy. More work is needed to further elucidate its independent role in relation to other factors involved in the treatment response. Potential areas of consideration could include brain imagining studies. Brain activity during and after a state of hypnosis and potential changes to neural pathways would be worthwhile although these studies are often fraught with complicated methodologies and can be quite costly to conduct.

8.9.2. Predictors of response

It seems realistic to offer gut-directed hypnotherapy to those patients most likely to respond and an alternative therapy to those which hypnotherapy is unlikely to be beneficial. However, work conducted to identify predictors of response has produced inconclusive results. The focus in Chapter 7 was based primarily on demographic characteristics such as age and gender but bowel habit subtype was also considered. Predictors of response were not identified in this investigation but the study was not of sufficient size to do this effectively. Work produced by others has, however, suggested that women with IBS respond more favourably to hypnosis than men.[157, 165] For example, in an audit of 1,000 consecutive patients, 80% of women responded as opposed to 62% of men, However, the observable improvement in men was still encouraging when compared with that obtained in pharmacological studies.[165] Bowel habit subtype according to ROME Foundation criteria was similarly not shown to have apparent influence on outcomes.[165] Personality traits, imaginative ability and

expectancy have also been explored with some demonstrable effect.[174, 175] As it currently stands, no conclusive predictors applicable to routine practice have been identified but future work identifying these would help with knowing in whom gut-directed hypnotherapy should be applied.[252]

8.9.3. How important is hypnotic susceptibility?

Several scales have been developed to determine how easily a person can be hypnotised. The most common scales of hypnotic susceptibility include the Harvard Group Scale of Hypnotic Susceptibility[261] and the Stanford Hypnotic Susceptibility Scale[262] both of which can be applied with relative ease. Despite the ease of application, the usefulness of such scales is questionable. While it is well established that people differ in their hypnotic capacities, and despite the great majority of people being able to experience hypnosis, not everyone is equally responsive.[252] For example, hypnotic susceptibility has not been shown to correlate with the effectiveness of therapy amongst IBS populations.[144, 165] While no specific scale for hypnotic susceptibility was employed in Chapter 7, the random allocation of participants to the three treatment conditions would have resulted in having patients with varying levels of hypnotic susceptibility within each group. It may be, therefore, that the percentage of people who didn't respond in either the gut-directed hypnotherapy (28%) or combined treatments (28%) happened to be those with poor hypnotic susceptibility.

8.9.4. Availability of suitably trained hypnotherapists

Very few professionals are trained for the specific implementation of gut-directed hypnotherapy and, therefore, their services can be difficult to access. Importantly, once suitably trained, there does not appear to be any operator-related influence on outcomes.[146] Despite this, the practice of hypnotherapy is both time-consuming and expensive. With no available data one can only speculate that while the time and cost associated with a course of

gut-directed hypnotherapy can be considerable, it may help to reduce the totalling cost to the economy when patients are repeatedly seeking health care professionals often with limited success.

A possible solution to this issue is to offer group, as opposed to individual, gut-directed hypnotherapy sessions. Gut-directed hypnotherapy has been used successfully in group settings where improvement was observed for overall gastrointestinal symptoms and psychological manifestations.[148, 171, 177] This observable improvement was found to be directly comparable to individual gut-directed hypnosis in one study, albeit with small participant numbers.[171] It may be that implementing group gut-directed hypnotherapy will make it accessible and affordable without reducing the overall effectiveness of the treatment.

Providing gut-directed hypnotherapy via other means such as online or through the development of a smartphone app may also be worthwhile. It may be that these interactive measures will be as effective as one-on-one sessions in which case the limitations regarding time and cost would be abolished. This needs considerable investigation benefit before the development or recommendation of these mediums is made to patients.

8.9.5. Timing of gut-directed hypnotherapy relative to other treatment modalities

Comparison of the rate of response to gut-directed hypnotherapy in patients with IBS suggests that it is at least as good as some of the new and expensive pharmacological treatment options[146] and was shown to be equally as efficacious as the low FODMAP diet. This together with the fact that there are no known side effects of hypnotherapy make gut-directed hypnotherapy a competitive treatment option.[146] Aside from the limitations imposed by the lack of hypnotherapists skilled in gut-directed techniques, the financial burden of a therapeutic course and the time commitment needed (usually between 6-12 one-hour

sessions), one could argue that, in patients who are willing to undertake such a course of therapy, it should be offered early in the management of IBS. However, there are no data for or against such a speculative contention. In practice outside expert, investigative centres, it appears to be most often offered in those who are unresponsive to other treatments, an extremely challenging group.

8.9.6. Using gut-directed in other functional gastrointestinal disorders

That gut-directed hypnotherapy improves overall and individual gastrointestinal symptoms, as well as psychological indices and quality of life, and that these improvements are maintained in the long-term, warrants this therapy to be given consideration in the treatment of other FGID. Considering that symptoms associated with individual FGIDs differ, although some symptoms commonly overlap, further investigations should be considered in specific patient populations. Notably, however, is that these disorders are all functional in nature and it would be expected that they would observed similar improvements to that observed in IBS populations following a course of gut-directed hypnotherapy.

8.10. Conclusions

The current thesis has contributed to our understanding of the role of mental health in patients with FGIDs. Our understanding of extraintestinal manifestations in patients with NCGS has increased albeit by contrasting findings. This thesis has also been the first to compare the efficacy of gut-directed hypnotherapy to that of the low FODMAP diet, the ‘gold standard’ dietary therapy in IBS populations.

Chapters 4 and 5 assessed the effects of gluten on extraintestinal symptoms in patients with self-reported NCGS where coeliac disease had been definitively excluded and who had reported symptomatic improvement on a gluten-free diet. Chapter 4 showed that short-term exposure to gluten specifically induced current feelings of depression with no effect on other indices or on emotional disposition. Gluten-specific induction of gastrointestinal symptoms was not observed. Chapter 5, using a longer and more detailed examination of psychological indices, showed no evidence of specific effects of gluten, with the exception of small increased response times on the Subtle Cognitive Impairment Test (SCIT). Similarly, gluten-specific induction of gastrointestinal symptoms was not observed. It may be that a lack of power and study design contributed to the lack of confirmatory psychological effects in Chapter 5. Future studies are warranted with more frequent measures of psychological outcomes.

Given these inconsistent results, however, and the high co-existence of gluten and FODMAPs in commonly consumed grain and cereal products, it is possible that any observed improvements psychological manifestations and/or gastrointestinal symptoms are the result of restricted poorly-absorbed short-chain carbohydrates, not gluten, since gluten restriction automatically reduces dietary FODMAP intake.

That gluten may not be a specific trigger of symptoms once dietary FODMAPs are reduced suggests a potential overlap between NCGS and IBS where other treatments can be applied. Additional therapeutic interventions in the IBS arsenal are warranted and built the rationale for Chapter 7 where the efficacy of gut-directed hypnotherapy was compared to that of the low FODMAP diet and showed comparative effectiveness. Gut-directed hypnotherapy should be considered a viable modality as primary therapy for patients with IBS. Whether NCGS exists as distinct entity from IBS needs to be established and warrants further exploration.

Reference List

1. Ong D, Mitchell S, Barrett J, *et al.* Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. *J Gastroenterol Hepatol* 2010; 25: 1366-73.

2. Drossman DA, Dumitrascu DL. Rome III: New standard for functional gastrointestinal disorders. *J Gastrointestin Liver Dis* 2006; 15: 237-41.

3. Hungin A, Whorwell P, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: An international survey of 40,000 subjects. *Aliment Pharm Ther* 2003; 17: 643-50.

4. Hillilä M, Färkkilä M. Prevalence of irritable bowel syndrome according to different diagnostic criteria in a non-selected adult population. *Aliment Pharm Ther* 2004; 20: 339-45.

5. Quigley E, Fried M, Gwee K, Olano C, Guarner F, Khalif I. Irritable bowel syndrome: A global perspective. *WGO Practice Guideline* 2009.

6. Serra J, Azpiroz F, Malagelada J. Impaired transit and tolerance of intestinal gas in the irritable bowel syndrome. *Gut* 2001; 48: 14-9.

7. Mayer EA. Irritable bowel syndrome. *New Engl J Med* 2008; 358: 1692-9.

8. Mertz H, Morgan V, Tanner G, *et al.* Regional cerebral activation in irritable bowel syndrome and control subjects with painful and nonpainful rectal distention. *Gastroenterology* 2000; 118: 842-8.

9. Buhner S, Li Q, Vignali S, *et al.* Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* 2009; 137: 1425-34.

10. Carroll IM, Ringel-Kulka T, Siddle JP, Ringel Y. Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroent Motil* 2012; 24: 521-48.

11. Duboc H, Rainteau D, Rajca S, *et al.* Increase in fecal primary bile acids and dysbiosis in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroent Motil* 2012; 24: 513-47.

12. Jeffery IB, O'Toole PW, Öhman L, *et al.* An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut* 2012; 61: 997-1006.

13. Kassinen A, Krogius-Kurikka L, Mäkivuokko H, *et al.* The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* 2007; 133: 24-33.

14. Ford AC, Talley NJ, Spiegel BM, *et al.* Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: Systematic review and meta-analysis. *BMJ* 2008; 337.

15. Hovdenak N. Loperamide treatment of the irritable bowel syndrome. *Scand J Gastroenterol* 1987; 22: 81-4.

16. Cann P, Read N, Holdsworth C, Barends D. Role of loperamide and placebo in management of irritable bowel syndrome (IBS). *Digest Dis Sci* 1984; 29: 239-47.

17. Efskind P, Bernklev T, Vatn M. A double-blind placebo-controlled trial with loperamide in irritable bowel syndrome. *Scand J Gastroenterol* 1996; 31: 463-8.

18. Ford A, Talley N, Schoenfeld P, Quigley E, Moayyedi P. Efficacy of antidepressants and psychological therapies in irritable bowel syndrome: Systematic review and meta-analysis. *Gut* 2009; 58: 367-78.

19. Clouse R. Antidepressants for irritable bowel syndrome. *Gut* 2003; 52: 598-9.

20. Ford AC, Brandt LJ, Young C, Chey WD, Foxx-Orenstein AE, Moayyedi P. Efficacy of 5-HT3 antagonists and 5-HT4 agonists in irritable bowel syndrome: Systematic review and meta-analysis. *American J Gastroenterol* 2009; 104: 1831-43.

21. Salonen A, de Vos WM, Palva A. Gastrointestinal microbiota in irritable bowel syndrome: Present state and perspectives. *Microbiology* 2010; 156: 3205-15.

22. Jeffery IB, Quigley EM, Öhman L, Simrén M, O'Toole PW. The microbiota link to irritable bowel syndrome: An emerging story. *Gut Microbes* 2012; 3: 572-6.

23. Rajilić–Stojanović M, Biagi E, Heilig HG, *et al.* Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* 2011; 141: 1792-801.

24. Hungin A, Mulligan C, Pot B, *et al.* Systematic review: Probiotics in the management of lower gastrointestinal symptoms in clinical practice: An evidence-based international guide. *Aliment Pharm Ther* 2013; 38: 864-86.

25. Whelan K. Probiotics and prebiotics in the management of irritable bowel syndrome: A review of recent clinical trials and systematic reviews. *Curr Opin Clin Nutr* 2011; 14: 581-7.

26. Floch MH. Recommendations for probiotic use in humans: A 2014 update. *Pharmaceuticals* 2014; 7: 999-1007.

27. Hoveyda N, Heneghan C, Mahtani KR, Perera R, Roberts N, Glasziou P. A systematic review and meta-analysis: Probiotics in the treatment of irritable bowel syndrome. *BMC Gastroenterology* 2009; 9: 15.

28. Moayyedi P, Ford AC, Talley NJ, *et al.* The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut* 2010; 59: 325-32.

29. Didari T, Mozaffari S, Nikfar S, Abdollahi M. Effectiveness of probiotics in irritable bowel syndrome: Updated systematic review with meta-analysis. *World J Gastroentero* 2015; 21: 3072-84.

30. Whorwell PJ, Altringer L, Morel J, *et al.* Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *American J Gastroenterol* 2006; 101: 1581-90.

31. O'Mahony L, McCarthy J, Kelly P, *et al.* Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; 128: 541-51.

32. Roberfroid M, Gibson GR, Hoyles L, *et al.* Prebiotic effects: Metabolic and health benefits. *Brit J Nutr* 2010; 104: S1-S63.

33. Olesen M, Gudmand-Høyer E. Efficacy, safety, and tolerability of fructooligosaccharides in the treatment of irritable bowel syndrome. *Am J Clin Nutr* 2000; 72: 1570-5.

34. Hunter J, Tuffnell Q, Lee A. Controlled trial of oligofructose in the management of irritable bowel syndrome. *J Nutr* 1999; 129: 1451-3.

35. Paineau D, Payen F, Panserieu S, *et al.* The effects of regular consumption of short-chain fructo-oligosaccharides on digestive comfort of subjects with minor functional bowel disorders. *Brit J Nutr* 2008; 99: 311-8.

36. Silk D, Davis A, Vulevic J, Tzortzis G, Gibson G. Clinical trial: The effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharm Ther* 2009; 29: 508-18.

37. Mendall MA, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). *Eur J Gastroen Hepat* 1998; 10: 59-62.

38. Pimentel M, Park S, Mirocha J, Kane SV, Kong Y. The effect of a nonabsorbed oral antibiotic (rifaximin) on the symptoms of the irritable bowel syndrome: A randomized trial. *Ann Intern Med* 2006; 145: 557-63.

39. Sharara AI, Aoun E, Abdul-Baki H, Mounzer R, Sidani S, ElHajj I. A randomized double-blind placebo-controlled trial of rifaximin in patients with abdominal bloating and flatulence. *American J Gastroenterol* 2006; 101: 326-33.

40. Barrett J, Yao C, Canale K, Philpott H, Gibson P. Poor reproducibility of lactulose and fructose breath testing: Impact on clinical management. *J Gastro Hepatol* 2013; 28: 118.

41. Lauritano EC, Gabrielli M, Scarpellini E, *et al.* Small intestinal bacterial overgrowth recurrence after antibiotic therapy. *Am J Gastroenterol* 2008; 103: 2031-5.

42. Halmos EP. Dietary FODMAPs and the pathogenesis of functional gastrointestinal symptoms: Monash University; 2013.

43. Golley S, Corsini N, Topping D, Morell M, Mohr P. Motivations for avoiding wheat consumption in Australia: Results from a population survey. *Public Health Nutr* 2015; 18: 490-9.

44. Aziz I, Branchi F, Pearson K, Priest J, Sanders DS. A Study Evaluating the Bidirectional Relationship Between Inflammatory Bowel Disease and Self-reported Non-celiac Gluten Sensitivity. *Inflamm Bowel Dis* 2015; 21: 847-53.

45. Moayyedi P, Quigley EM, Lacy BE, *et al.* The effect of fiber supplementation on irritable bowel syndrome: A systematic review and meta-analysis. *American J Gastroenterol* 2014; 109: 1367-74.

46. Atkinson W, Sheldon T, Shaath N, Whorwell P. Food elimination based on IgG antibodies in irritable bowel syndrome: A randomised controlled trial. *Gut* 2004; 53: 1459-64.

47. Zar S, Mincher L, Benson MJ, Kumar D. Food-specific IgG4 antibody-guided exclusion diet improves symptoms and rectal compliance in irritable bowel syndrome. *Scand J Gastroenterol* 2005; 40: 800-7.

48. Philpott H, Nandurkar S, Lubel J, Gibson PR. Alternative investigations for irritable bowel syndrome. *J Gastroenterol Hepatol* 2013; 28: 73-7.

49. Loblay RH, Swain AR. Food intolerance. *Rec Adv Clin Nutr* 1986; 2: 169-77.

50. Bogaerts K, Van Oudenhove L. Psychological treatment for irritable bowel syndrome: Future Medicine Ltd 2013.131-148.

51. Bengtsson M, Ulander K, Börgdal EB, Christensson A-C, Ohlsson B. A course of instruction for women with irritable bowel syndrome. *Patient Educ Couns* 2006; 62: 118-25.

52. Colwell L, Prather C, Phillips S, Zinsmeister A. Effects of an irritable bowel syndrome educational class on health-promoting behaviors and symptoms. *American J Gastroenterol* 1998; 93: 901-5.

53. Saito YA, Prather CM, Van Dyke CT, Fett S, Zinsmeister AR, Locke GR. Effects of multidisciplinary education on outcomes in patients with irritable bowel syndrome. *Clin Gastroenterol H* 2004; 2: 576-84.

54. Greene B, Blanchard EB. Cognitive therapy for irritable bowel syndrome. *J Consult Clin Psych* 1994; 62: 576.

55. Payne A, Blanchard EB. A controlled comparison of cognitive therapy and self-help support groups in the treatment of irritable bowel syndrome. *J Consult Clin Psych* 1995; 63: 779-86.

56. Vollmer A, Blanchard EB. Controlled comparison of individual versus group cognitive therapy for irritable bowel syndrome. *Behav Ther* 1999; 29: 19-33.

57. Heymann-Mönnikes I, Arnold R, Florin I, Herda C, Melfsen S, Mönnikes H. The combination of medical treatment plus multicomponent behavioral therapy is superior to medical treatment alone in the therapy of irritable bowel syndrome. *Am J Gastroenterol* 2000; 95: 981-94.

58. Boyce PM, Talley NJ, Balaam B, Koloski NA, Truman G. A randomized controlled trial of cognitive behavior therapy, relaxation training, and routine clinical care for the irritable bowel syndrome. *Am J Gastroenterol* 2003; 98: 2209-18.

59. Drossman DA, Toner BB, Whitehead WE, *et al.* Cognitive-behavioral therapy versus education and desipramine versus placebo for moderate to severe functional bowel disorders. *Gastroenterology* 2003; 125: 19-31.

60. Tkachuk GA, Graff LA, Martin GL, Bernstein CN. Randomized controlled trial of cognitive–behavioral group therapy for irritable bowel syndrome in a medical setting. *J Clin Psychol Med S* 2003; 10: 57-69.

61. Kennedy TM, Chalder T, McCrone P, *et al.* Cognitive behavioural therapy in addition to antispasmodic therapy for irritable bowel syndrome in primary care: Randomised controlled trial: York Publishing Services 2006.

62. Sanders KA, Blanchard EB, Sykes MA. Preliminary study of a self-administered treatment for irritable bowel syndrome: Comparison to a wait list control group. *Appl Psychophys Biof* 2007; 32: 111-9.

63. Blanchard EB, Lackner JM, Sanders K, *et al.* A controlled evaluation of group cognitive therapy in the treatment of irritable bowel syndrome. *Behav Res Ther* 2007; 45: 633-48.

64. Hunt MG, Moshier S, Milonova M. Brief cognitive-behavioral internet therapy for irritable bowel syndrome. *Behav Res Ther* 2009; 47: 797-802.

65. Ljótsson B, Falk L, Vesterlund AW, *et al.* Internet-delivered exposure and mindfulness based therapy for irritable bowel syndrome: A randomized controlled trial. *Behav Res Ther* 2010; 48: 531-9.

66. Moss-Morris R, McAlpine L, Didsbury L, Spence M. A randomized controlled trial of a cognitive behavioural therapy-based self-management intervention for irritable bowel syndrome in primary care. *Psychol Med* 2010; 40: 85-94.

67. Ljótsson B, Hedman E, Lindfors P, *et al.* Long-term follow-up of internet-delivered exposure and mindfulness based treatment for irritable bowel syndrome. *Behav Res Ther* 2011; 49: 58-61.

68. Oerlemans S, van Cranenburgh O, Herremans P-J, Spreeuwenberg P, van Dulmen S. Intervening on cognitions and behavior in irritable bowel syndrome: A feasibility trial using PDAs. *J Psychosom Res* 2011; 70: 267-77.

69. Mahvi-Shirazi M, Fathi-Ashtiani A, Rasoolzade-Tabatabaei S-K, Amini M. Irritable bowel syndrome treatment: Cognitive behavioral therapy versus medical treatment. *Arch Med Sci* 2008; 8: 123-9.

70. Chiarioni G, Whitehead WE. The role of biofeedback in the treatment of gastrointestinal disorders. *Nat Clin Pract Gastr* 2008; 5: 371-82.

71. Furman S. Intestinal biofeedback in functional diarrhea: A preliminary report. *J Behav Ther Exp Psy* 1973; 4: 317-21.

72. Radnitz CL, Blanchard EB. Bowel sound biofeedback as a treatment for irritable bowel syndrome. *Biofeedback Self-Reg* 1988; 13: 169-79.

73. Leahy A, Clayman C, Mason I, Lloyd G, Epstein O. Computerised biofeedback games: A new method for teaching stress management and its use in irritable bowel syndrome. *J Roy Coll Phys Lond* 1997; 32: 552-6.

74. Neff DF, Blanchard EB. A multi-component treatment for irritable bowel syndrome. *Behav Ther* 1988; 18: 70-83.

75. Blanchard EB, Schwarz SP. Adaptation of a multicomponent treatment for irritable bowel syndrome to a small-group format. *Biofeedback Self-Reg* 1987; 12: 63-9.

76. Schwarz SP, Blanchard EB, Neff DF. Behavioral treatment of irritable bowel syndrome: A 1-year follow-up study. *Biofeedback Self-Reg* 1986; 11: 189-98.

77. Blanchard EB, Schwarz SP, Suls JM, *et al.* Two controlled evaluations of multicomponent psychological treatment of irritable bowel syndrome. *Behav Res Ther* 1992; 30: 175-89.

78. Gibson PR, Varney J, Malakar S, Muir JG. Food Components and Irritable Bowel Syndrome. *Gastroenterology* 2015; 148: 1158-74. e4.

79. Shepherd SJ, Gibson PR. Fructose malabsorption and symptoms of irritable bowel syndrome: Guidelines for effective dietary management. *J Am Diet Assoc* 2006; 106: 1631-9.

80. Shepherd S, Parker F, Muir J, Gibson P. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: Randomized placebo-controlled evidence. *Clin Gastroenterol H* 2008; 6: 765-71.

81. Staudacher HM, Lomer MC, Anderson JL, *et al.* Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J Nutr* 2012; 142: 1510-8.

82. Halmos E, Power V, Shepherd S, Gibson P, Muir J. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; 146: 67-75.

83. Böhn L, Störsrud S, Liljebo T, *et al.* Diet low in FODMAPs Reduces Symptoms of Irritable Bowel Syndrome as Well as Traditional Dietary Advice: A Randomized Controlled Trial. *Gastroenterology* 2015.

84. Chumpitazi B, Cope J, Hollister E, *et al.* Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome. *Aliment Pharm Ther* 2015; 42: 418-27.

85. Martin L, van Vuuren C, Seamark L, *et al.* Long term effectiveness of short chain fermentable carbohydrate (FODMAP) restriction in patients with irritable bowel syndrome. *Gut* 2015; 64: A51-2.

86. Biesiekierski J, Peters S, Newnham E, Rosella O, Muir J, Gibson P. No effects of gluten in patients with self-reported non-celiac gluten sensitivity following dietary reduction of low-fermentable, poorly-absorbed, short-chain carbohydrates. *Gastroenterology* 2013; 145: 320-8.

87. Staudacher H, Whelan K, Irving P, Lomer M. Comparison of symptom response following advice for a diet low in fermentable carbohydrates (FODMAPs) versus standard dietary advice in patients with irritable bowel syndrome. *J Hum Nutr Diet* 2011; 24: 487-95.

88. De Roest R, Dobbs B, Chapman B, *et al.* The low FODMAP diet improves gastrointestinal symptoms in patients with irritable bowel syndrome: A prospective study. *Int J Clin Pract* 2013; 67: 895-903.

89. Berghouse L, Hori S, Hill M, Hudson M, Lennard-Jones J, Rogers E. Comparison between the bacterial and oligosaccharide content of ileostomy effluent in subjects taking diets rich in refined or unrefined carbohydrate. *Gut* 1984; 25: 1071-7.

90. Langkilde A, Andersson H, Schweizer T, Würsch P. Digestion and absorption of sorbitol, maltitol and isomalt from the small bowel. A study in ileostomy subjects. *Eur J Clin Nutr* 1994; 48: 768-75.

91. Barrett J, Gearry R, Muir J, *et al.* Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon. *Aliment Pharm Ther* 2010; 31: 874-82.

92. Marciani L, Cox E, Hoad C, *et al.* Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology* 2010; 138: 469-77.

93. Murray K, Wilkinson-Smith V, Hoad C, *et al.* Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. *American J Gastroenterol* 2014; 109: 110-9.

94. Zhu Y, Zheng X, Cong Y, *et al.* Bloating and distention in irritable bowel syndrome: the role of gas production and visceral sensation after lactose ingestion in a population with lactase deficiency. *American J Gastroenterol* 2013; 108: 1516-25.

95. Clausen MR, Jorgensen J, Mortensen PB. Comparison of diarrhea induced by ingestion of fructooligosaccharide idolax and disaccharide lactulose (role of osmolarity versus fermentation of malabsorbed carbohydrate). *Digest Dis Sci* 1998; 43: 2696-707.

96. Madsen JL, Linnet J, Rumessen JJ. Effect of nonabsorbed amounts of a fructose–sorbitol mixture on small intestinal transit in healthy volunteers. *Digest Dis Sci* 2006; 51: 147-53.

97. Sapone A, Bai J, Ciacci C, *et al.* Spectrum of gluten-related disorders: Consensus on new nomenclature and classification. *BMC Med* 2012; 10: 13.

98. Biesiekierski J. Understanding gluten sensitivity: The role of gluten and dietary carbohydrates in the genesis of gastrointestinal symptoms in individuals who do not have coeliac disease. Monash University: Monash University; 2012.

99. Wieser H. Chemistry of gluten proteins. *Food Microbiol* 2007; 24: 115-9.

100. Mayerle J, Tilg H. Clinical Update on Inflammatory Disorders of the Gastrointestinal Tract: Karger Medical and Scientific Publishers 2010.

101. Angéla Juhász FBaCWW. Applied food protein chemistry. Chapter 11: John Wiley & Sons, Ltd 2015.

102. Masci S, Rovelli L, Kasarda D, Vensel W, Lafiandra D. Characterisation and chromosomal localisation of C-type low-molecular-weight glutenin subunits in the bread wheat cultivar Chinese Spring. *Theor Appl Genet* 2002; 104: 422-8.

103. Gibson PR, Muir J, Newnham E. Other dietary confounders: FODMAPS et al. *Digestive Diseases* 2015; 33: 269-76.

104. Ludvigsson J, Leffler D, Bai J, *et al.* The Oslo definitions for coeliac disease and related terms. *Gut* 2013; 62: 43-52.

105. Makharia G, Mulder C, Goh K, *et al.* Issues associated with the emergence of coeliac disease in the Asia-Pacific region: A Working Party Report of the World Gastroenterology Organisation and the Asian Pacific Gastroenterology Association *J Gastroenterol Hepatol* 2014; 29: 666-77.

106. Holtmeier W, Caspary W. Celiac disease. *Orphanet J Rare Dis* 2006; 1: 1-8.

107. Catassi C, Elli L, Bonaz B, *et al.* Diagnosis of Non-Celiac Gluten Sensitivity (NCGS): The Salerno Experts' Criteria. *Nutrients* 2015; 7: 4966-77.

108. Green P, Cellier C. Celiac disease. *New Engl J Med* 2007; 357: 1731-43.

109. Kagnoff MF. Overview and pathogenesis of celiac disease. *Gastroenterology* 2005; 128: S10-S8.

110. Lundin K, Scott H, Hansen T, *et al.* Gliadin-specific, HLA-DQ (alpha 1* 0501, beta 1* 0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med* 1993; 178: 187-96.

111. Nilsen E, Lundin K, Krajci P, Scott H, Sollid L, Brandtzaeg P. Gluten specific, HLA-DQ restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon gamma. *Gut* 1995; 37: 766-76.

112. Vader W, Kooy Y, van Veelen P, *et al.* The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology* 2002; 122: 1729-37.

113. Lebenthal E, Branski D. Celiac disease: An emerging global problem. *J Pediatr Gastr Nutr* 2002; 35: 472-4.

114. Clemente M, De Virgiliis S, Kang J, *et al.* Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. *Gut* 2003; 52: 218-23.

115. Sollid LM. Coeliac disease: Dissecting a complex inflammatory disorder. *Nat Rev Immunol* 2002; 2: 647-55.

116. Arentz–Hansen H, Mcadam SN, Molberg Ø, *et al.* Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues. *Gastroenterology* 2002; 123: 803-9.

117. Koning F. The molecular basis of celiac disease. *J Mol Recognit* 2003; 16: 333-6.

118. Sapone A, Lammers KM, Mazzarella G, *et al.* Differential mucosal IL-17 expression in two gliadin-induced disorders: gluten sensitivity and the autoimmune enteropathy celiac disease. *Int Arch Allergy Imm* 2010; 152: 75-80.

119. Sapone A, Lammers K, Casolaro V, *et al.* Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: Celiac disease and gluten sensitivity. *BMC Med* 2011; 9: 23.

120. Brottveit M, Beitnes A-CR, Tollefsen S, *et al.* Mucosal cytokine response after short-term gluten challenge in celiac disease and non-celiac gluten sensitivity. *Am J Gastroenterol* 2013; 108: 842-50.

121. Bucci C, Zingone F, Russo I, *et al.* Gliadin does not induce mucosal inflammation or basophil activation in patients with nonceliac gluten sensitivity. *Clin Gastroenterol H* 2013; 11: 1294-9. e1.

122. Molina-Infante J, Santolaria Piedrafita S, Fernández Bañares F. Non-Celiac Gluten Sensitivity. *OmniaScience Monographs* 2015.

123. Carroccio A, D'Alcamo A, Cavataio F, *et al.* High Proportions of People with Non-Celiac Wheat Sensitivity Have Autoimmune Disease or Anti-nuclear Antibodies. *Gastroenterology* 2015; 149: 596-603.

124. Drago S, El Asmar R, Di Pierro M, *et al.* Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol* 2006; 41: 408-19.

125. Biesiekierski J, Newnham E, Irving P, *et al.* Gluten causes gastrointestinal symptoms in subjects without celiac disease: A double-blind randomized placebo-controlled trial. *Am J Gastroenterol* 2011; 106: 508-14.

126. Carroccio A, Mansueto P, D'Alcamo A, Iacono G. Non-celiac wheat sensitivity as an allergic condition: personal experience and narrative review. *Am J Gastroenterol* 2013; 108: 1845-52.

127. Vazquez-Roque M, Camilleri M, Smyrk T, *et al.* A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function. *Gastroenterology* 2013; 144: 903-11.

128. Hollon J, Puppa EL, Greenwald B, Goldberg E, Guerrerio A, Fasano A. Effect of gliadin on permeability of intestinal biopsy explants from celiac disease patients and patients with non-celiac gluten sensitivity. *Nutrients* 2015; 7: 1565-76.

129. Jones V, Shorthouse M, McLaughlan P, Workman E, Hunter J. Food intolerance: A major factor in the pathogenesis of irritable bowel syndrome. *Lancet* 1982; 320: 1115-7.

130. Carroccio A, Mansueto P, Iacono G, *et al.* Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: Exploring a new clinical entity. *Am J Gastroenterol* 2012; 107: 1898-906.

131. Cooper B, Holmes G, Ferguson R, Thompson R, Allan R, Cooke W. Gluten-sensitive diarrhea without evidence of celiac disease. *Gastroenterology* 1981; 81: 192-3.

132. Di Sabatino A, Volta U, Salvatore C. Low gluten doses in patients suspected for nonceliac gluten sensitivity: A randomized, placebo-controlled, cross-over trial. *Clin Gastroenterol H* 2015; 13: 1604-12.

133. Shahbazkhani B, Sadeghi A, Malekzadeh R, *et al.* Non-Celiac Gluten Sensitivity Has Narrowed the Spectrum of Irritable Bowel Syndrome: A Double-Blind Randomized Placebo-Controlled Trial. *Nutrients* 2015; 7: 4542-54.

134. Zanini B, Baschè R, Ferraresi A, *et al.* Randomised clinical study: Gluten challenge induces symptom recurrence in only a minority of patients who meet clinical criteria for non-coeliac gluten sensitivity. *Aliment Pharm Ther* 2015; 42: 968-76.

135. Gibson P. Editorial: noncoeliac gluten sensitivity–the controversy rages on. *Aliment Pharm Ther* 2015; 42: 1234-.

136. De Giorgio R, Volta U, Gibson PR. Sensitivity to wheat, gluten and FODMAPs in IBS: facts or fiction? *Gut* 2015; 5: 3839-53.

137. Zanini B, Marullo M, Ricci C, Lanzarotto F, Lanzini A. Sa1989 non celiac gluten sensitivity (NCGS) is outnumbered by FODMAPs sensitivity in patients spontaneously adhering to gluten free diet (GFD): A two stage double blind prospective study. *Gastroenterology* 2014; 5: S-348.

138. Brottveit M, Vandvik P, Wojniusz S, Løvik A, Lundin K, Boye B. Absence of somatization in non-coeliac gluten sensitivity. *Scand J Gastroenterol* 2012; 47: 770-7.

139. Biesiekierski J, Rosella O, Rose R, *et al.* Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals. *J Hum Nutr Diet* 2011; 24: 154-76.

140. Gonsalkorale WM. Gut-directed hypnotherapy: The Manchester approach for treatment of irritable bowel syndrome. *Int J Clin Exp Hyp* 2006; 54: 27-50.

141. Mayer EA, Tillisch K. The brain-gut axis in abdominal pain syndromes. *Annu Rev Med* 2011; 62.

142. Koloski N, Jones M, Kalantar J, Weltman M, Zaguirre J, Talley N. The brain–gut pathway in functional gastrointestinal disorders is bidirectional: A 12-year prospective population-based study. *Gut* 2012; 61: 1284-90.

143. Whorwell P, Prior A, Faragher E. Controlled trial of hypnotherapy in the treatment of severe refractory irritable-bowel syndrome. *Lancet* 1984; 324: 1232-4.

144. Galovski T, Blanchard E. The treatment of irritable bowel syndrome with hypnotherapy. *Appl Psychophys Biof* 1998; 23: 219-32.

145. Palsson OS, Turner MJ, Johnson DA, Burnett CK, Whitehead WE. Hypnosis treatment for severe irritable bowel syndrome: Investigation of mechanism and effects on symptoms. *Digest Dis Sci* 2002; 47: 2605-14.

146. Lindfors P, Unge P, Arvidsson P, *et al.* Effects of gut-directed hypnotherapy on IBS in different clinical settings- results from two randomized, controlled trials. *American J Gastroenterol* 2011; 107: 276-85.

147. Roberts L, Wilson S, Singh S, Roalfe A, Greenfield S. Gut-directed hypnotherapy for irritable bowel syndrome: Piloting a primary care-based randomised controlled trial. *Brit J Gen Pract* 2006; 56: 115-21.

148. Moser G, Trägner S, Gajowniczek EE, *et al.* Long-term success of gut-directed group hypnosis for patients with refractory irritable bowel syndrome: A randomized controlled trial. *Am J Gastroenterol* 2013; 108: 602-9.

149. Simrén M, Ringström G, Björnsson ES, Abrahamsson H. Treatment with hypnotherapy reduces the sensory and motor component of the gastrocolonic response in irritable bowel syndrome. *Psychosom Med* 2004; 66: 233-8.

150. Dobbin A, Dobbin J, Ross S, Graham C, Ford M. Randomised controlled trial of brief intervention with biofeedback and hypnotherapy in patients with refractory irritable bowel syndrome. *The journal of the Royal College of Physicians of Edinburgh* 2012; 43: 15-23.

151. Lowén M, Mayer E, Sjöberg M, *et al.* Effect of hypnotherapy and educational intervention on brain response to visceral stimulus in the irritable bowel syndrome. *Aliment Pharm Ther* 2013; 37: 1184-97.

152. Lindfors P, Unge P, Nyhlin H, *et al.* Long-term effects of hypnotherapy in patients with refractory irritable bowel syndrome. *Scand J Gastroenterol* 2012; 47: 414-21.

153. Smith GD. Effect of nurse-led gut-directed hypnotherapy upon health-related quality of life in patients with irritable bowel syndrome. *J Clin Nurs* 2006; 15: 678-84.

154. Palsson OS, Turner MJ, Whitehead WE. Hypnosis home treatment for irritable bowel syndrome: A pilot study. *Int J Clin Exp Hyp* 2006; 54: 85-99.

155. Al Sughayir M. Hypnotherapy for irritable bowel syndrome in Saudi Arabian patients. *East Med Health J* 2007; 13: 301-7.

156. Gonsalkorale W, Miller V, Afzal A, Whorwell P. Long term benefits of hypnotherapy for irritable bowel syndrome. *Gut* 2003; 52: 1623-9.

157. Gonsalkorale WM, Houghton LA, Whorwell PJ. Hypnotherapy in irritable bowel syndrome: A large-scale audit of a clinical service with examination of factors influencing responsiveness. *Am J Gastroenterol* 2002; 97: 954-61.

158. Gonsalkorale WM, Toner BB, Whorwell PJ. Cognitive change in patients undergoing hypnotherapy for irritable bowel syndrome. *J Psychosom Res* 2004; 56: 271-8.

159. Lea R, Houghton L, Calvert E, *et al.* Gut-focused hypnotherapy normalizes disordered rectal sensitivity in patients with irritable bowel syndrome. *Aliment Pharm Ther* 2003; 17: 635-42.

160. Houghton L, Heyman D, Whorwell P. Symptomatology, quality of life and economic features of irritable bowel syndrome-the effect of hypnotherapy. *Aliment Pharm Ther* 1996; 10: 91-5.

161. Prior A, Colgan S, Whorwell P. Changes in rectal sensitivity after hypnotherapy in patients with irritable bowel syndrome. *Gut* 1990; 31: 896-8.

162. Whorwell P, Prior A, Colgan S. Hypnotherapy in severe irritable bowel syndrome: Further experience. *Gut* 1987; 28: 423-5.

163. Taylor EE, Read NW, Hills HM. Combined group cognitive-behaviour therapy and hypnotherapy in the management of the irritable bowel syndrome: The feasibility of clinical provision. *Behav Cogn Psychoth* 2004; 32: 99-106.

164. Vidakovic-Vukic M. Hypnotherapy in the treatment of irritable bowel syndrome: Methods and results in Amsterdam. *Scand J Gastroenterol* 1999; 34: 49-51.

165. Miller V, Carruthers HR, Morris J, Hasan SS, Archbold S, Whorwell PJ. Hypnotherapy for irritable bowel syndrome: An audit of one thousand adult patients. *Aliment Pharm Ther* 2015; 41: 844-55.

166. Galovski TE, Blanchard EB. Hypnotherapy and refractory irritable bowel syndrome: A single case study. *Am J Clin Hypn* 2002; 45: 31-7.

167. Walters VJ, Oakley DA. Hypnotic imagery as an adjunct to therapy for irritable bowel syndrome: An experimental case report. *Contemp Hypnosis* 2006; 23: 141-9.

168. Zimmerman J. Cleaning up the river: A metaphor for functional digestive disorders. *Am J Clin Hypn* 2003; 45: 353-9.

169. Forbes A, MacAuley S, Chiotakakou-Faliakou E. Hypnotherapy and therapeutic audiotape: Effective in previously unsuccessfully treated irritable bowel syndrome? *Int J Colorectal Dis* 2000; 15: 328-34.

170. Barabasz A, Barabasz M. Effects of tailored and manualized hypnotic inductions for complicated irritable bowel syndrome patients. *Int J Clin Exp Hyp* 2006; 54: 100-12.

171. Harvey R, Gunary R, Hinton R, Barry R. Individual and group hypnotherapy in treatment of refractory irritable bowel syndrome. *Lancet* 1989; 333: 424-5.

172. Vlieger AM, van den Berg MM, Menko-Frankenhuis C, Bongers ME, Tromp E, Benninga M. No change in rectal sensitivity after gut-directed hypnotherapy in children with functional abdominal pain or irritable bowel syndrome. *Am J Gastroenterol* 2009; 105: 213-8.

173. Whorwell P, Houghton L, Taylor E, Maxton D. Physiological effects of emotion: Assessment via hypnosis. *Lancet* 1992; 340: 69-72.

174. Carruthers HR, Miller V, Morris J, Evans R, Tarrier N, Whorwell PJ. Using art to help understand the imagery of irritable bowel syndrome and its response to hypnotherapy. *Int J Clin Exp Hyp* 2009; 57: 162-73.

175. Carruthers HR, Morris J, Tarrier N, Whorwell PJ. Mood color choice helps to predict response to hypnotherapy in patients with irritable bowel syndrome. *BMC Complem Altern M* 2010; 10: 75.

176. Vlieger AM, Rutten JM, Govers AM, Frankenhuis C, Benninga MA. Long-term follow-up of gut-directed hypnotherapy vs. standard care in children with functional abdominal pain or irritable bowel syndrome. *Am J Gastroenterol* 2012; 107: 627-31.

177. Gerson CD, Gerson J, Gerson M-J. Group hypnotherapy for irritable bowel syndrome with long-term follow-up. *Int J Clin Exp Hyp* 2013; 61: 38-54.

178. Houghton L, Larder S, Lee R, *et al.* Gut focused hypnotherapy normalises rectal hypersensitivity in patients with irritable bowel syndrome (IBS). *Gastroenterology* 1999; 116: 1009.

179. Gruzelier J, Smith F, Nagy A, Henderson D. Cellular and humoral immunity, mood and exam stress: The influences of self-hypnosis and personality predictors. *Int J Psychophysiol* 2001; 42: 55-71.

180. Kiecolt-Glaser JK, Marucha PT, Atkinson C, Glaser R. Hypnosis as a modulator of cellular immune dysregulation during acute stress. *J Consult Clin Psych* 2001; 69: 674.

181. Mawdsley JE, Jenkins DG, Macey MG, Langmead L, Rampton DS. The effect of hypnosis on systemic and rectal mucosal measures of inflammation in ulcerative colitis. *American J Gastroenterol* 2008; 103: 1460-9.

182. Mertz H. Altered CNS processing of visceral pain in IBS. *IBS: Diagnosis Treat* 2002: 55-68.

183. Boot WR, Simons DJ, Stothart C, Stutts C. The pervasive problem with placebos in psychology why active control groups are not sufficient to rule out placebo effects. *Perspect Psychol Sci* 2013; 8: 445-54.

184. The CONSORT Group. The CONSORT Statement. 2007.

185. Francis C, Morris J, Whorwell P. The irritable bowel severity scoring system: A simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharm Ther* 1997; 11: 395-402.

186. Spielberger C. State-Trait Personality Inventory (STPI) research manual sampler set: Mind Garden Inc 1995.

187. Lovibond S, Lovibond PF. Manual for the depression anxiety stress scales: Psychology Foundation of Australia 1996.

188. Snaith R, Zigmond A. The Hospital Anxiety and Depression Scale manual. Windsor, Berkshire (UK): Nfer-Nelson 1994.

189. Herrmann C. International experiences with the Hospital Anxiety and Depression Scale: A review of validation data and clinical results. *J Psychosom Res* 1997; 42: 17-41.

190. Yelland G, Robinson S, Friedman T, Hutchison C. Detecting subtle cognitive impairment: Patent No. AU2004203679. Australia 2004.

191. Lichtwark I, Newnham E, Robinson S, *et al.* Cognitive impairment in coeliac disease improves on a gluten-free diet and correlates with histological and serological indices of disease severity. *Aliment Pharm Ther* 2014; 40: 160-70.

192. Friedman TW, Robinson SR, Yelland GW. Impaired perceptual judgment at low blood alcohol concentrations. *Alcohol* 2011; 45: 711-8.

193. Friedman TW, Yelland GW, Robinson SR. Subtle cognitive impairment in elders with Mini-Mental State Examination scores within the 'normal'range. *Int J Geriatr Psych* 2012; 27: 463-71.

194. Speirs SJ, Rinehart NJ, Robinson SR, Tonge BJ, Yelland GW. Efficacy of cognitive processes in young people with high-functioning autism spectrum disorder using a novel visual information-processing task. *J Autism Dev Disord* 2014; 44: 2809-19.

195. Bruce KM, Robinson SR, Smith JA, Yelland GW. Validity of a screening tool for detecting subtle cognitive impairment in the middle-aged and elderly. *Clin Intervent Age* 2014; 9: 2165.

196. Patrick D, Drossman D, Frederick I. A quality of life measure for persons with irritable bowel syndrome (IBS-QOL): User's manual and scoring diskette for United States1997.

197. Drossman DA, Patrick DL, Whitehead WE, *et al.* Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire. *American J Gastroenterol* 2000; 95: 999-1007.

198. Fisk JD, Doble SE. Construction and validation of a fatigue impact scale for daily administration (D-FIS). *Qual Life Res* 2002; 11: 263-72.

199. Anderson R, van Heel D, Tye-Din J, Jewell D, Hill A. Antagonists and non-toxic variants of the dominant wheat gliadin T cell epitope in coeliac disease. *Gut* 2006; 55: 485-91.

200. Verdu E, Armstrong D, Murray J. Between celiac disease and irritable bowel syndrome: the “no man's land” of gluten sensitivity. *Am J Gastroenterol* 2009; 104: 1587-94.

201. Wahnschaffe U, Schulzke JD, Zeitz M, Ullrich R. Predictors of clinical response to gluten-free diet in patients diagnosed with diarrhea-predominant irritable bowel syndrome. *Clin Gastroenterol H* 2007; 5: 844-50.

202. Wahnschaffe U, Ullrich R, Riecken E, D. Schulzke J. Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome. *Gastroenterology* 2001; 121: 1329-38.

203. Picarelli A, Maiuri L, Mazzilli M, *et al.* Gluten-sensitive disease with mild enteropathy. *Gastroenterology* 1996; 111: 608-16.

204. De Santis A, Addolorato G, Romito A, Caputo S, Giordano A, Gambassi G. Psychiatric schizophrenia symptoms regression and single photon emission computed

tomography normalization in a celiac disease after gluten free diet. *J Intern Med* 1997; 242: 421-3.

205. Gobbi G, Ambrosetto P, Zaniboni M, Lambertini A, Ambrosioni G, Tassinari C. Celiac disease, posterior cerebral calcifications and epilepsy. *Brain Dev* 1992; 14: 23-9.

206. Addolorato G. Anxiety but not depression decreases in coeliac patients after one-year gluten-free diet: A longitudinal study. *Scand J Gastroenterol* 2001; 36: 502-6.

207. Addolorato G, Stefanini G, Capristo E, Caputo F, Gasbarrini A, Gasbarrini G. Anxiety and depression in adult untreated celiac subjects and in patients affected by inflammatory bowel disease: A personality trait or a rective illness? *Hepato-gastroenterol* 1996; 43: 1513-7.

208. Ciacci C, Iavarone A, Mazzacca G, De Rosa A. Depressive symptoms in adult coeliac disease. *Scand J Gastroenterol* 1998; 33: 247-50.

209. Hallert C, Åström J. Psychic disturbances in adult coeliac disease: II. Psychological findings. *Scand J Gastroenterol* 1982; 17: 21-4.

210. Biesiekierski J, Newnham E, Shepherd S, Muir J, Gibson P. Self-diagnosis of non-coeliac gluten intolerance by Australian adults: Failure to exclude coeliac disease or benefit from a gluten-free diet. *J Gastroenterol Hepatol* 2011; 26: 70.

211. Boirie Y, Dangin M, Gachon P, Vasson M-P, Maubois J-L, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *P Natl Acad Sci* 1997; 94: 14930-5.

212. Mahe S, Roos N, Benamouzig R, *et al.* Gastrojejunal kinetics and the digestion of [15N] beta-lactoglobulin and casein in humans: The influence of the nature and quantity of the protein. *Am J Clin Nutr* 1996; 63: 546-52.

213. Swain A, Soutter V, Loblay RH. RPAH Elimination Diet Handbook: Camperdown, NSW, Australia: Allergy Unit, Royal Prince Alfred Hospital 2009.

214. Muir J, Rose R, Rosella O, *et al.* Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J Agr Food Chem* 2009; 57: 554-65.

215. Muir J, Shepherd S, Rosella O, Rose R, Barrett J, Gibson P. Fructan and free fructose content of common Australian vegetables and fruit. *J Agr Food Chem* 2007; 55: 6619-27.

216. Biagi F, Andrealli A, Bianchi P, Marchese A, Klersy C, Corazza G. A gluten-free diet score to evaluate dietary compliance in patients with coeliac. *Brit J Nutr* 2009; 102: 882-7.

217. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc B Met* 1995: 289-300.

218. Howell D. Statistical methods for psychology: Wadsworth, NY : Wadsworth Cengage Learning 2013.

219. Ludvigsson J, Reutfors J, Ösby U, Ekbom A, Montgomery S. Coeliac disease and risk of mood disorders: A general population-based cohort study. *J Affect Disorders* 2007; 99: 117-26.

220. Cohen R, Swerdlik M. Psychological testing and assessment : An introduction to tests and measurement 6th ed: Boston: McGraw-Hill 2005.

221. Polk D, Cohen S, Doyle W, Skoner D, Kirschbaum C. State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrino* 2005; 30: 261-72.

222. Morley J, Levine A, Yamada T, *et al.* Effect of exorphins on gastrointestinal function, hormonal release, and appetite. *Gastroenterology* 1983; 84: 1517.

223. Dorn L, Kolko D, Susman E, *et al.* Salivary gonadal and adrenal hormone differences in boys and girls with and without disruptive behavior disorders: Contextual variants. *Biol Psychol* 2009; 81: 31-9.

224. Eatough E, Shirtcliff E, Hanson J, Pollak S. Hormonal reactivity to MRI scanning in adolescents. *Psychoneuroendocrino* 2009; 34: 1242-6.

225. Cowen P. Cortisol, serotonin and depression: All stressed out? *Brit J Psychiat* 2002; 180: 99-100.

226. Choi S, DiSilvio B, Fernstrom M, Fernstrom J. Meal ingestion, amino acids and brain neurotransmitters: effects of dietary protein source on serotonin and catecholamine synthesis rates. *Physiology & behavior* 2009; 98: 156-62.

227. Young S, Smith S, Pihl R, Ervin F. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology* 1985; 87: 173-7.

228. Young S, Leyton M. The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels. *Pharmacol Biochem Be* 2002; 71: 857-65.

229. Klaassen T, Riedel W, van Someren A, Deutz N, Honig A, van Praag H. Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiat* 1999; 46: 489-97.

230. Murphy F, Smith K, Cowen P, Robbins T, Sahakian B. The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology* 2002; 163: 42-53.

231. Takahashi M, Fukunaga H, Kaneto H, Fukudome S-i, Yoshikawa M. Behavioral and pharmacological studies on gluten exorphin A5, a newly isolated bioactive food protein fragment, in mice. *Jpn J Pharmacol* 2000; 84: 259-65.

232. Sanz Y, De Palma G, Laparra M. Unraveling the ties between celiac disease and intestinal microbiota. *Int Rev Immunol* 2011; 30: 207-18.

233. Neufeld K, Kang N, Bienenstock J, Foster J. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroent Motil* 2011; 23: 255-e119.

234. Bercik P, Denou E, Collins J, *et al.* The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* 2011; 141: 599-609.

235. Heijtz R, Wang S, Anuar F, *et al.* Normal gut microbiota modulates brain development and behavior. *P Natl Acad Sci* 2011; 108: 3047-52.

236. Tillisch K, Labus J, Kilpatrick L, *et al.* Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 2013; 144: 1394-401.

237. Klein K. Controlled treatment trials in the irritable bowel syndrome: A critique. *Gastroenterology* 1988; 95: 232.

238. Yao CK, Gibson PR, Shepherd SJ. Design of clinical trials evaluating dietary interventions in patients with functional gastrointestinal disorders. *American J Gastroenterol* 2013; 108: 748-58.

239. Peters S, Biesiekierski J, Yelland G, Muir J, Gibson P. Randomised clinical trial: Gluten may cause depression in subjects with non-coeliac gluten sensitivity–An exploratory clinical study. *Aliment Pharm Ther* 2014; 39: 1104-12.

240. Ledochowski M, Widner B, Bair H, Probst T, Fuchs D. Fructose-and sorbitol-reduced diet improves mood and gastrointestinal disturbances in fructose malabsorbers. *Scand J Gastroenterol* 2000; 35: 1048-52.

241. Bailey C. A translation of Beccari's lecture 'Concerning grain'(1728). *Cereal Chem* 1941; 18: 555-61.

242. Wrigley C. Gluten as the key to wheat quality—a brief history. *Cereal Foods World* 2002; 47: 336-8.

243. Van Eckert R, Berghofer E, Ciclitira P, *et al.* Towards a new gliadin reference material–isolation and characterisation. *J Cereal Sci* 2006; 43: 331-41.

244. Gustin GM. A simple, rapid automatic micro-Dumas apparatus for nitrogen determination. *Microchem J* 1960; 4: 43-54.

245. Batey I, Gupta R, MacRitchie F. Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. *Cereal Chem (USA)* 1991.

246. Singh NK, Donovan GR, Batey I, MacRitchie F. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chem* 1990; 67: 150-61.

247. Junker Y, Zeissig S, Kim S-J, *et al.* Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *J Exp Med* 2012; 209: 2395-408.

248. Peters S, Muir J, Gibson P. Review article: Gut-directed hypnotherapy in the management of irritable bowel syndrome and inflammatory bowel disease. *Aliment Pharm Ther* 2015; 41: 1104-15.

249. Patrick DL, Drossman DA, Frederick IO, Dicesare J, Puder KL. Quality of life in persons with irritable bowel syndrome (development and validation of a new measure). *Digest Dis Sci* 1998; 43: 400-11.

250. Ledochowski M, Sperner-Unterweger B, Widner B, Fuchs D. Fructose malabsorption is associated with early signs of mental depression. *Eur J Med Res* 1998; 3: 295-8.

251. Ledochowski M, Widner B, Murr C, Sperner-Unterweger B, Fuchs D. Fructose malabsorption is associated with decreased plasma tryptophan. *Scand J Gastroenterol* 2001; 36: 367-71.

252. Yapko MD. Trancework: An introduction to the practice of clinical hypnosis. 4th ed. ed: New York: Routledge 2012.

253. Halmos EP, Christophersen CT, Bird AR, Shepherd SJ, Gibson PR, Muir JG. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut* 2015; 64: 93-100.

254. Koven NS, Abry AW. The clinical basis of orthorexia nervosa: Emerging perspectives. *Neuropsychiat Dis Treat* 2015; 11: 385.

255. Mittag D, Niggemann B, Sander I, *et al.* Immunoglobulin E-reactivity of wheat-allergic subjects (baker's asthma, food allergy, wheat-dependent, exercise-induced anaphylaxis) to wheat protein fractions with different solubility and digestibility. *Mol Nutr Food Res* 2004; 48: 380-9.

256. de Punder K, Pruimboom L. The Dietary Intake of Wheat and other Cereal Grains and Their Role in Inflammation. *Nutrients* 2013; 5: 771-87.

257. Jacobs GA, Latham LE, Brown MS. Test-retest reliability of the state-trait personality inventory and the anger expression scale. *Anxiety Research* 1988; 1: 263-5.

258. Group GE. Therapeutic Guidelines: Gastrointestinal. Version 5: Melbourne Australia: Therapeutic Guidelines Limited 2011.

259. Shepherd S, Gibson P. Nutritional inadequacies of the gluten-free diet in both recently‐diagnosed and long‐term patients with coeliac disease. *J Hum Nutr Diet* 2013; 26: 349-58.

260. Green PH, Jabri B. Coeliac disease. *Lancet* 2003; 362: 383-91.

261. Shor RE, Orne EC, Press CP. Harvard group scale of hypnotic susceptibility: Form A: Consulting Psychologists Press 1962.

262. Weitzenhoffer AM, Hilgard ER. Stanford hypnotic susceptibility scale, form C: Palo Alto, CA: Consulting Psychologists Press 1962.

List of Appendices

Appendix 1. Gastrointestinal Visual Analogue Scale

Please place a (X) anywhere on the line in order to indicate the severity of your symptom

Overall gastrointestinal symptoms:

Excellent, none at all — Very severe

Abdominal pain/discomfort:

Excellent, none at all — Very severe

Abdominal bloating/distention:

Excellent, none at all — Very severe

Passage of wind:

(ie. *flatulence*)

Excellent, none at all — Very severe

Satisfaction with stool consistency:

Very happy — Very unhappy

Tiredness and lethargy:

Excellent, none at all — Very severe

Nausea:

Excellent, none at all — Very severe

Appendix 2. Spielberger State Trait Personality Inventory

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I am in a questioning mood	1	2	3	4
3. I am furious	1	2	3	4
4. I feel strong	1	2	3	4
5. I am tense	1	2	3	4
6. I feel curious	1	2	3	4
7. I feel like banging on the table	1	2	3	4
8. I feel blue	1	2	3	4
9. I feel at ease	1	2	3	4
10. I feel interested	1	2	3	4
11. I feel angry	1	2	3	4
12. I feel miserable	1	2	3	4
13. I am presently worrying over possible misfortunes	1	2	3	4
14. I feel inquisitive	1	2	3	4
15. I feel like kicking somebody	1	2	3	4
16. I feel downhearted	1	2	3	4
17. I feel nervous	1	2	3	4
18. I feel like exploring my environment	1	2	3	4
19. I feel like breaking things	1	2	3	4
20. I feel alive	1	2	3	4

SELF-ANALYSIS QUESTIONNAIRE

STPI Form Y-1 Continued

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
21. I am jittery	1	2	3	4
22. I feel stimulated	1	2	3	4
23. I am mad	1	2	3	4
24. I feel sad	1	2	3	4
25. I am relaxed	1	2	3	4
26. I feel mentally active	1	2	3	4
27. I feel irritated	1	2	3	4
28. I feel safe	1	2	3	4
29. I am worried	1	2	3	4
30. I feel bored	1	2	3	4
31. I feel like hitting someone	1	2	3	4
32. I feel gloomy	1	2	3	4
33. I feel steady	1	2	3	4
34. I feel eager	1	2	3	4
35. I feel annoyed	1	2	3	4
36. I feel healthy	1	2	3	4
37. I feel frightened	1	2	3	4
38. I feel disinterested	1	2	3	4
39. I feel like swearing	1	2	3	4
40. I feel hopeful about the future	1	2	3	4

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you *generally* feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
41. I am a steady person	1	2	3	4
42. I feel like exploring my environment	1	2	3	4
43. I am quick tempered	1	2	3	4
44. I feel gloomy	1	2	3	4
45. I feel satisfied with myself	1	2	3	4
46. I am curious	1	2	3	4
47. I have a fiery temper	1	2	3	4
48. I feel happy	1	2	3	4
49. I get in a state of tension or turmoil as I think over my recent concerns & interests	1	2	3	4
50. I feel interested	1	2	3	4
51. I am a hot-headed person	1	2	3	4
52. I feel depressed	1	2	3	4
53. I wish I could be as happy as others seem to be	1	2	3	4
54. I feel inquisitive	1	2	3	4
55. I get angry when I'm slowed down by others mistakes	1	2	3	4
56. I feel sad	1	2	3	4
57. I feel like a failure	1	2	3	4
58. I feel eager	1	2	3	4
59. I feel annoyed when I am not given recognition for doing good work	1	2	3	4
60. I feel hopeless	1	2	3	4

SELF-ANALYSIS QUESTIONNAIRE

STPI Form Y-2 Continued

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
61. I feel nervous and restless	1	2	3	4
62. I am in a questioning mood	1	2	3	4
63. I fly off the handle	1	2	3	4
64. I feel low	1	2	3	4
65. I feel secure	1	2	3	4
66. I feel stimulated	1	2	3	4
67. When I get mad I say nasty things	1	2	3	4
68. I feel whole	1	2	3	4
69. I lack self-confidence	1	2	3	4
70. I feel disinterested	1	2	3	4
71. It makes me furious when I am criticized in front of others	1	2	3	4
72. I feel safe	1	2	3	4
73. I feel inadequate	1	2	3	4
74. I feel mentally active	1	2	3	4
75. When I get frustrated, I feel like hitting someone	1	2	3	4
76. I feel peaceful	1	2	3	4
77. I worry too much over something that really does not matter	1	2	3	4
78. I feel bored	1	2	3	4
79. I feel infuriated when I do a good job and get a poor evaluation	1	2	3	4
80. I enjoy life	1	2	3	4

Appendix 3. Depression Anxiety Stress Scale

DASS21

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all

1 Applied to me to some degree, or some of the time

2 Applied to me to a considerable degree, or a good part of time

3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3

19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

Appendix 4. Hospital Anxiety and Depression Scale

Hospital Anxiety and Depression Scale (HADS)

Please tick the most correct answer. Don't spend too long thinking about each answer. Answer as you are feeling now.

I feel tense or 'wound up':	Most of the time	
	A lot of the time	
	From time to time, occasionally	
	Not at all	

I still enjoy the things I used to enjoy:	Definitely as much	
	Not quite so much	
	Only a little	
	Hardly at all	

I get a sort of frightened feeling as if something awful is about to happen:	Very definitely and quite badly	
	Yes, but not too badly	
	A little, but it doesn't worry me	
	Not at all	

I can laugh and see the funny side of things:	As much as I always could	
	Not quite so much now	
	Definitely not so much now	
	Not at all	

Worrying thoughts go through my mind:	A great deal of the time	
	A lot of the time	
	From time to time, but not too often	
	Only occasionally	

I feel cheerful:	Not at all	
	Not often	
	Sometimes	
	Most of the time	

I can sit at ease and feel relaxed:	Definitely	
	Usually	
	Not often	
	Not at all	

Appendix 5. IBS-Quality of Life

IBS-Quality of Life

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

Q1. 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

Q2. 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

Q3. 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

Q4. 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

Q5. I feel fat/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

Q6. I feel like I'm 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

Q7. I feel 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

Q8. 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

Q9. 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

Q10. I feel isolated from others because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

Q11. I have to watch 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

Q12. 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

Q13. 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

Q14. I feel like 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

Q15. 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

Q16. 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

Q17. 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

Q18. I feel 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

Q19. 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

Q20. 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

Q21. 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

Q22. 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

Q23. I have to watch 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

Q24. Because of my bowel problems, I have difficulty being around people I do not know well. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 A GREAT DEAL

Q25. 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

Q26. 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

Q27. 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

Q28. I feel frustrated that I cannot eat when I want because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL

2 SLIGHTLY

3 MODERATELY

4 QUITE A BIT

5 EXTREMELY

Q29. 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

Q30. 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

Q31. 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

Q32. I fear 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

Q33. 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

Q34. 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

Appendix 6. Daily Fatigue Impact Scale

Daily Fatigue Impact Scale

Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. In certain medical conditions, feelings of fatigue can be more frequent and more of a problem than usual. The following questionnaire has been designed to help us understand how you experience fatigue and how it has affected your life. Below is a list of statements that describe how fatigue may cause problems in people's lives.

Please read each statement carefully and place an "**X**" in the box that indicates best **HOW MUCH OF A PROBLEM FATIGUE HAS BEEN FOR YOU TODAY.** Please check ONE box for each statement and do not skip any items.

	No Problem 0	Small Problem 1	Moderate Problem 2	Big Problem 3	Extreme Problem 4
1. Because of fatigue, I feel less alert.					
2. Because of fatigue, I have to reduce my workload or responsibilities.					
3. Because of fatigue, I am less motivated to do anything that requires physical effort.					
4. Because of fatigue, I have trouble maintaining physical effort for long periods.					
5. Because of fatigue, I find it difficult to make decisions.					

	No Problem 0	Small Problem 1	Moderate Problem 2	Big Problem 3	Extreme Problem 4
6. Because of fatigue, I am less able to finish tasks that require thinking.					
7. Because of fatigue, I feel slowed down in my thinking.					
8. Because of fatigue, I have to limit my physical activities.					