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MRI ASSESSMENT ON THE IMPACT OF A LOW FODMAP DIET ON THE GASTROINTESTINAL TRACT

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ABSTRACT

Introduction: Poorly absorbed fermentable carbohydrates (FODMAPs) escape digestion in the small bowel, and enter the colon to be fermented by gut bacteria thus increasing luminal volume. However, in susceptible individuals e.g. Irritable Bowel Syndrome (IBS) sufferers, this may result in gastrointestinal symptoms, due to the rapid emptying of the small bowel producing increased luminal water and distension in the colon from gas producing bacteria. Previous studies have demonstrated the effect of fermentation on FODMAPs by assessing microbial concentration and breath hydrogen. However, the physiological changes in the lumen, particularly in the colon, remain unclear.

Aims: The main purpose of this thesis is to assess the effects of FODMAPs on luminal volume, using magnetic resonance imaging, and how they impact appetite, gastrointestinal symptoms and function.

The hypotheses generated and tested were that:

i) FODMAPs increase luminal volume, causing symptoms in IBS patients

ii) Restriction of FODMAPs reduces luminal volume in patients and healthy volunteers

Methods: Four studies were undertaken, with the first being an observational study and the remainder being RCTs. The first study was a pilot study to compare a validated dietary assessment method with a reference method to quantify FODMAP intake in healthy volunteers (n=37). The second study assessed the effect of a low FODMAP diet versus a high FODMAP diet on gastrointestinal physiology and function in healthy volunteers (n=37; FOG). The

third study measured the effect of different physical forms of FODMAPs on gastrointestinal physiology in healthy volunteers (n=18; APPLE). The final study compared the effects of a low FODMAP diet versus habitual diet in patients with IBS (n=27; CRAIGS).

Results: Findings from the first study suggested that the 7-day food diary is more reliable in assessing energy, nutrient and FODMAP intake within the UK population. In the FOG study, colonic volume increased in both groups and fasting breath hydrogen levels declined on the low FODMAP diet. However, fasting breath hydrogen levels increased in those on a high FODMAP diet when supplemented with oligofructose. In the third study, altering the physical form of a meal had an effect on small bowel water content only during the late phase, whereas gastric emptying in healthy volunteers was fastest with apple juice, followed by apple puree and whole apples respectively. In the CRAIGS study, the low FODMAP diet lowered the increase in colonic volume when compared with the habitual diet.

Conclusions: A high FODMAP diet, whether from dietary intake or supplementation, can lead to an increase in colonic volume. In IBS patients this impacts on their overall global symptoms. The unexpected increase with a low FODMAP diet, and the inconclusive effect of fermentation as a result of low breath hydrogen levels, suggests that the role of foods substituted as part of the low FODMAP diet needs further investigation. Meanwhile, altering physical properties of FODMAPs has an impact on luminal volume, but this also needs further investigation. A detailed dietary intake using a 7 day food diary during pre and post dietary intervention is recommended as a measure of compliance.

PUBLICATION ARISING FROM THESIS

Sloan et al., 2018. A low FODMAP diet is associated with changes in the microbiota and reduction in breath hydrogen but not colonic volume in healthy subjects. PLOS one, 13(7): e0201410. (publication from FOG study, Chapter 3).

PRESENTATIONS ARISING FROM THESIS

Oral presentations

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Effect of different physical forms of fermentable carbohydrates (FODMAPs) on gastric emptying, appetite and symptoms in healthy volunteers. (The Apple study). Sue Watson Postgraduate Presentation, School of Medicine, University of Nottingham (UK), 2016.

Poster Presentation

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CANDIDATE STATEMENT

All the work in this thesis was performed by the candidate under guidance from the supervisory team (Prof Robin Spiller and Dr Miranda Lomer) except where otherwise stated; additional contributors are listed below.

Study 1: Pilot Study to Quantify and Evaluate FODMAP Intake in Healthy Volunteers using a Validated Food Frequency Questionnaire against a 7-day Food Diary

	Author's contribution
Ethics	30% as this study was part of Study 3 which was jointly carried out with Giles Major (GM) another fellow PhD candidate.
Study design	30% to the study design
Recruitment	40% of recruitment
Intervention	100% involved in guiding participants to complete the online questionnaire and food diaries.
Data collection	100% of all dietary data.
Statistical analysis	SK carried out all statistical analysis, in consultation with Khalid Weissam (KW), a research fellow at NDDC.
Interpretation of results	This was done by SK with assistance from ML and KW.

Study 2: A Randomised Controlled Trial of the Effect of the Low FODMAP Diet and Dietary Oligofructose on Gastrointestinal Form and Function of Healthy Volunteers

	Author's contribution
Ethics	30% as this study was part of Study 3 which was jointly carried out with Giles Major (GM) another fellow PhD candidate.
Study design	40% of study design.
Recruitment	40% of recruitment.
Intervention	SK purchased all the study test meals and supplements with assistance from Emma Bradley (EB), staff member of NDDC. SK undertook all planning and preparation for all test meals throughout the study as well as ordering lunch for volunteers during their MRI study days. SK also prepared all study supplements used in the study prior to randomisation. Randomisation of study supplements were done by Antonella Ghezzi (AG), nurse manager of NDDC, and Rebecca Harris (RH), a fellow PhD candidate. 40% of transit pill preparation. 100% of the dietary intervention was carried out by SK for all 37 participants.
Data collection	All dietary data was collected by SK.
Statistical analysis	Dietary data entry together with statistical analysis for both dietary and MRI data was done by SK.
Interpretation of results	SK interpreted all the results with assistance from RS and ML.

Study 3: Effect of Different Physical Forms of Fermentable Carbohydrates (fodmaps) on Gastrointestinal Volume, Appetite and Symptoms in Healthy Volunteers. (The Apple Study)

	Author's contribution
Ethics	80% of the ethics of this study, with guidance from RS and LM.
Study design	70% of the study design with assistance from LM, RS, and Sue Pritchard (SP), senior postdoctoral fellow at NDDC.
Recruitment	SK recruited all patients for the study.
Intervention	SK sourced the test meals and was assisted by EB in the purchase. The apple puree and apple juice were prepared EB, LM and SK at Campden BRI, a research and technology institute.
Data collection	SK prepared the MRI setting for all study days, administered the questionnaires, and carried out the breath hydrogen test during the study day. The solid test meal was prepared by SP during the study day when SK was running the study.
Statistical analysis	All data except imaging data was collected and compiled by SK.
Interpretation of results	SK carried out all statistical analysis with guidance from RS and Caroline Hoad (CH), senior research fellow at SPMIC.

Study 4:A pilot randomised controlled trial comparing the effects of low FODMAP diet versus habitual diet, on colonic volume and symptoms in IBS patients. (CRAIGS Study)

	Author's contribution
Ethics	90% of ethics with guidance from RS and ML.
Study design	80% of study design with assistance from RS and ML.
Recruitment	SK recruited all the patients through consultations with General Practitioners approached through the Primary Care Network Team.
Intervention	SK randomised the participants and planned their visits. As part of the screening criteria, SK organised all patient blood sampling and testing. During the study day, SK administered all the questionnaires and food diary, provided all dietary intervention to all 27 patients and carried out the breath hydrogen tests. In addition to organising their meals on scan days, SK also prepared the patients for their MRI scans.
Data collection	All dietary data was collected by SK.
Statistical analysis	SK carried out all statistical analysis with guidance from RS, ML and KW.
Interpretation of results	SK interpreted all results with assistance from RS and LM.

Image Analysis

LM and Dr Caroline Hoad (CH) devised the MRI sequence to measure the physiological variables for all three studies. Postdoctoral fellow and MRI expert Sue Pritchard (SP) processed the MRI data for all three studies. SP also performed analysis of colon volume for the FOG, Apple and CRAIGS studies as well as the WGTT for the FOG study. Joe Alapaddan (also a PhD candidate with SPMIC) performed the analysis for the Apple study gastric volume. SK carried

out the SBWC analysis for the Apple study. In addition, SK also learned how to perform the analysis for gastric volume and colon volumes.

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ABBREVIATIONS

a.u.	Arbitrary units
ANOVA	Analysis of variance
AUC	Area under the curve
CNAQ	Comprehensive Nutrition Assessment Questionnaire
DRV	Dietary Reference Values
DP	Degree of polymerisation
FD	Food diary
FFQ	Food frequency questionnaire
FGID	Functional gastrointestinal disorder
FODMAP	Fermentable Oligo-Di-Monosaccharides and Polyols
GE	Gastric emptying
HADS	Hospital anxiety and depression score
IBS	IBS
IBS-C	Constipation predominant IBS
IBS-D	Diarrhea predominant IBS
IBS-GIS	IBS global improvement score
IBS-M	Mixed diarrhea and constipation predominant IBS
IBS-SSS	IBS severity score
IQR	Interquartile range
MD	Maltodextrin
MRI	Magnetic resonance imaging
NIHR	National Institute of Health Research

NDDC	Nottingham Digestive Diseases Centre
NDNS	National dietary nutrition survey
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
OF	Oligofructose
REC	Research Ethics Committee
RNI	Recommended nutrition intake
T_{50}	half emptying time
T_{rate}	half emptying rate
UoN	University of Nottingham
SCFA	Short chain fatty acids
SPMIC	Sir Peter Mansfield Imaging Centre
VAS	Visual analogue scale
WAPS	Weighted average position score
WGTT	Whole gut transit

CHAPTER 1 INTRODUCTION

1.1 Background

Fermentable Oligo-di-monosaccharide and Polyols (FODMAPs) are a group of poorly absorbed, short-chain carbohydrates that have been found to trigger gastrointestinal symptoms in IBS patients)(Ong et al., 2010). They comprise of free fructose and sorbitol, lactose, oligosaccharides and polyols (mannitol, xylitol and maltitol) (Gibson, 2017)(Varney et al., 2017) (Shepherd et al.,2008). The low molecular weight FODMAPs are poorly absorbed and as a consequence exert substantial osmotic effects particularly fructose, lactose and polyols. (Shepherd et al, 2013)(Barrett and Gibson, 2012). Their osmotic effect increases small bowel water content in the intestinal lumen as demonstrated in imaging studies.(Murray et al., 2014) (Placidi et al., 2012). Other FODMAPs such as oligosaccharides are of higher molecular weight and hence less osmotic effects but are non digestible resisting hydrolysis in the small intestine. They undergo rapid fermentation in the colon to produce hydrogen, carbon dioxide, and short chain fatty acids (Schaafsma and Slavin, 2015) (Gibson and Roberfroid, 1995). The digestibility and chain length of oligosaccharides may dictate the speed of fermentation. It has also been observed that oligofructose, a subgroup of inulin with a degree of polymerization (DP) between 3-9 is more rapidly fermented compared to soluble dietary fibre, a polysaccharide polymer with DP > 10.(Mann et al., 2007),(Wong et al., 2006).

It is believed that the osmotic and fermentative effect of FODMAPs induces functional gut symptoms such as diarrhoea, bloating and abdominal discomfort (Shepherd et al., 2008). Due to the combined effect of FODMAPs on functional gut symptoms, dietary intervention is initially targeted at lowering the amount

of FODMAPs, followed by gradual reintroduction into the diet which is as tolerated (Whelan et al, 2018). In addition, the low FODMAP diet has been successful in reducing symptoms in IBS patients as demonstrated by several RCT's (Heidi Maria Staudacher et al., 2017),(Eswaran et al., 2016) Current population studies involving IBS patients and healthy volunteers have measured macronutrient and micronutrient content of the diet (Böhn et al., 2013),(Bohn et al., 2013),(Williams et al., 2011), (Saito et al., 2005). Meanwhile, other studies have focused on the dietary perceptions and food avoidance between these groups to alleviate gastrointestinal symptoms (Hayes et al., 2014) , (Ligaarden et al., 2012) , (Østgaard et al., 2012). These investigations were useful as they probed into the dietary habits of IBS patients. However, due to poor study design and dietary assessment methods, the results are debatable in showing a difference in intake between two groups as well as self-reported food intolerance. Additionally, there was no attempt to measure FODMAP intakes in these studies. Nevertheless, a study conducted in the United Kingdom has measured FODMAP intake in IBS patients taking a habitual diet (Staudacher et al., 2012). However, there is no recent data on average FODMAP intake in IBS patients compared to that of a healthy population.

Oligosaccharides are known to induce prebiotic effect by enhancing the growth of bifidobacteria (Meyer & Stasse-Wolthuis, 2009). It has been argued that a long-term low FODMAP diet has an unfavourable effect on colonic health due to the reduction of prebiotic effect. By reducing the dietary intake of FODMAPs for 3-4 weeks, short-term studies have shown a reduction of *bifidobacteria* spp. (Halmos et al., 2014), (Staudacher et al., 2012). Therefore, more double

blinded, RCT's studies are needed to define underlying mechanisms and establish efficacy of the diet in the long term.

1.2 Functional Gastrointestinal Disorder

A functional gastrointestinal disorder (FGID) is a chronic condition that affects the gastrointestinal system with a heterogenous etiology which include biological, psychological and environmental factors (Spiller, 2016),(Spiller et al., 2007), (Whitehead et al., 2002). As there is no organic causes or diagnostic tests to confirm the condition, FGID is often initially misdiagnosed by most physicians. (Sayuk et al., 2017). Consequently, additional procedures or diagnostic tests are usually carried out which are often unnecessary.

As a result, up to 75% of IBS patients are probably undiagnosed, as reported by a study in the United States (Hungin et al., 2005). In addition, IBS symptoms often overlap with functional and psychiatric disorders (Whitehead et al., 2002) and patients also often have multiple comorbidities such as somatic pain syndromes experienced due to fibromyalgia, chronic fatigue syndrome and chronic pelvic pain (Lovell and Ford, 2012) , (Riedl et al., 2008).

Therefore, the ROME working group, formed by a group of researchers and clinicians with expertise in this disorder, reached a consensus on the definition of FGID and its classification, and formed the criteria for diagnosis. (Drossman, 2006), (Longstreth et al., 2006). The most current criteria was published in 2016, and is known as the ROME IV criteria (Lacy et al., 2016), but since the studies forming this thesis were carried out between 2014 and 2017, ROME III

(Drossman, 2006) was used instead and will be discussed in the following section.

One of the main differences between the criteria is that the word discomfort was removed from the current guideline, since it does not have the same meaning globally and does not exist in some languages (Lacy et al., 2016). In addition, the latest criteria are more strict when applied to diagnosing symptoms, with patients being required to report more frequent abdominal pain compared to the previous criteria (Simrén, 2017), (Whitehead et al., 2017).

Error! Reference source not found. shows the ROME III criteria to classify FGID a ccording to six domains. The condition of particular interest is IBS, categorised in the third domain (bowel). Symptoms between C1-C4 domains tend to overlap, such as functional diarrhoea characterised by those experiencing loose stools without pain and functional bloating where abdominal distension is present, but without changes in bowel habit.

Therefore, IBS was characterised as pain associated with change in bowel habit (Longstreth et al., 2006). However, the ROME III criteria is mostly used in research settings; it is not commonly known or applied in the primary care as General Practitioners (GPs) tend to concentrate on presenting problems (Spiller et al., 2007), (Longstreth et al., 2006).

Table 1-1: Rome III criteria for classification of FGID according to domains

Domains	**Category**
Esophageal	A
Gastroduodenal	B
Bowel	C
i) IBS	C1
ii) Functional bloating	C2
iii) Functional constipation	C3
iv) Functional diarrhoea	C4
Functional abdominal pain syndrome (FAPS)	D
Biliary	E
Anorectal	F

(Drossman, 2006),(Longstreth et al., 2006)

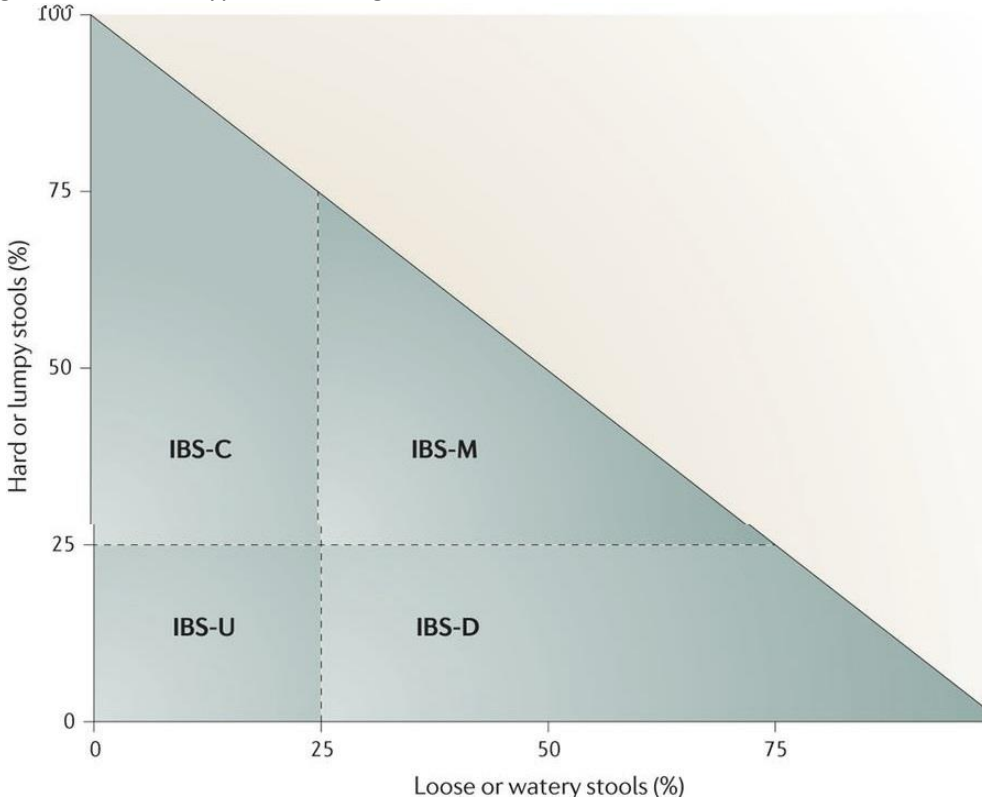
1.3 Definition of IBS

IBS is classified as FGID and characterised by symptoms such as abdominal pain and or distension, diarrhoea and constipation, which improves with defecation (Chey et al, 2015)(Longstreth et al., 2006). Patients are classified into 4 different subtypes: diarrhoea predominant (IBS-D), constipation predominant (IBS-C), mixed diarrhoea and constipation (IBSM), and unspecified (IBSU)

Figure 1-1 depicts IBS subtypes according to bowel form at a particular point in time and the percentage of time the bowel form has to be present to meet the criteria for IBS subtypes. As an example IBS-M is characterised as individuals who suffer from both diarrhoea and constipation which constitutes more than 25% of bowel movements. (Cordain et al., 2005)(Wall,et al., 2014)(Longstreth et al., 2006). Subtyping patients according to their predominant symptoms is important selecting the appropriate diagnostic tests and treatments.(Chey et al., 2015). However, evidence suggests that patient's symptoms vary over time indicating a switch to a different subtype. The most common switch is in IBS-C

and ISB-D both to IBS-M. Switches between IBS-C to IBS-D or vice versa is less common (Olafsdottir et al., 2011), (Drossman et al., 2005).

Figure 1-1: IBS subtypes according to the ROME III criteria



Adapted from(Enck P et al, 2016)

1.4 Prevalence of IBS

This chronic and debilitating condition affects approximately 7-21% of the global population (Chey et al., 2015). Demographic predictors of IBS include gender, age and socioeconomic status (Spiegel, 2009). Most Western population studies have reported IBS as a condition with female predominance (Lovell and Ford, 2012), (Heitkemper and Jarrett, 2008). On the contrary, there are more male IBS sufferers compared to female in non-western countries suggesting lack of female predominance (Quigley, 2006), (Miwa, 2008).

Almost 30% of people reporting IBS symptoms are seen by physicians (Canavan, et al., 2014). In the UK alone, around 50% are seen as outpatients (Hungin et al., 2005). This suggests that IBS has a large impact on quality of life and viewed as a healthcare burden both directly and indirectly (Canavan et al., 2014)

The direct cost in the UK, is mainly related to incorrect diagnosis leading to inappropriate cholecystectomy and hysterectomy (Kennedy and Jones, 2000). Also, inappropriate referrals for non-GI symptoms and prescription drugs adds to this cost (Dalrymple and Bullock, 2008). The indirect cost is often due to work absenteeism and reduced productivity (Maxion-Bergemann, et al.,2006).

1.5 Diagnosis of IBS

The diagnosis of IBS in clinical practice is largely by exclusion of, organic diseases which may mimic symptoms of IBS and alarm features such as onset of symptoms after the age of 50, unexplained weight loss, family history of Gastrointestinal disease and colorectal cancer screening (Chey et al., 2015).

In a research setting IBS is diagnosed according to stricter Rome III criteria (see Table 1-2) focusing on key features such as abdominal pain or discomfort, which may be relieved by defecation or changes in stool frequency or consistency (Spiller et al. 2007), (Longstreth et al., 2006) in addition to exclusion of organic diseases and alarm features.

More recently selected tests such as complete blood cell count, C-reactive protein or faecal calprotectin, serologic testing for ceoliac disease are also being performed as a criterion of exclusion for IBS. (Enck et al., 2016), (Chey et al., 2015).

1.6 Etiology of IBS

IBS has multifactorial etiology which includes environmental and host factors as shown in Figure 1-2. (Chey W et al, 2015), (Whitehead et al., 2002). Examples of environmental factors are stressful events during early life such as abuse and psychosocial stressors, food intolerance, frequent antibiotics use and enteric infection which occurs after a period of infectious gastroenteritis (Enck et al, 2016).

Meanwhile host factors include alteration in pain perception, altered brain gut function (Aziz and Thompson, 1998), dysbiosis (Kassinen et al., 2007), increased intestinal permeability, (Camilleri et al.,2012) gut mucosal immune activation (O'Sullivan et al., 2000) and visceral hypersensitivity (Elsenbruch, 2011), (Chang, 2005), (Quigley, 2005)

Therefore, besides gastrointestinal symptoms, IBS patients also suffer from somatic syndrome such as chronic fatigue syndrome and fibromyalgia (Riedl et al., 2008), (Whitehead et al., 2002). However, there is also an increased prevalence psychiatric disorder among patients such as anxiety and depression (Fond et al., 2014) suggesting a relationship with psychological and physiological factors.

Meanwhile, IBS patients with no comorbid somatic disorders are more likely to suffer from motility disturbances and visceral hyperalgesia which stems from a biological etiology. (Whitehead et al., 2002). Recently it has been demonstrated that structural abnormality is also present in IBS-D patients whereby alteration in tight junction and permeability resulted in mast cell

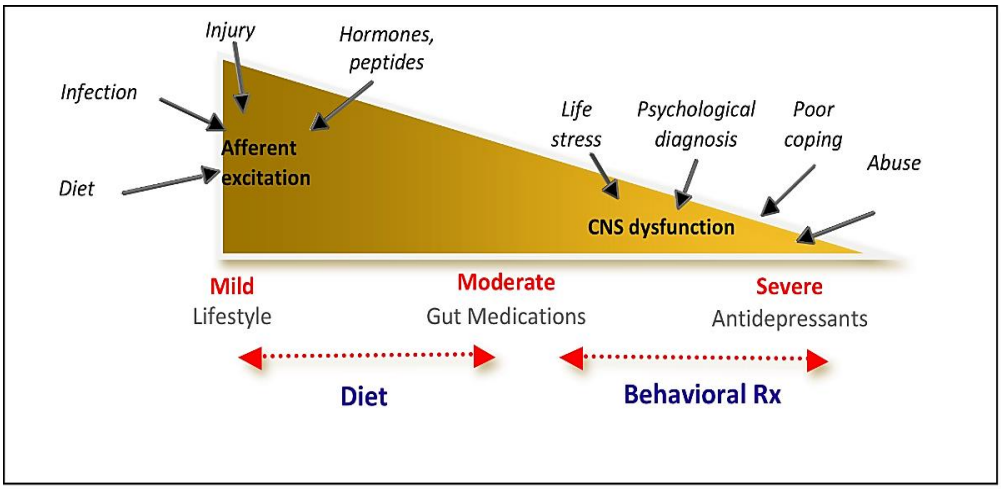
activation in the jejunal mucosa (Martínez et al., 2013). Biopsies from IBS patients showed an increased mast cell activation and mast cell mediators such as protease and histamine indicating that these immune cells are significantly increased in most patients with IBS (O'Sullivan et al., 2000) as well as those with post infectious IBS . (Dunlop et al., 2003).

Table 1-2: ROME III diagnostic criteria for IBS

Recurrent abdominal pain or discomfort at least 3 days a month in the past 3 months associated with two of more of the following:
a) Improvement with defecation b) Onset associated with a change in frequency of stool c) Onset associated with a change in stool form/appearance
All criteria are fulfilled for the past 3 months with symptom onset at least 6 months prior to diagnosis.

Adapted from (Drossman et al., 2010)

Figure 1-2: Symptom severity and multifactorial etiology



Adapted from (Whitehead et al., 2002).

1.7 Treatment for IBS

Current treatment options for IBS include: dietary lifestyle modification (McKenzie et al., 2016), (Spiller, 2016), hypnotherapy, motility agents, serotogenic agents, bulking agents, and anti-depressants,(Saha, 2014), (Ruepert et al., 2011) targeted towards severity of symptoms.

The first line therapy in IBS patients are aimed at improving bowel habits. Antidiarrheal medications such a loperamide is recommended for IBS-D patients as they reduce stool frequency and increase stool consistency; fibre, supplements and laxatives are targeted to those with constipation. These medications are widely available and prescribed by physicians, but they do not reduce overall global symptoms such as bloating, pain and diarrhoea in IBS patients (Spiller et al., 2007). A recent randomised controlled trial demonstrated an improvement in stool consistency, urgency and bloating ($p < 0.002$) with ondansetron a 5 HT_3 antagonist (Garsed et al., 2014). Other treatments include antispasmodic and antidepressants but effectiveness depends on the individual patient (Ruepert et al., 2011).

1.8 Relationship between IBS and Diet

As food intolerance appeared to be evident among IBS patients, dietary strategies were targeted around elimination of offending food followed by reintroduction (Simrén et al., 2001) ,(Goldstein et al., 2000) .However, these studies only looked at individual foods, and there was no mechanistic evidence relating offending food to gastrointestinal symptoms (J. S. Barrett & Gibson, 2012),(Shepherd et al., 2008)

Observational studies, RCT's and re-challenge studies began emerging, focusing on short chain carbohydrates mainly fructose, lactose, galacto-oligosaccharides, fructan (inulin) and polyols. There was sufficient evidence indicating poorly absorbed short chain carbohydrates as the cause of gastrointestinal symptoms in IBS patients from RCTs (Ong et al., 2010), (Shepherd et al., 2008) In addition, the effects of these fermentable carbohydrates were investigated using MRI; it was observed that fructose distends the small bowel with water, and inulin distends the colon with gas (Murray et al., 2014).

King et al demonstrated that patients with IBS have abnormal colonic fermentation as they produced more hydrogen gas compared to healthy controls suggesting that the gut bacteria were not utilising the fermentation by product efficiently.(King, et al, 1998)

1.9 Chemical properties of carbohydrate

Carbohydrates contribute 46% of energy intake in the UK adult population. (Bates et al., 2011). They are classified in general according to their chemical properties, digestion and absorption in the small bowel.

Chemically, carbohydrates are classified based on individual monomers (see Table 1-3) such as sugar (monosaccharides and disaccharides), polyols, oligosaccharides (malto-oligosaccharides and non digestible oligosaccharides and polysaccharides (starch and non starch polysaccharides) (Cummings and Stephen, 2007).

Glucose, fructose and galactose are six-carbon sugars which serve as the building block of di-oligo and polysaccharides. Glucose and fructose are present in fruits, plant juices and honey whereas galactose is naturally present in milk in combination of glucose and lactose. (Cummings and Stephen, 2007).

However, sugars can also be found in non starch polysaccharides (hemicelluloses and pectin) in polymeric form. The degree of polymerisation (DP) which is the bond between each monomer and type of glycosidic linkage (α or β) divides carbohydrates into three main group which are mono-and disaccharides with a DP of 1-2, oligosaccharides with DP between 3-9.and polysaccharides with a DP ≥10, (Mann et al., 2007)

1.10 Physiological properties of carbohydrates

Based on its physiological properties, carbohydrates can also be categorised as digestible carbohydrates which are readily digested and absorbed in the small intestine and non digestible carbohydrates which are resistant to hydrolysis thus reaches the colon intact to be fermented by gut bacteria.

Most short chain carbohydrates (mono-di-oligosaccharides) are poorly digested and absorbed compared to polysaccharides (Cummings and Stephen, 2007b). However, in some people, lactose a disaccharide and fructose a monosaccharide is poorly absorbed in the small intestine. They then end up being fermented in the colon causing gastrointestinal symptoms.

Resistant carbohydrates are categorised as non starch polysaccharides (NSP), resistant starch (RS), resistant short chain carbohydrates (fructan, oligosaccharide and synthetic or hydrolysed polysaccharide) and sugar

alcohols. As they resist digestion they end up being fermented in the colon. The most common resistant carbohydrates are found in the plant cell wall polysaccharides which occurs naturally but there is also a small quantity of them being used as functional ingredients which have been known to resist digestion. These include extracted polysaccharide such as gums, oligosaccharides, polydextrose, resistant maltodextrin, and high resistant starch (Englyst et al., 2007).

Table 1-3: Classification of carbohydrates

Class	Components	Sources
Sugar (DP 1-2)	Glucose, fructose, galactose, sucrose, lactose, maltose and trehalose	**Glucose** Fruits, plants, honey and juices **Fructose** Present with glucose in apples, mango, watermelon, honey and tomatoes. Commercially added to food products such as jam, baked goods, cereals and soft drinks **Sucrose** Composed of the monosaccharides glucose and fructose **Galactose** In milk in combination with glucose as lactose and maltose
Polyols (DP 1-2)	Erythritol, xylitol, mannitol, sorbitol, lactitol, isomalt, maltitol	**Sorbitol** Present in stone fruits, apples, pears, sugar free mints/gum **Mannitol** Present mostly in vegetables such as mushroom and cauliflower. Also added to sugar free mint/gum
Oligosaccharides DP (3-9)	Fructo- oligosaccharides (FOS), oligofructose (OF)	**Fructan** Found in wheat, rye, asparagus, onion, leek and garlic. Commercially added to yogurt as prebiotic
	Galacto-oligosaccharides (GOS), raffinose, stachyose and polydextrose	**GOS** Legumes such as baked beans, kidney beans, soy beans, borlotti beans, lentils and chickpeas
Polysaccharides (DP>9)	Amylose and amylopectin, modified starches, cellulose, hemicellulose, pectin, beta-glucan, fructans, plant gums and mucilages, hydrocolloids and inulin	Found in plant material includes vegetables and fruit skin

Adapted from (Shepherd et al, 2013)

1.11 Short chain carbohydrates

The variability in DP affects digestibility and absorption.(Shepherd et al.,2013)

1.11.1 Fructose

Glucose and galactose are actively absorbed in the human small intestine via the sodium dependent transporter (SGLT) (Thorens and Mueckler, 2010). Fructose a six carbon monosaccharide are absorbed via glucose-independent facilitated transport and glucose-dependent fructose co-transport. GLUT 5 is a glucose independent pathway for fructose absorption which is dependent on concentration gradient for movement of fructose across membrane.

Meanwhile, GLUT 2 a glucose dependent pathway is a low affinity facultative transporter which carries both fructose and glucose in which glucose facilitates uptake of fructose (Gibson and Shepherd, 2010).

However, the GLUT-5 pathway is impaired in those with fructose malabsorption and it is concentration dependent. Only 10% of people with FGID malabsorb 25g of fructose but when the concentration is doubled to 50g, almost 80% of them malabsorb fructose. (Rao et al., 2007)

It is unlikely that anyone would consume 50g of fructose at one sitting as it is equivalent to drinking a 10 litre mango juice (Muir et al., 2009).

A fructose: glucose ratio of 1:1 is optimal for fructose absorption (Murray et al., 2014), (Latulippe and Skoog, 2011), (Truswell et al, 1988). However, some studies have demonstrated incomplete absorption of fructose in healthy volunteers when given with glucose (Tuck et al., 2016) ,(Truswell et al., 1988).

Fruits such as blackberry, grapes, kiwifruit, melon, nectarine and oranges have more glucose compared to fructose (Muir et al., 2009) and they are absorbed using the GLUT-2 pathway. However, when they are consumed as concentrated juices or dried fruits, they end up being malabsorbed (Fedewa and Rao, 2014), (Putkonen et al., 2013)

Fructose intake has increased globally over the past 40 years and 80% of intake is in the form of high fructose corn syrup a processed starch, present in sugared beverages, restaurant and fast foods (Popkin and Nielsen, 2003). On average fructose intake varies between 11g to 54g around the world (Gibson et al.,2007) The mean fructose intake in the US population is around 41g/day (Marriot et al., 2009). There is no data for the UK population but IBS patients in UK have a lower fructose intake 17g/d (Staudacher et al., 2012) and it is much lower among Australian patients around 12.7g (Halmos et al., 2015). High amounts of fructose in excess of glucose is present in honey, apple, pear, mango, watermelon, high fructose corn syrup and fruit juices (Shepherd and Gibson, 2006).

1.11.2 Lactose

It is present as a disaccharide with glucose and galactose as constituent monosaccharide units. Lactase enzyme is required to hydrolyse disaccharide into its monosaccharide units to be absorbed but most humans lack this enzyme due to genetic predisposition. Almost 90% of Chinese are lactase deficient compared to 20% of Caucasians (Itan, et al ,2010). Some older adults also experience a loss of lactase activity (Lomer et al., 2008).

Lactose deficiency is determined from intestinal biopsies as well as a food challenge whereby high lactose containing food is administered when diagnostic tests are inconclusive. In addition, lactose breath test is also carried out however the results may be inconclusive if patients do not suffer from lactose intolerance (Deng et al., 2015).

IBS patients have a reduced lactose intake as most of them avoid milk and milk products. Patients in the UK consume 7g/day of lactose (Hayes, et al., 2014) compared to 12g/d in the Swedish population (Larsson, et al,, 2004). However, up to 240 ml of milk or 12g of lactose could be tolerated by those who are lactase deficient if its consumption is spread throughout the day (Suarez, et al., 1995) which enables adequate calcium intake.

Besides naturally present in mammalian milk, lactose is also found in processed dairy products such as cheese and yogurt as well being added to commercial foods such as bread and cakes (Shepherd et al., 2013).

1.11.3 Polyols

They are also known as sugar alcohols and occur as hydrogenated mono-disaccharides (Livesey, 2001). Sorbitol and mannitol are naturally occurring sugar alcohols, made up of six carbon polyol isomers. They are similar in molecular weight and size but differ in the orientation of their hydroxyl group (Cummings and Stephen, 2007).

Polyols are absorbed by passive diffusion via small intestinal pores. However, the size of the pores determines absorption whereby large pores found proximally absorbs polyols better than to the small pores found distally causing

some to be poorly absorbed. The size of polyol molecule also influences its absorption with an A 4- carbon polyol better absorbed compared to a 6-carbon polyol (Lenhart and Chey, 2017), (Cummings and Stephen, 2007). Erythritol is completely absorbed compared to partially absorbed sorbitol and non absorbed lactitol (Livesey, 2003). Other factors such as fast transit and mucosal damage causes polyols to be inefficiently absorbed.

About 60-70% of healthy individuals malabsorb 10g dose of sorbitol (Hyams, 1983). The malabsorption is dose dependent whereby a 20g sorbitol solution was malabsorbed by 100% of healthy volunteers compared to 90% who malabsorbed 10g solution detected using breath hydrogen meter (Corazza et al., 1988). Interestingly mannitol is absorbed better compared to sorbitol in IBS patients (Yao et al., 2014).

Sorbitol naturally occurs in fruits whereas mannitol occurs in vegetables in small quantities. However, sugar free chewing gum contains 10 times more sorbitol compared to fruits and vegetables (Yao et al., 2014).

In the UK, polyols are added as sweeteners to milk and milk products, jellies, candy, gums, tablets, energy reduced product such cocoa, chewing gum, cereal and energy bars, biscuits/cookies, fruit and vegetable based desserts. IBS patients in the UK have a lower mean intake of polyol <1g /day (Heidi M Staudacher et al., 2012) compared to healthy adults 5.6g/day in adults (Tennant, 2014).

1.11.4 Oligosaccharides

Oligosaccharides with a DP 3-9 are hydrolysed to form monosaccharides before being absorbed but those containing glycosidic linkage resist enzymatic reaction and are fermented by colonic bacteria (Macfarlane and Cummings, 2006), (Loo et al., 1999).

Maltodextrin is a short chain oligosaccharide derived from starch hydrolysis. It is digested and absorbed in the small intestine therefore commonly used in food industry for food texture modification as well as energy drinks and supplements (Cummings and Stephen, 2007).

Apart from malto-oligosaccharide all other oligosaccharides; fructan and galacto-oligosaccharides (GOS) are malabsorbed in the small intestine. This is mainly due to the presence of glycosidic linkages in them that are resistant to cleavage by the pancreatic and brush border hydrolase enzymes.

1.11.5 Fructans

Fructans are fructose polymers with a glucose terminal with a β (1-2) and (2-6) bond. It is hydrolysed by enzyme fructanase and measured as fructose and glucose components.(Englyst et al., 2007). They are also known as fructo-oligosaccharides (FOS) or oligofructose (OF) both with a DP<10 and inulin which is a subgroup of fructan with a DP between 2-60.

As humans do not produce hydrolase they are unable to break the glycosidic β (1-2) bond. Ileostomy study demonstrated that 90% of ingested fructans can be recovered from small intestine (Bach and Hessov, 1995). Thus, fructans reaches the colon unabsorbed to be fermented, by gut bacteria.

Fructans are storage carbohydrate commonly found in artichokes, chicory, wheat, rye, asparagus and members of the onion, leek and garlic family (Cummings and Stephen, 2007). Although onion and wheat have low fructan content, they are consumed in large quantities. Commercial fructans from chicory roots or sucrose are added by food manufacturers to most processed foods, yogurts and even protein powder due to its prebiotic effect (Loo et al., 1995) mouthfeel, and low calorific content (Crittenden and Playne, 1996).

The mean inulin intake in IBS patients around 3.6g/day (Heidi M Staudacher et al., 2012) which is similar to healthy individual in the UK which is around 4g/day (Dunn et al., 2011).

1.11.6 Galacto-oligosaccharides

Raffinose, stachyose and verbascose are sugar polymers which consists of sucrose molecules joined to galactose molecules. As humans do not produce galactosidases, hydrolysis of galactosidic linkages of stachyose and raffinose does not take place to form monosaccharides units, (Macfarlane and Cummings, 2006). GOS naturally occur as plant seeds in lentils and legumes such peas and beans.

Human milk also contains oligosaccharides with galactose as the predominant molecule (Bode and Jantscher-krenn, 2012). They may serve as substrate for colonic fermentation (Kunz et al., 1998). Total GOS in human milk is 5.0-8.0g/l but only trace amounts are present in cow's milk (Boehm et al., 2005). GOS intake within UK IBS patients are 2g/day (Staudacher et al., 2012) but not reported in healthy individuals.

1.12 Long chain carbohydrates

They are non-starch polysaccharide which consists of resistant starch, pectin, guar gum, inulin. The digestibility and fermentation vary between long chain carbohydrates with non-starch polysaccharide being rapidly fermented, followed by intermediate fermentable soluble fibre (psyllium/ispaghula and oats), insoluble slowly fermentable fibre (wheat bran, lignin, fruit and vegetables) and finally insoluble non-fermentable fibre (cellulose, methylcellulose, sterculia) (Eswaran et al., 2013).

1.12.1 Non Starch Polysaccharide (NSP)

These are carbohydrate polymers with ≥ 10 monomeric units that escapes digestion in the small intestine. It is part of all plant polysaccharides present in plant cell walls and also occur as gums and mucilages which are often used in food processing as additives (Englyst,et al., 2007).

Chemically, they are often categorised as dietary fibre, with variable monomers bonded by a β linkage unlike starch which only has glucose monomers with α 1-4 linkage (Cummings and Stephen, 2007). The variability of NSP is due to its sugar composition and glycosidic linkages same as SCC which determines its physico-chemical properties.

NSP escapes digestion and exerts its prebiotic effect to be fermented by bacteria to produce SCFA. It is also known for its physiological effect of GI tract and immunomodulation. As a result, NSP may reduce/limit the risks of infectious diarrhoea, obesity, diabetes, colorectal cancer, neonatal necrotising enterocolitis and inflammatory bowel disease (Kumar et al., 2012).

1.12.2 Resistant starch

It is the sum of starch and starch degradation products that escapes digestion in the small intestine (Champ et al., 2003), (Englyst and Hudson, 1996). RS is classified into 5 different forms due its physical and chemical properties (Englyst and Hudson, 1996). RS1 are starches which are physically inaccessible to enzymes as it is entrapped within cell wall and matrices. They are usually present in bread, seeds and pulses.

RS2 are native starch granules which are raw and has an organised crystalline structure. They are usually present in potatoes and bananas with high amounts present in raw starch produce and unripe fruits.

RS3 are retrograded starch which are a result of cooking and cooling effect of starch. Foods such as potato and pasta will have this effect (Cummings and Stephen, 2007).

RS4 are chemically modified starch which forms new chemical bonds such as crosslinking, esterification and etherisation. These are used in food processing for bread and cakes and are listed as modified potato starch, modified tapioca starch and modified maize starch (Dura et al., 2017).

RS5 are amylose-lipid complexes and resistant maltodextrin which are formed during food processing or modification which changes the arrangement of starch molecules (Watanabe et al., 2018), (Pereira et al., 2014).

The physio-chemical aspects of RS may influence rate and site of fermentation in the colon as RS resists digestion when it is chemically modified prior to being added to processed foods. Overall RS is found largely in legumes, tubers and

cereals. However only data on raw RS content is available on the UK food composition tables although its content may vary during cooking and manufacturing process. Findings from an Indian study reported about 5% of RS present in boiled and pressure cooked slightly higher than 1.5% found in tubers (Yadav et al., 2010).

As measuring RS is not very cost effective in the UK this leads to underestimation of RS when food composition data is used (Englyst,et al., 2007). This poses a problem in those with FGID as RS also causes gastrointestinal symptoms. Retrograded starch has been known to cause pain and bloating (Suarez and Levitt, 2000) as it rapidly fermented in the colon and produces a laxative effect if consumed in large quantity (Brouns et al., 2002)

Therefore, to manage their symptoms IBS patients are advised to avoid intake of reheated starch.(Irritable bowel syndrome in adults: diagnosis and management (CG61), 2008).

1.13 Prebiotic Effect of Short Chain Carbohydrates

A prebiotic is a safe food ingredient which is neither digested nor absorbed in the small intestine but undergoes fermentation by beneficial bacteria in the colon. It is able to alter the composition of colonic microbiota in the host, to a healthier composition (Van Loo, 2012)(Zdun´, 2004).

Fermentation occurs in the large bowel, dependent on the amount and type of substrate available to be fermented by anaerobic bacteria. Fermentable carbohydrate substrates are then broken down, to produce short chain fatty acids yielding energy (Cummings and Englyst, 1987).

Certain FODMAPs and non starch polysaccharides produce SCFA which consists of acetate, propionate, butyrate and lactate. These by products have numerous health benefits such as maintaining normal GI structure and function as well as preventing and alleviating colonic diarrhoea.

In addition, the presence of SCFA lowers colonic pH, inhibits growth of pathogenic microbiota whilst improving mineral utilisation. (Topping and Clifton, 2001).

Inulin and oligofructose which is a part of FODMAPs are often avoided or reduced during a low FODMAP diet (Shepherd. et al, 2013). However, in the human diet, inulin and oligofructose particularly, have been shown to improve the symbiotic relationship between host and gut bacteria. Over the years, they have gained worldwide recognition as prebiotics; fermentable food ingredients which have been known to simulate growth of bifidobacteria and lactobacilli in the colon (Cummings and Stephen, 2007). This potentially provides a healthy balance of gut microbiota (Macfarlane, et al., 2006) besides increasing calcium absorption and bone mineral density in adolescents (Elia and Cummings, 2007).

A randomised controlled trial in the UK demonstrated a reduction of bifidobacteria in the stool content of IBS patients who were on a 4 week, dietitian-led low FODMAP diet, compared to patients on a habitual diet (Staudacher et al., 2012).

This supports previous investigations, showing evidence of altered gut microbiota in IBS patients compared to healthy controls (Kerckhoffs et al., 2011),(Kassinen et al., 2007). Therefore, the long-term effect of a low FODMAP

diet may have an adverse effect on health-promoting gut microbiota, due to a low concentration of bifidobacteria (Shepherd et al., 2013).

1.14 The Low FODMAP Diet

As most IBS patients report multiple food intolerance leading to symptoms such as diarrhoea and pain avoiding one single food may not produce symptomatic relief thus an exclusion of all offending groups of foods from the diet seems more practical and useful. (Burden, 2001).

Therefore, the low FODMAP diet was developed by the Monash group which restricts intake of fructan, galacto-oligosaccharides, lactose, fructose and polyols (Barrett et al., 2010). Efficacy studies showed global improvement in symptoms in most patients. The initial retrospective study by Shepherd et al 2006, investigated the effect of this diet in 62 IBS patients with fructose malabsorption (FM). (Shepherd and Gibson, 2006).

Although the diet was successful, with 75% of patients reporting improvement in overall gastrointestinal symptoms, efficacy is questionable as it could be due to placebo effect (the patients were not blinded). Nevertheless, a new diet limiting FODMAPs was emerging through this study (Shepherd and Gibson, 2006).

Therefore, a placebo-controlled, randomised, re-challenge trial was conducted, to determine if symptom improvement in IBS patients with FM was related to fructose restriction; and also, if symptoms were associated with either free fructose or poorly absorbed short-chain carbohydrates in general. Subjects who had low FODMAP dietary education at least 3 months before the

study, were given low FODMAP test meals and blinded solutions of fructose, fructans, fructose plus fructans, and glucose (placebo) for 2 weeks. Almost 80% of patients had symptoms induced with each of the challenge substances, compared with placebo, where only 14% of patients reporting inadequately controlled symptoms.

Results from this study justified that symptom induction was associated with all the blinded solutions except placebo, which had a lower effect. This provided strong evidence that global restriction of poorly absorbed short chain carbohydrates, or FODMAP, rather than a single component, is beneficial in IBS patients with FM (Shepherd et al., 2008)

Two efficacy studies were carried out manipulating FODMAP content in the diet, to investigate the effect of the low FODMAP diet on gastrointestinal symptoms. The first study by Ong et al 2010, measured hydrogen and methane gas production in healthy controls and IBS patients, following a 9g low FODMAP diet or a 50g high FODMAP diet. Participants were provided with their randomised diet for 2 days, then were crossed over to the alternate diet and subjected to a breath test. As expected, breath hydrogen was higher in both groups with the high FODMAP diet; but interestingly, methane production was reduced in healthy volunteers following a high FODMAP diet. There was also significant difference in GI symptoms induced in IBS patients compared to the other group.

This concludes that manipulating dietary FODMAP content may induce gastrointestinal symptoms in IBS patients, evidenced by hydrogen gas

production in IBS patients (Ong et al., 2010). Although this study was important in evaluating the efficacy of the low FODMAP diet in unselected IBS patients, there were limitations due to the short trial duration of only 2 days and the high FODMAP diet not reflecting that habitual dietary intake of Australians.

Consequently, another efficacy study was carried out, comparing the effect of a low FODMAP diet against a habitual Australian diet. 30 unselected IBS patients and 8 healthy volunteers, in this single blind, cross-over study were randomised to a 3g low FODMAP diet or a 23.7g habitual diet high in FODMAP content, for 3 weeks. IBS patients following the low FODMAP diet showed a significant improvement in symptoms and stool consistency (Emma P Halmos et al., 2014).

However, major limitations of this study were the small sample size, possible unblinding of participants and unmatched cases and controls. Researchers in this study have improvised methods from the previous study to investigate efficacy of the diet and were successful in preparing test meals containing 0.5g FODMAP per meal. Alternatively, this may not reflect true intake if patients were consuming a low FODMAP diet at home.

When the low FODMAP diet was compared to a modified diet based on the UK, dietary guidelines for IBS patients (McKenzie et al., 2016) (IBS in adults: diagnosis and management (CG61), 2008) more patients reported adequate improvement with the low FODMAP diet. (Eswaran et al., 2016), (Staudacher et al., 2011).

Although, the efficacy of the diet in symptom reduction is clear it is still not widely accepted as first line therapy for IBS patients. The diet is highly restrictive and has little effect on those with IBS-C and long term FODMAP restriction potentially reduces the prebiotic effect of FODMAPs. Also, only those who strictly follow the diet experience overall symptom improvement.

As IBS patients are a heterogenous group they may have different tolerance threshold whereby some patients may be able to tolerate more FODMAPs than the other. Therefore, exploratory and mechanistic studies were conducted to understand the effect of the diet on gut microbiota and luminal volumes. This will be briefly summarised in the next two sections.

Table 1-4: Fodmaps intervention trials

Author/Study Design	Participants	Intervention	Outcome measures	Findings	Limitations
(Zahedi et al., 2018) RCT, single blind	IBS-D n=110	Low FODMAP diet (LFD) General Healthy Diet (GHD) 6 weeks	Changes in symptom scores Changes in stool frequency and consistency	LFD improves overall GI symptoms scores ($p<0.01$), stool frequency ($p<0.01$) and consistency ($p=0.003$) versus GHD	Free fructose and fructan was not measured. Only IBS-D patients were studied
(Eswaran et al., 2018) RCT, open label	IBS-D n-84	Low FODMAP diet mNICE diet 4weeks	1) Proportion of patients reporting adequate relief 2) Reduction in abdominal pain and stool consistency	Adequate relief in 52% (LFD) vs 41% (mNICE) ($p=0.13$) Abdominal pain 51% (LFD) vs. 23% (mNICE), $p=0.008$) Significant reduction in abdominal pain, bloating, stool consistency and frequency and urgency in LFD compared to mNICE at 4 weeks	Free fructose and fructan not measured Only IBS-D patients were studied
(Hustoff et al., 2016) RCT, double blind, place controlled crossover	IBD-D n=11 IBS-Mixed n=9	Low FODMAP diet (LFD) with maltodextrin supplementation versus High FODMAP diet (HFD) with fructo-oligosaccharides supplementation for 6 weeks	Changes in symptom scores Changes in cytokines and interleukin and tumor necrosis factor Gut microbiota, composition Short chain fatty acids	Symptom relief 80% (LFD) versus 30% (HFD) ($p=0.013$) Interleukin levels decreased significantly after 3 weeks of LFD Actinobacteria, Bifidobacterium, and Faecalibacterium praus- nitzii, total SCFAs, and n- butyric acid, decreased significantly on LFD as compared to baseline. Only microbiota concentration increased in HFD.	Total FODMAP intake was not assessed at baseline or intervention
(Valeur et al., 2016) RCT, open label	IBS D=34 IBS-C=19 IBS M=10	LFD for 4 weeks	Symptom severity scores Short Chain Fatty acids (SCFA) in fecal samples	More than 50% reduction in symptom severity in 32 patients SCFA reduced at baseline and after fermentation	FODMAP intake was not assessed at baseline Transit time was not measured

Continuation of Table 1.4 Fodmaps intervention trials

Author/Study Design	Participants	Intervention	Outcome measures	Findings	Limitations
McIntosh et al.,2017 RCT, single blind	IBS D n=10 IBS M n=23 IBS C n =2 IBS U n =1	Low FODMAP diet (LFD) versus High FODMAP diet (HFD) for 3 weeks	Changes in symptom severity score Changes in breath hydrogen Changes in metabolomes	Symptoms reduced in LFD ($p<0.001$) Breath hydrogen reduced in LFD Histamine reduced in LFD ($p<0.05$)	FODMAP intake was assessed but not reported
Bohn et al., 2015 RCT, Single blind	IBS-C n=22 IBS-D n=18 IBS M/U n=25	Low FODMAP diet (LFD) versus Traditional IBS diet for 4 weeks	Changes in symptom severity score Dietary Intake Changes in stool frequency and consistency	Symptom severity reduced in both groups Reduction of energy, protein and fat intake during intervention but energy significantly reduced in low FODMAP group	Low response rates Study was not powered to detect differences in response post intervention between IBS subtypes
Halmos et al.,2014 RCT single blind crossover	IBS all subtypes n=30 Healthy volunteers n=8	Low FODMAP diet (LFD) versus typical Australian diet for 3 weeks	Overall symptom scores Fecal water content between groups Adherence to diet	Overall symptom severity scores reduced on LFD Reduced bloating, wind and pain Reduced stool frequency on LFD IBS-D had altered fecal frequency	Adherence to diet was due to provision of foods which were low in FODMAPs Inequal comparison between volunteers and patients
Staudacher et al., 2012 RCT	IBS-D or IBS patient with bloating (n=35)	LFD diet versus Habitual diet for 4 weeks	Changes in symptom severity score Dietary intake Stool frequency and consistency Stool microbiota analysis	Adequate control of symptoms in the intervention group compared to controls ($p=0.001$) Significant difference in FODMAP intake between both groups ($p=0.001$) Reduction in stool frequency in intervention group ($p=0.008$) Lower concentrations of bifidobacteria in intervention group ($p=0.001$)	Patients with mild symptoms were excluded from the study Symptomatic response of intervention group could be due to placebo response

1.15 Low FODMAP intervention studies.

As the efficacy of the low FODMAP diet has been well demonstrated, more randomised controlled trials began looking at the effect of the diet on fermentation, microbial concentrations, stool frequency and consistency, fecal water as well as overall symptoms. Some of the trials have been outlined on table 1.4.

Overall, only one study was a double blind placebo controlled trial (Hustoft et al., 2017). Four others were single blind trials, followed by three open label trials (Eswaran et al., 2016), (Valeur et al., 2016) and (Staudacher et al., 2012). The sample size ranged from n=20 to n=100 participants. Almost all of the trials studied IBS patients with the exception of one crossover study which included health volunteers (Halmos et al., 2014). Of these, three studies only recruited patients with IBS-D (Zahedi, et al., 2018), (Eswaran et al., 2016) and (Hustoft et al., 2017).

The low FODMAP diet was the intervention diet and the comparator ranged from a high FODMAP diet, high FODMAP diet with supplementation of oligofructose, traditional IBS diet, habitual diet, healthy diet and a modified diet based on the NICE guidelines (m NICE) (Irritable bowel syndrome in adults: diagnosis and management (CG61), 2008). Duration of the intervention ranged from 3 weeks to 6 weeks.

Similar to the results from the efficacy studies, the low FODMAP diet reduced overall gastrointestinal symptoms particularly gas, bloating and pain. However, when compared with the mNICE diet (Eswaran et al., 2016), there was a

significant reduction in abdominal pain, bloating, stool consistency and frequency and urgency in low FODMAP diet. However, there was no significant difference in adequate relief between both groups in this particular study. Although patients did not have significant improvement of diarrheal symptoms their stool frequency was significantly reduced. However a more recent trial demonstrated that patients on a low FODMAP diet versus general diet had a significantly lower stool frequency and improved stool consistency (Zahedi et al., 2018). In addition another Australian study also demonstrated a change in fecal frequency in patients with IBS-D while being on a low FODMAP diet (Halmos et al., 2014). A large proportion of IBS patients in previous studies have reported partial symptom relief whereas long term symptom control has been statistically significant in respect to abdominal pain, bloating and flatulence. However, this is due to the heterogenous symptom reporting in these studies. Therefore, in future studies it is recommended that overall symptom reporting be combined with objective measure, such as stool frequency and consistency over the period of intervention (Mitchell,et al., 2019).

A total of three studies outlined in table 1.4 looked at the fermentative effect of FODMAP. Of these, two of the studies measured fecal microbiota concentration and short chain fatty acid concentration (Hustoft et al., 2017), (Staudacher et al., 2012), whereas the other study only measured short chain fatty acid concentration (Valeur et al., 2016) .The results from these studies demonstrate a reduction in bifidobacteria concentration and SCFA concentration following a low FODMAP diet. There was no significant difference in total or individual fecal SCFA in the Staudacher et al., study which

only included patients with IBS-D. However, in the Valeur study which consisted of all subtypes of IBS patients, the difference in concentration between subgroups were lower post intervention (Valeur et al., 2016).The differences in the results are more likely due to the varying techniques used to sequence the microbiota as well as different comparator diets across the studies.

Although the results of these trials were quite promising, they must be interpreted carefully due to the limitations. Not many studies assessed or reported FODMAP intake, which questions the adherence of patients to the low FODMAP diet. When designing a trial with dietary intervention it is recommended to measure a baseline dietary intake especially of the food/group being challenged. However, assessment of adherence using subjective assessment methods such as self-reported intake or nutritional analysis are prone to error and need careful interpretation (Yao, et al.,2013).

Another major limitation in most trials is the study design due to difficulty in planning placebo controlled dietary trials. Only one study, Eswaran et al., provided placebo controlled diet by using a sham diet as the comparator. Meanwhile Halmos et al., provided placebo controlled foods by provision of low FODMAP foods which similar consistency and texture as high FODMAP foods.

Last but not least, blinding was also a major limitation in most studies. Even though Halmos et al., carried out a double blind study, the participants who were given the placebo controlled food were able to tell the difference due to the taste of the food.

Nonetheless, the results from these RCT's are quite promising when it comes to the role of the low FODMAP diet in management of symptoms and stool consistency and frequency. Despite some concerns regarding the long term impact of the diet on microbiota concentration (due to the reduction of prebiotics in the diet), this is the only dietary intervention available for patients who have persisting symptoms - particularly those with IBS-D and IBS-M.

1.16 Exploratory Study

Although efficacy studies provided strong evidence that reducing short chain carbohydrates improved gastrointestinal symptoms in IBS patients the effects of the diet on gut microbiota was unclear. This led to a UK study, in which 41 patients with IBS were randomised into intervention and control groups. 19 of the patients from the intervention group were advised to follow a low FODMAP diet, whereas 22 from the control group were advised to continue on their habitual diet for 4 weeks. Both groups were also advised to avoid probiotics and prebiotics. Participants were requested to complete a 7-day food diary and a stool diary, complete a symptom severity questionnaire and provide stool samples both at baseline and at the end of the study.

Although there were no changes in faecal short chain fatty acids, the low FODMAP diet significantly reduced concentration of bifidobacteria. Patients in the intervention group reported improvement of symptoms compared to control (Staudacher et al., 2012).

This was followed another study comparing the effect of an Australian diet versus the Low FODMAP diet whereby intake of a low FODMAP diet for 3

weeks, resulted in a significant higher faecal pH (7.37), similar short-chain fatty acid concentrations, greater microbial diversity but reduced total bacterial abundance compared with a typical Australian diet. Whereas the Australian diet increased the concentration of butyrate producing microbiota. (Halmos et al., 2015).

A more recent placebo controlled study in IBS patients demonstrated that long term low FODMAP decreased SCFA's, n-butyric acid and levels of faecal bacteria (*Actinobacteria*, *Bifidobacterium*, and *Faecalibacterium*) and decreased serum levels of proinflammatory ctyokines; IL-6 and IL-8. However, 10 days of FOS (16g/day) increased the level of these bacteria although SCFAs and cytokines levels were not altered.(Hustoft et al., 2017).

Due to altered metabolism of carbohydrates in IBS patients, there are possible changes that occur in the diversity of the bacterial genera (Jeffery et al., 2012). Previous studies in IBS patients with diarrhoea have shown a reduction in *Bifidobacteria* and *Verrucomicrobium*, and an increase in *Lactobacillus*, *Veillonella*, *Prevotella* and *Parasporo* (Rigsbee et al., 2012), (Carroll et al., 2010). However, it was uncertain if this was due to a long term effect.

All studies identified a reduced concentration of the luminal microbiota when FODMAPs were restricted between 3-4 weeks which is less compared to patients in a clinical setting who are advised to follow the diet for 6-8 weeks. Therefore, long term restriction may not be beneficial due to decreased abundance of protective microbiota as well as SCFA, so further research is needed to study the long term effect of the diet on luminal environment.

1.17 Mechanistic Study on Effects of FODMAP on Gastrointestinal Symptoms

Mechanistic studies using MRI technique have demonstrated changes in luminal volume in both healthy volunteers and patients when fermentable carbohydrate was consumed either orally or infused.

When, 11 healthy volunteers were given 350mls of test meal containing 5% mannitol and 5% glucose the small bowel content significantly increased ($p<0.001$) with mannitol but not glucose reaching 381mL and 47 mL respectively indicating that mannitol was undigested and poorly absorbed.

This was followed by another MRI study conducted by the same unit, to investigate differential effects of FODMAP in the small and large intestine contents of healthy volunteers. 16 volunteers in this randomised, single blind cross-over study were given 4 different challenge solutions containing glucose, fructose, inulin or a mixture of glucose and fructose. A series of MRI scans were carried out to assess gastric content, small bowel water content and colonic gas (Murray et al., 2014).

The results were able to prove the differential effects of FODMAPs, whereby free fructose distends the small bowel with water; the effects were reduced by adding glucose to fructose, supporting results from previous studies(Shepherd et al., 2008) Inulin distended the colon more with gas when compared to fructose, confirming no absorption in the small bowel, and only fermentation taking place. This concurs with a previous ileostomy study, which suggested that a high FODMAP diet increased volume of liquid and fermentation in the

proximal colon (Barrett et al., 2010). A more recent study demonstrated that 40g of inulin increased breath hydrogen levels, colonic gas and colonic volume in IBS patients compared to 40g of glucose intake,(Major et al., 2016).

The studies above provided an understanding on how fermentable carbohydrates alter luminal volume in both patients and healthy volunteers. However, the intervention solution had higher concentration of FODMAPs and did not reflect usual intakes amongst patients or volunteers. Therefore, intervention studies on the effect of the diet itself on luminal volume would provide a clear understanding.

1.18 Breath Testing

Breath test is commonly used to determine malabsorption whereby a high fructose or lactose load usually given. In some cases, it is also used to diagnose small intestinal bacterial overgrowth (SIBO) using lactulose and glucose. It is a quick, inexpensive, useful and safe diagnostic test to assess malabsorption in IBS patients.

1.18.1 Breath hydrogen

Luminal gases are either from swallowed air or produced from fermentable carbohydrates such as fructose, lactose, sorbitol, mannitol, fructo-oligosaccharide or inulin. Swallowed gases predominantly consist of oxygen and nitrogen whereas the latter are hydrogen and methane, (Carbonero, et al., 2012).

Hydrogen gases are either absorbed via diffusion or utilised by bacteria which then reduces carbon dioxide, a by product of the fermentable substrate to methane or acetate and reduces trace gases sulphates to sulphides.

Only a small volume of hydrogen is excreted through the breath due to presence of microbiota which utilises hydrogen leading to small amounts being eliminated via the lungs. However, around 15% of patients are methane producers due to the high concentration of methanogenic bacteria (Eisenmann, Amann, Said, Datta, & Ledochowski, 2008)(Romagnuolo et al, 2002).

A rise in breath hydrogen >20 ppm suggests malabsorption due to the fermentative effect of colonic bacteria. However not everyone who malabsorb fructose have symptoms as 60%-80% people have been shown to malabsorb fructose and they are asymptomatic. Nevertheless, breath hydrogen is used to diagnose patients who malabsorb fermentable carbohydrates particularly fructose and lactose.

Patients are usually given a challenge drink containing a single fermentable carbohydrate the most. A fructose load of 35g is suggested to diagnose fructose malabsorption (Skoog and Bharucha, 2004). However, a load of 50g of lactose is not recommended as in reality this is equivalent to 1 cup of milk and not many people would be having that amount at one time. The cut off value for hydrogen breath test is around 10-20ppm (Gibson et al., 2002).

Recently, the North American consensus group have determined that a breath hydrogen concentration ≥ 20ppm is more appropriate as a cut off point for malabsorption as fasting breath hydrogen is higher in IBS population compared

to healthy individuals. However, methane levels of ≥ 10ppm is considered to be methane positive. (Rezaie et al., 2017).

In preparation for breath testing subjects are usually asked to avoid fermentable carbohydrates such as pasta, breads, fibre rich products and legumes the night before their test. Smoking and physical activity may induce hyperventilation therefore it is also restricted at least 8-10 hours prior to the breath test. Lastly alcohol free antibacterial mouthwash is given to subjects before testing to prevent premature hydrogen or carbon dioxide production from oral flora on test substrate , (Romagnuolo et al, 2002).

Hydrogen breath test is non-invasive, simple, safe, easy to use and inexpensive to assess malabsorption. However, it needs to be interpreted with caution as false positive results test could occur due to rapid small bowel transit (Gibson et al., 2007).

Also, other factors such as oral bacteria flora needs to be taken into account. False negative results may also occur due to high concentration of non hydrogen producing bacteria or delayed gastric emptying. (Eisenmann et al., 2008),(Simrén & Stotzer, 2006)

Therefore, the breath hydrogen test combined with magnetic resonance imaging (MRI) is more useful in assessing malabsorption of fermentable carbohydrates.

1.19 MRI

The first clinical imaging using magnetic resonance was developed in Nottingham in the late 1980 by the late Sir Peter Mansfield with his team which earned him the Nobel Prize Award.

Since then, MRI has been used extensively in the field of gastroenterology with new techniques developed over time to measure gastric emptying (Gopirajah et al., 2016),(Kwiatek et al., 2009), small bowel water content (Murray et al., 2014) (Hoad et al., 2007a), colonic volume and motility (Gopirajah et al., 2016), (Marciani, 2011),(Goetze et al., 2006) ,both healthy individuals and patients with IBS, coeliac disease and Crohn's disease. (Major and Spiller, 2014).

The MRI's non invasive and non ionizing feature makes it favourable compared to other methods such as manometry which uses intraluminal catheter and CT scans which causes radiation. In addition, the MRI sequences are also readily available in most clinical scanners and image analysis could be done remotely saving time and cost. However, the unique feature of MRI is its good sensitivity to mobile water (Grover et al., 2015).

Despite its advantages the MRI is expensive compared to other alternative modalities but by combining multiple examinations into a single examination, the overall cost could be reduced to save time. (Puylaert et al., 2015). Also, several sequences can be used to study a large area of interest such as the stomach, small intestine and colon with just one operator thus replacing other labour intensive methods (Marciani, 2011).

The principles of MRI and its application within the studies carried out will be described briefly in the next section, (Grover et al., 2015),(Liang and Lauterbur, 2000).

1.19.1 Principles of MRI

In summary MRI transmits its strong magnetic field and energy in the form of radio wave which excites hydrogen nucleus (1H) which is bound to water in the body to release waves. It then records the locations of this wave with high accuracy. The hydrogen nucleus behaves like a small bar magnet spinning on its axis with a north-south pole inducing a magnetic moment as shown in Figure 1-3. This quantum mechanics description is similar to the classical mechanics of spinning objects

The magnetic field is generated one way but due to the dipole moment of the nuclei, they orientate either perpendicular to the magnetic field direction in high energy state or parallel to the magnetic field in low energy state. A radiofrequency (RF) magnetic field applies RF energy in short pulses which lasts for microseconds. This disrupt and misalign the protons with the constant field and when the pulse ends, the protons realign again resulting in emission of low energy radio frequency nuclei.

The hydrogen nuclei only absorb energy with specific frequency called the 'resonant frequency'. So, the MRI only sends the radio frequency (RF) energy wave at the resonant frequency of the hydrogen nuclei. This excites the nuclei with spin property and the nuclei transitions from high energy into lower energy and vice versa during relaxation. When the nuclei return to its resting

alignment it emits RF energy received by the 'receiver coils' which converts energy waves into signal.

The signal emitted from hydrogen nuclei is variable depending on the location of the tissue in the body. As an example, a returning signal from the hydrogen nuclei in the stomach is different than the returning signal from the hydrogen nuclei in the colon.

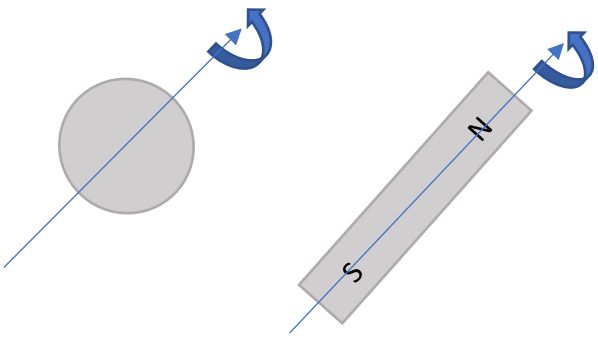
The RF energy is applied in short (microseconds) pulses so multiple transmitted radiofrequency pulses are used in a sequence to examine a specific tissue on a region of interest. Each time an RF pulse is applied at the resonance frequency, the MRI machine detects presence of hydrogen nuclei which is amplified and displayed as 'free induction decay'(FID).

An MRI examination is made up of a series of pulse sequences with different tissues (such as fat and water) having different relaxation times so, they are identified separately. As an example, by using a "fat suppression" pulse sequence, the signal from fat will be removed, leaving only the signal from any abnormalities lying within it.

The time between administered pulses is known as repetition time (TR) and the time that RF echo is received by the RF detector is known as echo time (TE) both controlled by the scanner operator. By adjusting TE and TR is adjusted according to a tissue's T1 and T2 tissue is the region of interest could be differentiated.

The parameters measured in this thesis were gastric emptying, small bowel water content, colonic volume and gas, and whole gut transit.

Figure 1-3: Spin property of the hydrogen nucleus



1.19.2 MRI in measuring Gastric Content and Air

The MRI is able to acquire images for abdominal volume by visualising the stomach within a single breath hold. By choosing the appropriate T1 or T2 weighting both intragastric meal and air volumes can be accurately measured over time after meal ingestion (Hoad et al., 2015a).

In T1 weighted imaging, tissues with high fat content appear bright and compartments with water appear dark and its vice versa in T2 weighted imaging. A varying TE (5-30) and flip angle could produce both T1 weighted and T2 weighted effect. As water has long T1 and T2, a pulse sequence which relies on T2 will result in image where water has high signal.

Echo planar imaging (EPI) is the fastest acquisition method in MRI (100ms/slice) and it is based on excitation pulse and continuous signal acquisition in the form of gradient echo in order to acquire a single shot or segmented acquisition. It produces high-resolution images in less than 100ms and is able to measure gastric volume as low as 50mls. A balanced gradient echo is used to image stomach content of food, foams and gas.

This gradient echo is generated by frequency echo gradient whereby it is used in reverse to enforce transverse dephasing spinning protons and then to realign dephased protons to acquire signal. This sequence is sensitive to small changes in T1 and T2. (Hykin et al., 1994) This imaging technique for gastric volume is validated and reproducible. (Hoad et al., 2015a).

Upon ingestion or infusion of test a meal, transverse rapid multislice set of EPI images serial volume are usually acquired from the heart to the kidneys to determine the position and measure the volume of the gastric lumen. The data is acquired until the stomach appears empty and volunteers hold their breath before each image acquisition to minimize diaphragmatic displacement. T2 data sets are then acquired on the gastric contents using a spin-echo EPI sequence to measure meal dilution.

These measurements are then repeated for the whole experimental session, which could last up to 1.5 hours however volunteers are only scanned for the short time necessary to acquire the images which is less than 10 minutes. When the transmitted radiofrequency pulse is switched off T1 relaxation occurs whereby the protons reorient resulting in recovery of longitudinal magnetisation. T2 relaxation is when protons diphase resulting in transverse magnetization.

The repeat

1.19.3 Small bowel water content

Initial research on water absorption and secretion on small bowel using MRI was done using a low field using echo-planar magnetic resonance imaging

(Adkin et al., 1995),(Hykin et al., 1994). Over time, changes to field strength, sequence design and coil design have been done to improve image quality.

One notable MRI study was by Schiller and his team. (Schiller et al., 2005) who measured fluid content of the stomach and small and large bowel using MRI using internal calibration of signal intensity levels via the gall bladder and cerebral spinal fluid (CSF) signals.

However, no validation or error analysis was presented in this work. Internal calibration using the gall bladder would not be appropriate for studies using high fat meal as intervention as the gall bladder would empty and refill over the time course of the experiment. The Gut MRI group in Nottingham have successfully monitored changes in small bowel water content (SBWC) upon feeding using a sequence of magnetic resonance cholangiopancreatography (MRCP) which was initially developed to study the biliary tract. By using a turbo spin-echo (TSE) sequence which is strongly T2-weighted and sensitive to mobile water. The team were able to detect and increase in SBWC induced by wheat bran.

The sequence used visualises the anatomy of small bowel by acquiring coronal plane and transverse images with in-plane resolution. This sequence produces high-intensity signals from areas with free mobile and dark signals from poorly mobile or bound water or body tissues (Hoad et al., 2007a).

1.19.4 Colonic volumes

Colonic volume is usually imaged using dual echo. Images with differences in weightings or echo time are used to obtain both proton density and T2

weighted images or in phase or out of phase gradient echo images simultaneously without increasing measurement time.

The first sequence is the abdominal coronal dual-echo and followed by a transverse dual echo fast field sequence. The field echo is gradient echo in non steady state imaging with fast relaxation time usually less than 100ms and small flip excitation angle. The varying echo time and flip angle produces a T1 weighted and T2 weighted effect.(Pritchard et al., 2014).

In line with this, I plan to conduct 3 studies during my PhD which are aimed to understand the mechanistic effect of FODMAPs among IBS patients and healthy volunteers by using magnetic resonance imaging. In order to evaluate and understand the mechanistic effects of FOMDAPs, changes in luminal volumes will be measured using magnetic resonance imaging technique.

As part of these studies, samples will be obtained which may be used to answer questions about the impact of such dietary interventions on luminal volume. A more detailed 'research aims, objectives and hypothesis' will be described in the methods section.

1.19.5 Reproducibility

The MRI methods for gastric emptying, small bowel water content and colon volumes have all been validated. Interobserver reproducibility was done for gastric volumes and gastric secretion volumes whereby three observers were given data of 10 heathy volunteers to be analysed. The results showed excellent reproducibility (Hoad et al., 2015).

This was similar for small bowel water content whereby 20 data sets which were selected randomly were analysed by three observers which was done on two occasions. The same data was analysed twice with an interval of two months to avoid bias.

In addition, intra-observer repeatability was only done by the one observer who measured a single data set ten times with only the position of region of interest that varied. It was ensured that poor positioning of the region of interest was avoided to avoid large error such as the whole spine being included during the measurement but with the region of interest not central. (Hoad et al., 2007).

Similar to small bowel water content, both inter observer and intra observer repeatability was done for colon volumes. Data set of three participants were analysed by two observers for inter observer reproducibility whereas data set of two different subject sets were chosen to be analysed. Almost 75% of the scored values were identical for within observer and between observer (Placidi, 2011).

CHAPTER 2 PILOT STUDY TO QUANTIFY AND EVALUATE FODMAP INTAKE IN HEALTHY VOLUNTEERS USING A VALIDATED FOOD FREQUENCY QUESTIONNAIRE AGAINST A 7-DAY FOOD DIARY

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2.1 Background

Fermentable carbohydrate (FODMAP) intake is associated with poor outcomes in those with IBS, as it triggers gastrointestinal symptoms such as diarrhoea, pain and bloating. (Staudacher et al., 2017),(Marsh, et al.,2016). Besides subjective data obtained from patients, an objective measure is needed to in order to support the hypothesis above. This could only be done by quantifying FODMAP intake of the population being studied using a validated dietary assessment tool.

FODMAP consumption has also been increasing over the years (Tennant, 2014)(Popkin & Nielsen, 2003),(Moshfegh, et al., 1999), as a result of food manufacturing practices. Most processed foods entering the food chain contains FODMAPs which are added as flavour enhancers, sugar and fat replacement (Crittenden & Playne, 1996) and also for its prebiotic effect. (Wilson and Whelan, 2017). However, there remains limited data on FODMAP intake within the UK and the rest of the world, Australia excepted,(Barrett & Gibson, 2012).

The Monash group have validated and developed a food frequency questionnaire to assess FODMAP intake. It is a semi-quantitative FFQ with 297-item, also known as the comprehensive nutrient assessment questionnaire (CNAQ). When the FFQ was compared to a 7-day food diary during validation, it correlated well with most nutrients and FODMAPs, (Barrett and Gibson, 2010). FFQ is not only quick, easy and inexpensive to administer, it is also most

commonly used in epidemiological studies due to its ability to measure all nutrients.(Thompson and Subar, 2008) ,(Willett et al., 1985).

Therefore, the FFQ (CNAQ) seemed to be the most suitable dietary assessment tool to quantify FODMAP intake although it has only been validated within the Australian population. As validation studies are costly and time consuming, for the purpose of this study, the FFQ was compared against a 7-day food diary which has been validated in the UK to measure energy, macronutrients and micronutrients.

The 7-day food diary is known to be the accepted gold standard for validating other dietary assessment methods, such as the FFQ and 24-hour recall. It has been shown to have a good level of agreement with 16-day weighted records (Bingham et al., 1994), and has good correlation with biomarkers such as urinary nitrogen (Bingham et al., 1997)and doubly labelled water,(Bingham et al., 1997). The 7-day food diary is very similar to the weighed food diary, except recorded food and beverages are estimated using household measures or food photographs. This of course decreases the respondent's burden, especially when eating out. Thus, it is widely used in cross-sectional studies in addition to intervention trials. However, when compared with biomarkers the 7-day food diary was still the preferred method as unlike biomarkers it was able to give estimates of absolute intake, (Prentice et al., 2011). Biomarkers may not always provide an accurate measure as they are affected by diseases, absorption and metabolism. Therefore, the present study used the 7-day food diary as the next best recommended method (Bingham et al., 1995).

2.2 Aim

The study aims to establish the limits of agreement between a validated food frequency questionnaire (CNAQ) and a 7-day food diary

2.3 Objective

The objective is to quantify and evaluate energy, nutrient and FODMAP intake in healthy volunteers by comparing the comprehensive nutrition assessment questionnaire (CNAQ), a validated food frequency questionnaire in Australia, against a 7-day food diary.

2.4 Hypothesis

There will be no difference in the energy, nutrient and FODMAP intake using either the food frequency questionnaire or the 7-day food diary.

2.5 Methods

2.5.1 Subjects

The study was advertised to healthy volunteers using posters throughout University of Nottingham (UoN) campuses, and the Sir Peter Mansfield Imaging Centre (SPMIC). Participants who had previously expressed an interest in volunteering for studies at the SPMIC or Nottingham Digestives Diseases Centre (NDDC) were also contacted through the healthy volunteer database. Participants were given a dedicated number with which to text NDDC to register interest. The study was also promoted using social media (See www.facebook.com/ UoN.Gastrointestinal.MRI). Participants were recruited

between September and October 2014, with the last participant completing the study in December 2014. This study was approved by the Research Ethics Committee (REC) of the School of Medicine (SoM), University of Nottingham (A14082014 FOG SoM NDDC SPMIC).

Table 2-1: Inclusion and Exclusion Criteria

Inclusion criteria
Aged 18 or older
Able to give informed consent
Exclusion criteria
Self-declared vegetarian, vegan or kosher/ halal diet who cannot eat carmine red dye
Pregnancy
History of pre-existing gastrointestinal disorder that may affect bowel function
A positive diagnosis of IBS based on Rome III criteria
Previous resection of the oesophagus, stomach or intestine (excluding appendix)
Intestinal stoma

2.5.2 Study Design

This was an observational study involving healthy volunteers.

2.5.3 Procedure and Intervention

Interventions took place at NDDC, Queen's Medical Centre (QMC), described below.

2.5.3.1 Visit 1

Visit 1 took place at the NDDC, lasting 45-60 minutes. Upon gaining their consent, participants were screened against eligibility criteria (see Table 2-1) and the ROME III criteria (Appendix B : Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders', 2010)] to exclude participants with IBS.

Those eligible were enrolled in the study. Participants were asked to complete the online FFQ (CNAQ) and were given a 7-Day Food Diary with which to record their food and beverage intake. Using a written guide containing information on portion sizes, the Research Investigator (SK) instructed participants on how to record their food and beverage intake utilising the above diary.

2.5.3.2 Structure of Comprehensive Nutrition Assessment Questionnaire (CNAQ)

Participants completed an online semi-quantitative FFQ at an allocated computer work station, which had the questionnaire displayed according to their study number. The questionnaire was divided into columns, the first of which contained common food items with average portion sizes.

Foods were categorised according to groups such as fresh and canned fruit, dried fruit, vegetables, legumes, egg, meat, fish and equivalents, dairy, breakfast cereals, breads, carbohydrate foods, sugar and spreads, fats and oils, condiments and sauces, drinks and miscelleaneous. Participants were asked to select only one of the next 8 columns to record the frequency for an average portion of each food item consumed over the past year, ranging from never, less than 1 per month to 6 per day. All 297 food items had to be individually selected in order to complete the questionnaire. Some of the food items in the FFQ were not commonly consumed in the UK, while others were known by different names, e.g. aubergine is known as eggplant and papaya as paw-paw in Australia. Such anomalies were explained to the participants prior to questionnaire completion. All participants completed the questionnaire within

30-40 minutes. Participants completed the FFQ online and the nutrient and FODMAP content was exported from the website using an excel spreadsheet.

2.5.3.3 Rationale for Recording Food Intake using an Estimated Food Diary

As individuals typically have day-to-day variety in their food intake, a 7-day food diary is essential to capture such variations. A review by Bingham in 1987 suggested that at least 5-10 days of prospective recording was important to quantify macronutrient intake (Bingham et al., 1995). However, this method can increase participant burden and may result in under-reporting of intake after several days of recording.

Based on the study by Basiotis et al., 1987, only a small number of days is needed to measure food energy, macronutrient and some micronutrient between small groups of males (n=13) and females (n=16). A 3 day food record is adequate to estimate food energy in males and females. Meanwhile a four to six day record is adequate is to estimate protein, carbohydrate, fat, niacin potassium and phosphorus, sodium and oleic acid in both groups. (Basiotis P, rt al., 1987). In addition, a food record of 3 days has been recommended by Bingham et al., to estimate average food intake and its distribution in a group of individuals as long as it randomised to cover seasonal and weekday variations. (Bingham, et al.,1988).

Therefore, it was determined that for incomplete diaries, if the first 4 days of recording were complete, they were assumed to be suitable for analysis.

2.5.3.4 Recording of Food Diary

Participants were given a food diary (Appendix 9.5.1) to prospectively record their daily intake of food and beverages for 7 consecutive days during their screening visit. In order to optimise accuracy of recording, they were given verbal instructions and written guidelines (Appendix 9.5.2) on how to complete the diaries.

Details such as portion sizes, product brand names, cooking and preparation methods were required to describe the food items recorded. If composite dishes were prepared at home, the recipe was also recorded`. This included dry or wet weight of each ingredient, cooking method, number of servings and portion size consumed. Participants who ate restaurant/takeaway meals were requested to provide a description of snack, meal or beverage consumed and the name of the establishment. This was to assist in obtaining information regarding the meal if the description was unclear. In some cases, individuals were asked to provide food/beverage packaging for products that may not found on the UK food composition table, e.g. noodles produced and manufactured in Thailand.

To quantify the amount consumed, portion sizes were estimated using household measures and photographs (Appendix 9.5.2). The photographs consisted of small, medium and large portion sizes of several food items representing all the food groups.

An example of a completed day's food recording within the guide was shown to each participant, in order to reiterate the level of required detail. Although the main purpose of this diary was to record estimated intake some

participants choose to weigh their food and beverage consumption, which was acceptable. During participant visits, completed diaries were reviewed by the researcher to ensure that all information was legible and complete to facilitate dietary data entry.

2.5.3.5 Dietary Data Entry and Analysis

Data from each food diary was manually entered into a dietary software package: Dietplan P3 (Forestfield Software Limited version 6.70.75) for nutrient analysis. This user-friendly software was contained in a universal serial bus (USB) stick. Food and beverage data from food diaries was entered into Dietplan by matching with appropriate items listed within the software. Each food item and portion size was entered into Dietplan. Where there was no match for a food item, a similar food item in terms of nutritional composition was used (e.g. Cheddar cheese was used for Red Leicester cheese). For composite dishes and recipes, each ingredient was entered separately to create a new food item based on that composite dish or recipe. For new foods (e.g. gluten-free bread and lactose-free milk) similar traditional products were cloned and the content of specific nutrients (e.g. lactose) were adapted accordingly for the new foods.

Portion sizes were estimated when they were not recorded in the food diary, and several assumptions were made. Firstly, if the food had already been entered into the food diary on a different occasion with a portion size, this portion size was used. Secondly, if the food had not been entered into the diary with a portion size, an age appropriate average portion size was used from

standard food portion size tables(Mills and Patel, 2002) or other available literature.

2.5.3.6 Food Diary

Energy, macronutrient and micronutrient and FODMAP intakes were calculated for all 37 participants. Completed data with total intake were exported on excel spreadsheets. Mean daily intakes and total daily intakes were calculated. Energy intakes were evaluated and compared to gender-matched NDNS data. Those whose intake was not within the 2.5-97.5th percentile (1058-3315 kcal for males, 769-2587 for females) were re-analysed and revised as necessary. Besides comparing energy, data was also sorted in descending order for macronutrient intakes in order to observe for any excessive intakes. These were assumed to be input error and where required, food diaries were examined and corrected. Some of the participants consumed high amounts of protein in a form of supplementation; this was also entered and measured.

2.5.3.7 Macronutrient, Micronutrient and FODMAP Quantification from food diary

Macronutrient and micronutrient analysis was based on McCance and Widdowson's food composition data, UK (McCance, Holland, Widdowson, Britain) and (Ministry of Agriculture, 1991).

FODMAP intake was estimated using a newly developed FODMAP database, produced by King's College London (KCL), UK. In brief, for lactose and free fructose, McCance and Widdowson data was used (McCance et al., 1991). For fructans, galacto-oligosaccharides, sorbitol and mannitol, published data was

used (Prichard et al., 2016),(Anderson et al., 2015), (Yao et al., 2014), (Whelan et al., 2011), (Biesiekierski et al., 2011), (Muir et al., 2009),(Muir et al., 2007,).

Foods with published data, e.g. apple, onion, and wheat were added to the database. For foods where only qualitative data was available, e.g. those known to be low in FODMAPs, e.g. meat and fish it was assumed that they contributed no FODMAPs; for foods known to contain FODMAPs without any published data, data for similar foods were used, e.g. data for kidney beans were used for pulses without published data. For composite dishes and recipes, the raw ingredients used to make the composite meals were used to calculate the FODMAP levels for that food taking into consideration effects that cooking would have on water content for each ingredient. The database that was developed contained estimated levels of FODMAPs for over 90% of foods in the database.

2.5.3.8 Macronutrient, Micronutrient and FODMAP Quantification from FFQ

The macronutrient and micronutrient data were obtained from the Australian food composition table, NUTTAB 2006. The FODMAP data were obtained from the Australian FODMAP database. Most of the FODMAP data were obtained from laboratory analysis using High Performance Liquid Chromatography (HPLC) and fructan essay. The remaining data were a combination of analytical values obtained from recipe calculation and borrowed data from other existing databases.(Barrett and Gibson, 2010).

2.5.3.9 Micronutrient intake estimated from both food diary and FFQ

It was reported in a Swedish study, that there was not much difference in energy and macronutrient intake among IBS patients and healthy control [n=374 (control) vs n=139 (IBS)], but there were significant differences in fibre and calcium intake between both groups with the controls having a higher intake. (Bohn et al., 2013). In another south African study, (n=122) dietary fibre intake, iron, folate and calcium were significantly lower than dietary reference intake among IBS patients. (Stevenson . et al, 2014).Therefore calcium, folate, and iron were assessed in all the dietary assessment studies throughout this thesis.

2.6 Outcome Measure

The difference in the mean nutrient and FODMAP intake at baseline was calculated to assess the agreement between the FFQ and food diary.

2.7 Statistical analysis

Data handling and cleaning was done by SK, with assistance from ML. Statistical analysis was carried out with advice from KW and from the Statistical consultancy service at the School of Medicine, University of Nottingham. All statistical analysis was performed using IBM SPSS Statistics for Windows Version 23 (SPSS, Chicago, Illinois, USA) and Graph Pad Prism for Windows Version 7.0 (GraphPad Software, La Jolla California USA). Data was assessed for normality using Shapiro Wilk test and histogram.

Differences for energy, macronutrients, micronutrients, individual FODMAPs and total FODMAPs between the FFQ and the food diary were analysed using four statistical methods:

1) Comparison of means using paired t-test to compare estimates of intakes. Mean crude and energy adjusted dietary intake was calculated for each dietary assessment method. The performance of FFQ estimating dietary intake at the group level was determined by a paired t-test.

2) Spearman ranked correlations were used to assess the relationship derived from different methods.

3) Bland-Altman limits of agreement to establish how closely the FFQ agreed with the food diary. This was also visualised by plotting the difference between the FFQ and food diary against the mean of the two methods.

Where data were normally distributed parametric tests were performed and when they were non-normally distributed non-parametric tests were undertaken using SPSS. Parametric analysis and non-normally distributed data were analysed using non-parametric tests. Graph pad Prism was used to create the graphs representing limits of agreement between both methods.

2.8 Sample size calculation

The sample size of this study was based on the third study, Chapter 3. The effect size was based on a previous MRI study therefore a sample size of 45 participants was derived.

2.9 Results

2.9.1 Subjects

Of the 45 subjects who were recruited, 37 subjects were eligible and were enrolled into the study. All subjects completed both the FFQ and food diary giving a response rate of 100%. There were more females (68%) to males (32%) and both groups were between 20-53 years. The gender ratio and age group was quite similar when compared with the NDNS data (Bates et al., 2011). as shown in Table 2-2).

Table 2-2: Demographics of participants compared with NDNS data of adults

	FFQ/ Food Diary	NDNS
Sample size	37	1288
Sex (male: female)	12:25	503:785
Age, range(years)	20-53	19-64

2.9.2 Comparison of energy, nutrient and FODMAP intakes

The FFQ was compared against the 7-day food diary, for the intake of energy, protein, total fat, carbohydrate, starch, sugar, fibre, sodium, calcium, folate, iron, magnesium and total FODMAPs which includes fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), lactose, excess fructose, mannitol and sorbitol. FODMAP intake recorded using the food diary was measured using available FODMAP data in the UK. Intakes from both FFQ and food diary were compared and presented as absolute intake in Table 2-3 and energy adjusted intakes in Table 2-4.

The FFQ overestimated energy adjusted nutrient and FODMAP intake with a range between 32% (starch) to 460% (sorbitol). All indexes of the FFQ were significantly different from the food diary ($p<0.001$) with the exception of total fat ($p=0.079$), iron ($p=0.792$) and FOS ($p=0.014$) (Table 2-3). The FFQ is considered valid if the spearman coefficient is > 0.5. The correlation coefficient between the FFQ and food diary ranged from -0.76 (Total FODMAPs) to 0.05 (lactose). Apart from total FODMAP intake, all other nutrients and individual FODMAP failed to meet the spearmen correlation.

The limits of agreement for all nutrients were very broad suggesting that the FFQ had poor agreement with the food diary and this was replicated even when

outliers were removed. Figure 2-1,Figure 2-2,Figure 2-3 and Figure 2-4 show the limits of agreement for energy, carbohydrates, fibre and total FODMAPs.

Intakes measured using FFQ and food diary were also compared to dietary reference values (DRV's)(Public Health England, 2016a) and national population intakes in age and gender-matched individuals in the UK (NDNS). (Public Health England and Food Standards Agency, 2014) (see Table 2-5).

Overall, both male and female participants met the recommended Reference Nutrient Intakes (RNI) for all nutrients using both FFQ and food diary apart from carbohydrate intake measured using the food diary. Carbohydrate intakes were lower in both male (280 g/d) and females (221 g/d) when compared to DRV (male: 344-370g/d, female: 280-290g/d) and gender-matched individual intakes in the UK. (male:249g/d, female:199g/d).

All micronutrient intakes measured using FFQ and food diary were much higher than the RNI and national intakes with the exception of folate intake in men which was slightly lower than the population intake (.

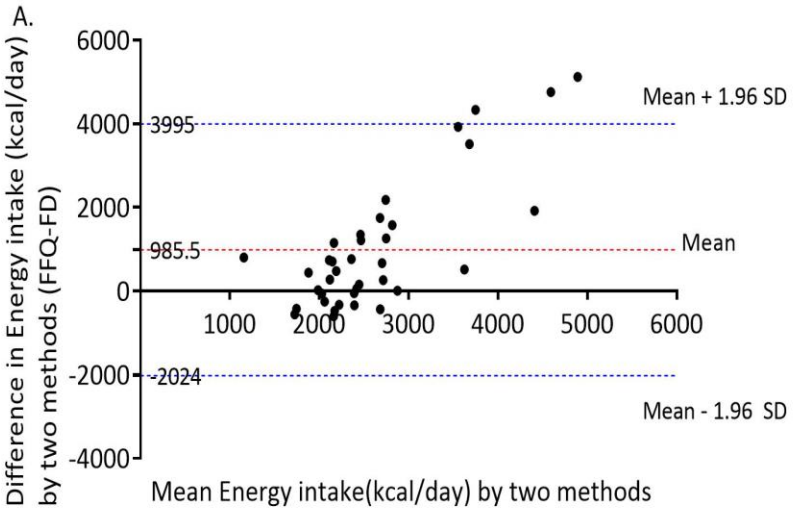
Table 2-3: Comparison between FFQ and food diary in assessing intake of macronutrients, micronutrients and FODMAPs

Parameter (n=37)	FFQ mean(SD)	Food Diary mean(SD)	Mean percentage (FFQ/FR)	Mean difference (FFQ-FE)	P value	Spearman correlation	Bland-Altman comparison of methods (FFQ versus Food Diary
Energy	3122(1500)	2137(510)	155.7	985.5	0.001	0.1	2075 - 4076
Protein	145(83)	88.3(25.6)	167.7	56.6	0.001	0.4	-91.22 – 204.5
Fat	103(46)	87.1(26.9)	133.9	15.9	0.259	-0.1(0.447)	-94 – 125.9
Carbohydrate	370(223)	240.1(61)	171.6	129.3	0.001	-0.2(0.912)	-325.7 – 584.4
Starch	185(102)	137.1(49.3)	153.7	47.8	0.095	0.2(0.371)	-161.6 – 257.2
Sugar	164(105)	97(33.7)	195.8	67.4	0.001	-.07(0.969)	-138.4 – 273.1
Fibre	42(27)	20(6.3)	230.9	21.8	0.001	0.3(0.066)	-35.5 - 76
Calcium	1559(1242)	909(316)	171.5	650.6	0.001	0.4(0.007)	-1572 - 2873
Folate	555(346)	257(79.4)	218.87	298.1	0.001	0.4(0.008)	-328.3 – 924.6
Iron	21.6(14.4)	19(32.6)	157.1	2.6	0.005	0.3(0.089)	-55.7 – 60.9
Magnesium	539(338)	310(86)	178.7	229.1	0.001	0.3(0.04)	-398.6 – 856.8
Total FODMAPs	41(29.1)	18.3(7.6)	292.9	22.6	0.001	-0.02(905)	-37.7 - 83
FOS	3.7(2.5)	2.7(1.2)	171.6	-0.9	0.082	0.2(0.314)	-6.4 – 4.6
GOS	2.2(1.9)	0.7(0.4)	458.3	-1.5	0.001	0.2(0.254)	-5.1 – 2.2
Excess fructose	7.3(9.7)	3.7(2.4)	292.9	22.6	0.095	-.03(0.856)	-16.6 – 23.8
Mannitol	1(0.9)	0.3(0.3)	1287.4	-0.8	0.001	0.3(0.038)	-2.3 – 0.8
Sorbitol	3.1(2.7)	0.7(0.6)	2525.4	-2.4	0.001	0.2(0.252)	-7.5 – 2.7
Lactose	23.5(23.2)	10.2(6.3)	171.6	-13.6	0.001	0.5(0.001)	-55.6 – 28.5

Table 2-4: Comparison between FFQ and food diary in assessing energy adjusted intake of macronutrients, micronutrients and FODMAPs

Parameter (n=37)	FFQ mean(SD)	Food Diary mean(SD)	Mean difference (FFQ-FR)	Mean percentage (FFQ/FR)	P value	Spearman correlation	Bland-Altman comparison of methods (FFQ versus Food Diary)
Energy	3122(1500)	2137(510)					
Protein	145(78)	88.2(21)	57.1	175.5	0.001	-0.16	100.1 – 214.3
Fat	104(37.5)	87(23)	16.6	129.1	0.079	-0.11	-67.1 – 100.2
Carbohydrate	368(204)	240(49)	127.6	159.2	0.001	-0.14	-277.8 - 533
Starch	185(91)	137(33)	-95.5	32.4	0.001	-0.15	-171 – 19.93
Sugar	164.2(92.3)	97(14)	66.2	170.9	0.001	-0.12	-114.9 – 247.4
Fibre	41.5(21.8)	20(3.4)	21.4	211.7	0.001	-0.14	-21.2 - 64.1
Calcium	1556(942)	908(177)	647.6	178.6	0.001	-0.15	-1225 - 2521
Folate	552(306)	257(36)	295.2	219.1	0.001	-0.16	-306.7 – 897.1
Iron	22(13)	19(4.6)	2.6	129.6	0.792	0.02	-25.4 – 30.7
Magnesium	537(315)	311(42)	225.5	175.4	0.001	-0.14	-394.5 – 845.4
Total FODMAPs	41(23.1)	18.3(0.7)	22.4	222.7	0.001	-0.76	-22.8 – 67.7
FOS	3.7(1.9)	2.7(0.1)	1.0	135.93	0.014	-0.03	-2.8 – 4.7
GOS	2.2(1.4)	0.7(0.1)	1.4	298.9	0.001	-0.009	-1.2 – 4.1
Excess fructose	7.3(6.1)	3.7(0.3)	3.6	198.1	0.002	-0.02	-8.4 – 15.6
Mannitol	1(0.5)	0.3(0.04)	0.8	371.7	0.001	-0.010	-0.1 – 1.7
Sorbitol	3.1(1.8)	0.7(0.2)	2.4	458.8	0.001	-0.113	-1.2 – 5.8
Lactose	24(12.2)	10(0.4)	13.3	230.7	0.001	0.05	-10.7 – 37.3

Figure 2-1: Level of Agreement between two methods (FFQ versus FD) for mean energy intake



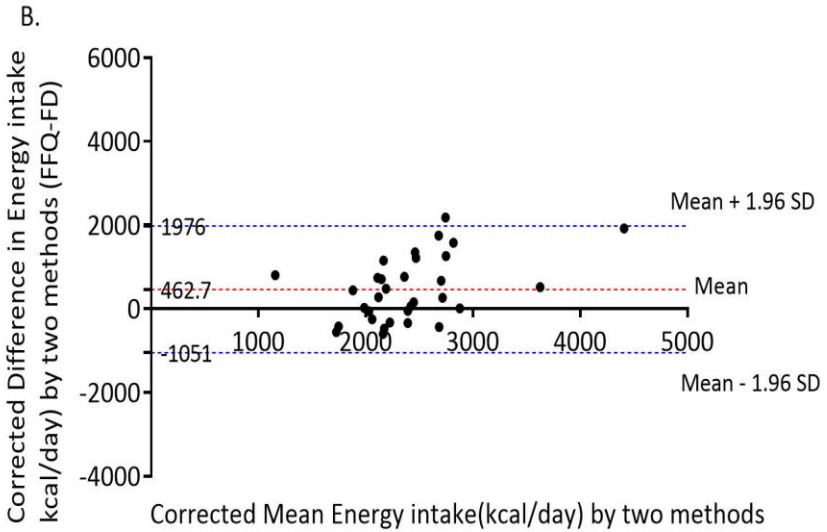


Figure 2-2: Level of Agreement between two methods (FFQ versus FD) for mean Fodmaps intake

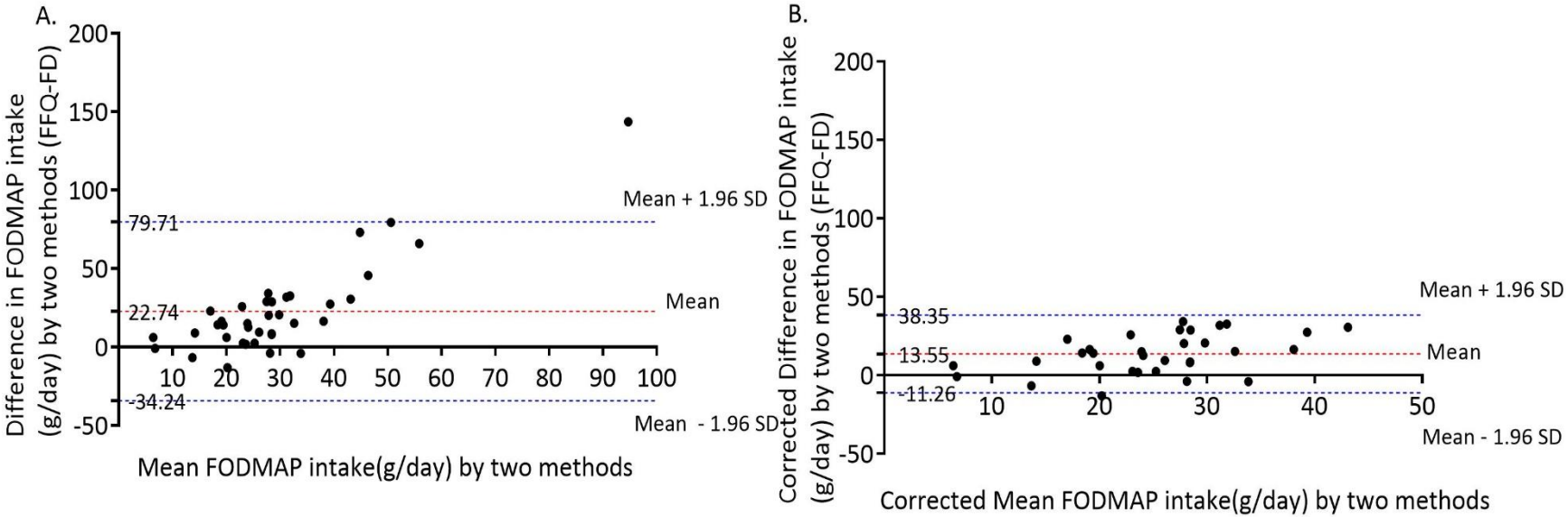


Figure 2-3: Level of Agreement between two methods (FFQ versus FD) for total carbohydrate intake

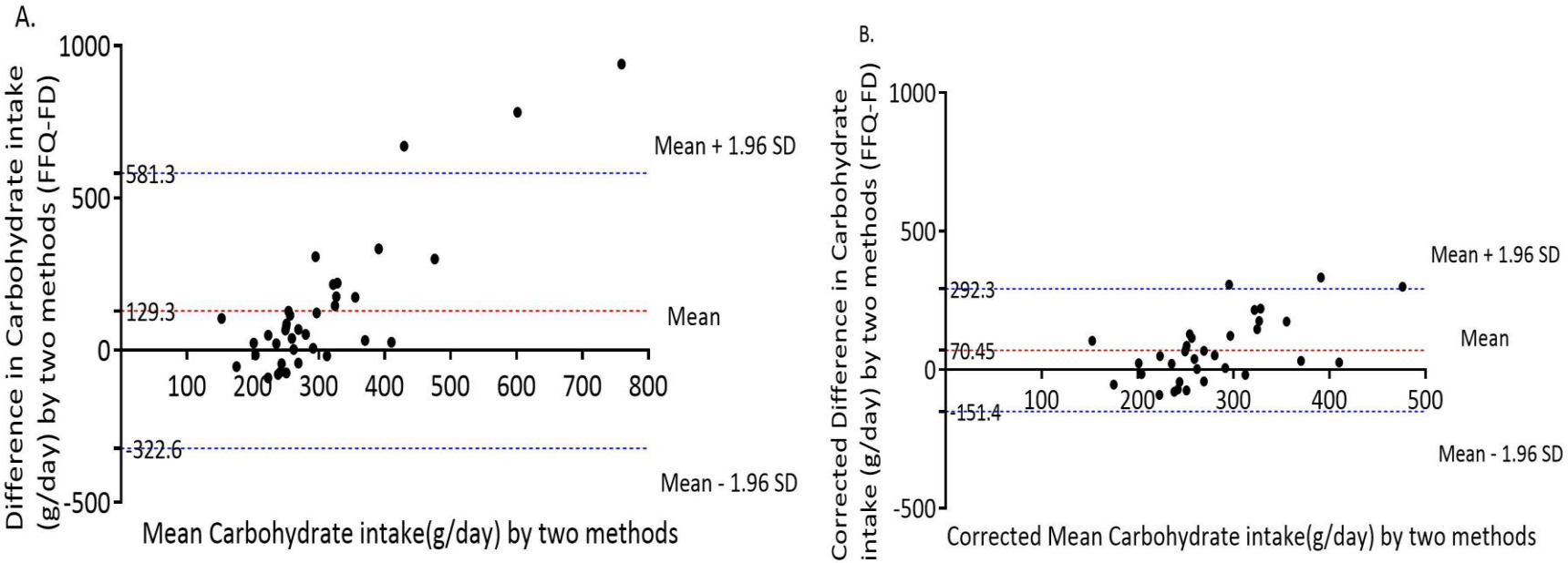


Figure 2-4: Level of Agreement between two methods (FFQ versus FD) for total fibre intake

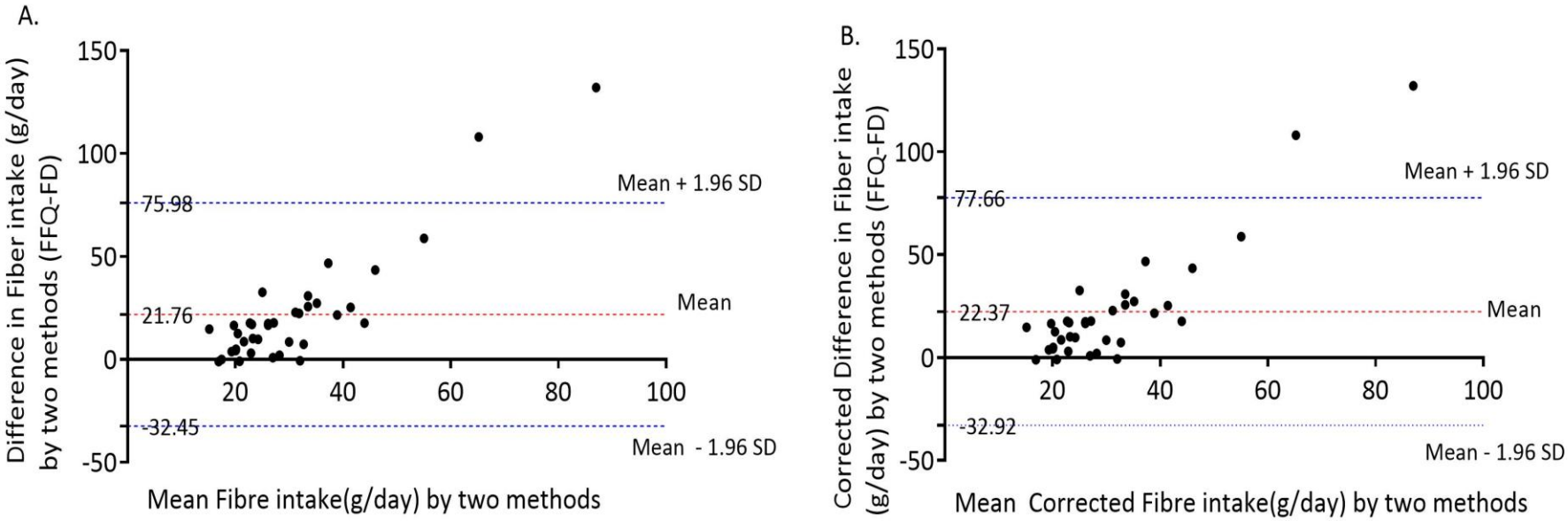


Table 2-5: Energy and nutrient intake measured using FFQ and FD with RNI and NDNS data

Parameter	**Female (n=25)**				**Male (n=12)**			
	FFQ	FD	DRV	NDNS	FFQ	FD	DRV	NDNS
Energy (kcal) EAR (kcal/d)	3007 (1670)	1951 (363)	2103-2175	1641 (504)	3363 (1103)	2524 (566)	2581-277	2100 (602)
Protein-RNI (g/day) (%) protein energy	133 (80.4)	79 (21)	45	66.6 (20.8) 16	170.4 (86.3)	108 (25)	55.5	87.4 (29.5) 17
Fat (g) % fat energy	96 (50.3)	79 (19)	33	62.4 (25.1) 34	118 (34)	104 (33)	34	76.6 (28.5) 33
Carbohydrate (g) (%) carbohydrate energy	369 (259)	221 (53)	280-290 47	199 (66) 46	370 (128)	280 (59)	45	249 (78) 45
Fibre Individual minimum	43 (32)	19.5 (6.5)	30	20.7 (7.1)	39.3 (13.4)	21.1 (6)	30	17 (6.2)
Calcium-RNI (mg/day)	1464 (1263)	825 (285)	700	746 (288)	1757 (1225)	1084 (317)	700	897 (381)
Folate -RNI (µg/d)	554 (397.3)	250 (82.4)	200	253 (209)	557.2 (219)	272 (74)	200	300 (327)
Iron -RNI (mg/day)	21.4 (16.6)	20.1 (40)	8.7-14.8	9.3 (3.2)	21.9 (8.5)	15 (4.2)	8.7	11.6 (4.3)

DRV: Dietary Reference Value,
NDNS: National Diet and Nutrition Survey (combined 2008/9 ,2011/12, 2013/14); Energy and nutrient intake for males at baseline and follow-up compared with the UK population intakes and dietary reference values.
*Energy, macronutrient intakes taken from 19-64 year cohort

2.10 Discussion

The study aimed to establish the limits of agreement between a validated food frequency questionnaire (CNAQ) and a 7-day food diary. The FFQ had poor correlation and level of agreement with the food diary for both energy adjusted macronutrients and micronutrients. In addition, it also overestimated energy and nutrient intake much more than the food diary in relation to national intakes of those aged between 19-64.

In comparison to population intake, the FFQ overestimated energy intake in females by 89% and in males by 60%. This was much higher compared to the food diary which overestimated energy by 39% in females. Whereas, energy intake among males were underestimated by 7.4%. Nutrient intake was also overestimated by FFQ between 20% to 160% in both males and females. The food diary overestimated nutrients within a range of 8% to 52% in females and also underestimated nutrients in males between 2.5% to 15%.

The current data supports previous work in the UK which showed that habitual intake met the RNI's for both macronutrients and micronutrients in IBS patients (Staudacher et al., 2012). This was also similar to habitual intakes among patients and healthy controls in a Swedish study (n=187) which met the Nordic dietary intake recommendations. (Bohn et al., 2013). Both studies used food diaries to record intake which ranged from four to seven days.

2.10.1 Reference method

This is the first study to assess FODMAP intakes using a UK FODMAP database as most studies have quantified FODMAPs using an Australian database (Halmos et al., 2015), (Staudacher et al., 2012), (Ong et al., 2010,). Currently, the only tool to quantify FODMAPs in the UK is limited to inulin and oligofructose (Dunn et al., 2011), therefore, it is more likely to underestimate FODMAP intakes.

Therefore, the food diary was chosen as a reference method to collect detailed description of food and beverage intake. Although, it is only valid to measure energy and nutrients in the UK, the food diary has been used in studies both in the UK and Australia to measure FODMAP intake. The UK study reported habitual FODMAP intake among IBS patients (n= 19) to be 29.6g/d (Staudacher et al., 2012). The Australian study reported intakes between 16.5g/d to 18.2g in healthy controls and 16.5g/d to 16.6g/d among IBS patients (Halmos et al., 2015), (Halmos et al,2014).

2.10.2 Limitations of reference method

A food diary is usually given so participants could describe and report their food intake. The data from a food diary is more comprehensive compared to a FFQ as it contains a detailed description of foods and beverages consumed and day to day variation if the record is kept for a week. However, people often tend to under report their intake over time also known as response bias or change their intake during the diary period known as response bias. This then leads to low energy reporters.(Brunner et al., 2001). In one of NDNS survey in the UK,

around 48% of female and 29% of male over 65 years and above were categorised as low energy reporters in the NDNS survey. This was similar to the 16-64 age group whereby 48% women and 29% males were under reporters. (Cook et al.,2000). Under reporters are usually obese or overweight individuals. In the EPIC-NORFOLK study, it was found that women under reported energy intake by 14% compared to 10% under reported by men.(Lentjes et al., 2014)

In addition, the food diary is also known to increase respondent burden and requires a highly motivated participant with high literacy rate (Thompson and Subar, 2008).

2.10.2.1 Identifying low energy reporter

In order to identify low energy reporters, the Goldberg cutoff could be utilised. This could be easily done by estimating individual energy requirement of participants based on their physical activity by multiplying their Basal Metabolic Rate (BMR) with physical activity level (PAL) to obtain energy expenditure. This would then be compared with energy intake (Black and Cole, 2000). This was done in this study as participants' physical activity was not assessed. However, it will be useful in planning future studies to assess self reported dietary intake.

2.10.2.2 Assessor error

There were some missing data on portion sizes for some food and beverages in the food diary. Therefore, the assessor had to estimate the portion sizes which has been described in section 2.5.3.5. This may have contributed to assessor error in estimating portion size by selecting a smaller or large portion size. There is a possibility is recipe calculation whereby weight of components of composite foods were underestimated or overestimated. Water gain or loss in

cooking was not taken into consideration. Finally selecting the wrong food code during data entry ie drinking chocolate was selected as diluted drink and not powder, underestimating energy intake.

2.10.2.3 Data error

The food composition table used to analyse the dietary data was only updated for certain food products therefore there was limited information on nutrient composition for newer products not yet included. Therefore, missing values and lack of information and out of date information would have led to data error. In addition, food label error may have also occurred whereby a difference between label and chemical analysis of processed foods.

The subject error could have been reduced by using a more appropriate method such as multiple-pass dietary recall method, conducted twice or thrice non-consecutively.

Meanwhile, assessor error could have been reduced by checking the details of food diary with the subject. This was done, although not necessarily thoroughly enough during the study.

Finally, data errors could have been reduced by checking on food composition table assumptions. This was done to a certain extent whereby nutrient details of processed food products were updated using the software. However, only major macronutrient data was available for updates and this may have underestimated micronutrient values.

2.10.2.4 Systematic Errors- Within and Between Individual Variation

Systematic error occurs when individuals with higher intake under-report or those with lower intake over-report dietary intake. In a 7 day food diary, this is usually corrected by averaging the total nutrient intake over 7 days to obtain mean daily nutrient intake.

However, a 2013 Japanese study reported that age and gender may affect the number of records required for adequate dietary assessment. A group of Japanese women and men completed a 4 -day non- consecutive food diary for each season (16 days in total). They were between 30-49 (both male and female) years old, 50-69 years old (female) and 50-76 years old (male). It was found that with younger women and older adults, fewer days were required to assess group or individual nutrient intake compared to younger men (Fukumoto et al., 2013).Given the age range of the participants in this study, which was 20-53 years of age and the male to female ratio of 12:25, perhaps a short 4-day food diary given over two occasions may have been able to reflect a representative micronutrient intake.

2.10.3 Test Method

Although the FFQ was the test method in this study, it overestimated energy, nutrient and FODMAP intakes as shown in Table 2.3. It is recommended that the reference method is completed first before the test method. In addition, the longer the gap taken between recording a food diary and filling a FFQ, the stronger the correlation between both methods to assess intake (Willett, et al., 1985).This was the opposite for this study due to the proposed study design. Participants completed their FFQ after consent and started their food diary

either on the same day or a week before their subsequent which may have influenced recording of their food diary. It is recommended that the FFQ is administered twice in a study period (Cade et al., 2002), however this was not carried out as the study duration was less than a year.

2.10.4 Limitations of the test method

The FFQ has been known to overestimate intakes particularly fruits and vegetables. This is possible due to social desirability bias whereby vegetarians have been shown to over report fruit and vegetable intake (Calvert et al., 1997).

2.10.4.1 Seasonal Variation

In order to capture FODMAP intake, it is pertinent to collect information on food and beverage consumed during different seasons as seasonal variation has an impact on FFQ response. (Fowke et al., 2004). An example would be stone fruits which are high in excess fructose and polyols which are seasonal and are only available during the summer. As both dietary assessments were, undertaken between September to December there is a high possibility that it did not reflect true FODMAP intake. Although some fruits may still be available all year round, the price may differ and since our subjects were mainly students, this may have been an additional factor.

2.10.4.2 Random error

During the study, the FFQ was administered online and was considered complete when all food items had been ticked. However, during data cleaning, a few participants had excessive food intakes within the same food group. When their FFQ was compared with their food diaries, intakes were much higher in the FFQ suggesting recall bias. This can be seen in Figure 2.2 and 2.3 which shows energy, carbohydrates, sodium and calcium as having the greatest mean difference between the two methods.

This could be classified as random error. In addition, the FFQ had fruits and vegetables that were not very common within the UK such as cumquat, jackfruit, kiwifruit, longan, lychee, nashi pear, sharon fruit, quince, tamarillo, tangelo; bok choy, bamboo shoots, bean sprouts, choko, kohlrabi and taro. This was another limitation as the initial study validating the FFQ had 20% of participants who were born overseas (Barrett et al.,2010). However, there were no missing foods for the most commonly eaten fruits and vegetables in the UK.

In summary both the FFQ and food diary had its limitations whereby people tend to over report foods deemed heathy in an FFQ and under-report energy, fat and alcohol intake in a food diary.

Perhaps a dietary recall method could have been used to reduce participant burden. This method has been shown to reduce underreporting due to self-reported intake. A 24-hour multiple pass method involves a structured interview between a participant and a trained investigator. The investigator

prompts the participant to recall foods consumed the day before. This interview is usually carried out face to face or by phone. This involves three passes with the first being a quick list whereby the participant needs to recall everything that has been consumed the day before. Then the same participant would need to give a detailed description of the foods which has been listed known as the second pass based on the interviewer's structured questions to determine the type of foods consumed, amount and cooking methods. Finally, a third pass is done whereby the interviewer reviews the list and probes of there is any other food/snacks that has been consumed clarifying the amount or portion size. (Johnson., et al., 1996).

Although it is time consuming, it is easy, inexpensive and could be done over the phone reducing the number of visits involved in the study. (Tran., et al., 2000). In order to assist in the recall, participants could be provided with an empty food record and a guide with estimated food portions to assist them during the phone call session and multiple recalls could be scheduled to record intake on weekdays and weekend. Despite being cost effective this method has its limitations. Participants may have a tendency to underreport when assessments are repeated and could also alter their intake prior to their scheduled assessment (Illner et al., 2012).

2.10.5 Conclusion

Although both dietary assessment methods had its limitations, the purpose of the study was to compare FODMAPs intake using a validated FFQ versus a food diary. Due to the fact that the FFQ overestimated energy and nutrient intake in

this UK study it is unsure if the FODMAP intake measured using FFQ is reliable to be used within the UK population. A validation study is needed to improve the FFQ to be used within the wider population. Until then, the 7 day food diary is probably a better method to assess energy, nutrient and FODMAP intake in IBS patients.

CHAPTER 3 A RANDOMISED CONTROLLED TRIAL OF THE EFFECT OF THE LOW FODMAP DIET AND DIETARY OLIGOFRUCTOSE ON GASTROINTESTINAL FORM AND FUNCTION OF HEALTHY VOLUNTEERS.

3.1 Background

The efficacy of the low FODMAP diet to treat the symptoms of IBS has been demonstrated in a number of RCTs (Varjú et al., 2017) (Marsh et al., 2016). Three main studies were those involving feeding trials, carried out in Australia involving healthy volunteers and patients, in which they were randomised to receive meals of high or low FODMAP content (Halmos et al., 2015),(Barrett et al., 2010), (Ong et al., 2010). Others involved patients receiving, as their intervention, low FODMAP dietary advice from dietitians (McIntosh et al., 2017,(Eswaran et al., 2016), (Staudacher et al., 2012),(Staudacher et al., 2011).

Besides symptom improvement, some studies started to assess luminal changes by measuring products of fermentation (Ong et al., 2010), faecal bacteria (Staudacher et al., 2017),(Halmos et al., 2015), and more recently, metabolome (McIntosh et al., 2017). This was pertinent, as FODMAPs have been shown to increase the small bowel water content of the lumen in ileostomates (Barrett et al., 2010), and quantity of colonic gas (Ong et al 2010).

To further understand the physiological changes, mechanistic studies using magnetic resonance imaging (MRI) techniques were carried out in Nottingham. One of the RCTs was a double blind, 4-way crossover study in healthy volunteers, which confirmed that FODMAPs in the form of fructose did increase luminal volume, whereas fructans increased colonic gas and therefore distended the large bowel (Murray et al., 2014). However, this was a proof of concept study and not a representation of habitual intake, as the fructan and

fructose doses were both 40g. This was certainly more than the habitual intakes of healthy volunteers or patients as reported by three studies.

The first was a UK study reporting 16.6g of fructose intake and 3.6 g of fructan intake in patients. The other two were Australian studies which reported 16.5-19.2g of fructose intake, and less than 4g of fructan intake. There was not much difference in intakes between healthy volunteers and patients.

The mechanistic action of FODMAPs is still unclear but the efficacy of the low FODMAP diet has been demonstrated and well accepted. Therefore, in this study the low FODMAP diet was used to control the FODMAP intake in order to measure the effect of FODMAPs (oligofructose) on colonic volume.

Two groups of healthy volunteers received as intervention a supplementation of oligofructose and maltodextrin on a background low FODMAP diet for 1 week. The idea for both groups was to lower FODMAP intake through dietary intervention, increase FODMAP intake through supplementation in one group whilst the other group served as a control. Changes from baseline in luminal volume were compared using MRI by measuring colonic volumes.

3.2 Aim

To assess whether FODMAP supplementation versus placebo affects fasting colonic volume in healthy volunteers while following a low FODMAP diet and investigate other physiological mechanisms that may explain any changes.

3.3 Primary Objective

1. To measure (by MRI) fasting colonic volume before and after supplementation and compare the differences between group before and after.

3.4 Secondary Objectives

1. To measure changes in whole gut transit (WGT) using MRI

2. To measure changes in colonic fermentation by assessing fasting breath hydrogen using a breath hydrogen meter

3.To determine compliance to a low FODMAP diet and supplementation by quantifying total FODMAP intake before and after intervention, using a 7 day food diary.

3.5 Hypotheses

A recent study from our MRI GI group reported an increased colonic volume, following an intake of 40g of inulin added to 500ml of water (Major et al., 2016). Individual data from a previous crossover study in healthy volunteers showed a within-individual coefficient of variation of 17% for fasting colon. (Murray et al., 2014). Therefore, there was an expected increase in colonic volume following oligofructose supplementation.

The null hypothesis of this study was that there is no difference from baseline in fasting colonic volume of healthy volunteers who consumed oligofructose supplementation, compared to those who consumed placebo (maltodextrin) for one week.

The alternative hypothesis of the study was that the increase in fasting colonic volume from baseline will be greater in healthy volunteers who consumed oligofructose supplementation compared to those who consumed placebo for one week.

3.6 Methods

3.6.1 Subjects

The participants recruited to the Study in Chapter 2 were included. Recruitment, inclusion and exclusion criteria have been described above in Chapter 2 (Table 2-1). Additional exclusion criteria for this study are described below:

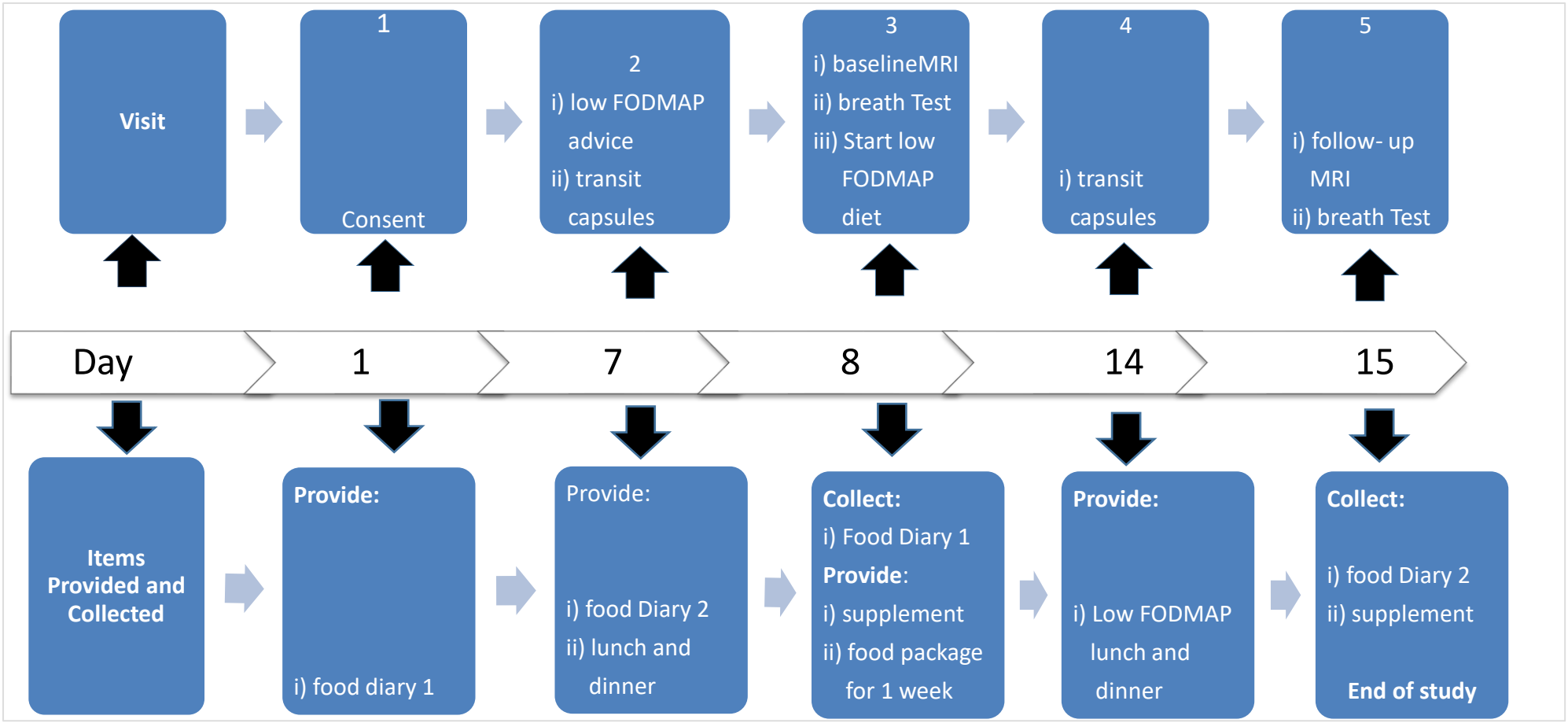
Table 3-1: Exclusion criteria for randomised controlled trial of the effect of the Low fodmap diet and dietary oligofructose on gastrointestinal form and function of healthy volunteers.

Exclusion criteria
Any medical condition compromising participation in the study. e.g. diabetes, respiratory disease limiting ability to lie in the scanner
Contraindications for MRI scanning e.g. metallic implant, pacemakers, history of metallic foreign body in eye(s), and penetrating eye injury
Unable to limit alcohol intake to <35units/week and ≤ 8 units. day during trial
Inability to stop drugs known to alter GI motility
Inability to lie flat on the scanner (cannot lie flat or > 120kg)

3.6.2 Study Design

This was a parallel group, double-blind, randomised controlled trial at the BRU and SPMMRC trial sites. All subjects were randomised at a ratio of 1:1 in blocks of 6 to prevent imbalance between groups. This study was approved by the Research Ethics Committee of the School of Medicine at the University of Nottingham (A14082014 FOG SoM NDDC SPMMRC). The study has been prospectively registered on clinical trials.org (NCT02259465).

Figure 3-1: Schematic diagram of study process and intervention



3.6.3 Summary of study

Participants completed 5 visits over 15 days at the BRU or SPMIC. Those who completed all visits and returned both food diaries and supplement container were regarded as having completed the study as shown in Figure 3-1.

All participants were advised to follow a low FODMAP diet for 1 week beginning from Visit 3 subject to availability of scanner.

3.6.3.1 Visit 1 (Consent)

Visit one (30 minutes) took place at the NDDC. The investigator fully explained the study, and screened participants for eligibility using the ROME III("Appendix B : Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders," 2010) criteria to exclude participants with functional gut disorders. Those who were eligible were then consented and enrolled in the study. An MRI safety screening questionnaire including a questionnaire regarding personal tattoos was completed as part of this process. Height, weight, medical history, current medication and smoking history were also recorded.

Participants were instructed on how to complete a 7-day food diary (see chapter 2) and were given a sample food diary with guide. They recorded their food and beverage intake during their habitual diet and proceeded to record another 7-day food diary during the intervention phase.

Participants were asked to restrain from excessive alcohol intake during the study, and to avoid consuming alcohol 48 hours before their MRI scan.

3.6.3.2 Randomisation

Randomisation was performed by an independent member of the NDDC staff, Dr Rebecca Harris (RH), using the online program www.randomization.com, seed 6565, in a 1.1 ratio. The code and seed number were retained by RH for the duration of the study, while a second copy was held by the Chief Investigator Prof. Spiller, in a sealed envelope until unblinding.

3.6.3.3 Blinding

Both participants and investigators in this study were blinded to the supplement, which consisted of oligofructose (intervention) or maltodextrin (placebo). Both supplements were prepared in opaque containers by the investigator (SK). The supplements were then given to RH for allocation of the appropriate randomised study number. The investigator (SK) was blinded to the randomisation codes. Both supplements were in a form of white powder identical in appearance. The only identification was the participant number. Participants were finally unblinded when the study was completed.

3.6.3.4 Compliance to supplement

Compliance to supplement was determined by collecting and weighing containers for each participant's final visit. It was assessed by measuring the gross weight of the supplement container before being given to the participant and following its return at the end of the study. Compliance was calculated as (actual weight change / expected weight change) * 100%, with an acceptable range of 70 – 140%. All participants demonstrated adequate compliance with most of them taking more than 100% of the required amount.

3.6.3.5 Details of products

Participants were randomised to receive either maltodextrin 7g twice daily (Hut Group, Northwich, UK) (placebo) or oligofructose 7g twice daily (Orafti P95® Beneo, Germany). Maltodextrin is a long-chain glucose polysaccharide with no known GI side effects and oligofructose is a short chain carbohydrate. The dose of oligofructose proposed in the study is considered not to have any significant adverse effects as healthy volunteers are able to tolerate up to 30g of Oligofructose/day (Windey et al., 2014). Participants were instructed to take whichever product for which they were randomised, twice daily for 7 days during intervention, using the scoop provided to add to their warm beverages or breakfast cereal.

3.6.3.6 Supplement preparation

The research investigator (SK) prepared both products using a sterile container and added 5g of Carmine Red food dye – E120 into each supplement container. The final weight of each supplement was approximately 200g exclusive of container.

Carmine Red was used as a marker for whole gut transit as it is non-absorbable in the gut. It has no side effects and improves masking both supplements by making them pink. This was used to aid compliance, since subjects were informed that this could be assessed in the stool samples they provided. The stool sample was collected as part of the outcome measure for GM a research colleague's thesis and will not be discussed here. The use of this dye in a previous study was approved by the University of Nottingham Medical School research ethics committee for a 2013 pilot study (A11072013 SCS MRI).

3.6.3.7 Packaging and Labelling

Both products were packed using an opaque plastic container in the NDDC in a food preparation area by the research investigator trained in food hygiene. Labelling was done by staff members who had access to the randomisation codes.

3.6.3.8 Dietary Intervention

This was ascertained by reviewing completed food diaries, recorded by participants during the intervention phase. A diary was regarded complete and subjected to analysis provided that the first four days of the diary were complete. This was deemed acceptable, as the first four days of a food diary would typically depict usual intake for the participant's age group.

3.6.3.9 Rationale for recording food intake using an estimated food diary

Participants were required to keep a second 7-day food diary to record their intake while being on a low FODMAP diet. In addition, the diary was used to assess adherence to the diet and compare FODMAP intake between groups.

As individuals have day-to-day variety in their food intake, this method captured such intake variations. A review by Bingham in 1987 suggested that at least 5-10 days of prospective recording was important to quantify macronutrient intake (Bingham et al., 1995). However, this method can increase participant burden and may result in under-reporting of intake after several days of recording. Therefore, for incomplete diaries, if the first 4 days of recording were complete it was assumed suitable for analysis (O'Neill et al ,2007).

3.6.3.10 Visit 2

Visit two took place at NDDC approximately 1-4 weeks after visit 1, lasting around 90 minutes. Each visit consisted of 6 participants.

Participants were instructed to fast from 8pm the night before, the exception being for a small amount of water with essential medication. They were requested to fast prior to swallowing 5 Gadolinium-filled MRI transit capsules. After swallowing their transit capsules, participants were offered breakfast

3.6.3.11 Transit capsules

The transit capsules (Figure 3-2)were made of polyoxymethylene and were the shape of a size 1 gelatine capsule (20mm by 7mm). They were filled with 0.4 ml of 15µM Gadoteric acid solution and sealed with cyanoacrylate superglue. They have previously been used in studies with healthy volunteers and patients without complication (NRES approvals 10/H0906/50 and 11/EM/0245).

Figure 3-2: Transit Capsule



Chaddock et al., 2014

3.6.3.12 Provision of meals to aid compliance

Prior to the MRI scans participants were provided with breakfast, lunch and dinner similar to a habitual diet (Table 3-1 and Table 3-2). The meals were provided to reduce interindividual and intraindividual variability in MRI parameters related to diet. In addition, participants were advised to avoid a meal high in fibre (especially lentils and pulses) prior to their MRI scan, as per standard protocol, to prevent accumulation of gas in the small and large bowel which would mask the gastrointestinal volume from the study product and placebo. However, participants were allowed to snack in addition to the meals provided during the visit, as long as they consumed the food supplied for their main meals.

3.6.3.13 Low FODMAP dietary education

All subjects were given low FODMAP dietary education by a registered dietitian. The session was conducted over 45 minutes within a group setting, using written material published by Guy's and St Thomas' NHS Foundation Trust. Subjects were given dietary advice using the "Reducing Fermentable Carbohydrates the Low FODMAP Way" booklets as well as the "Suitable Products for the Low FODMAP Diet" which was to be used as their shopping guide. As instructed, participants followed the dietary advice for a week.

Table 3-2: Test meals provided to participants during visit 2 and visit 4

	Breakfast	**Lunch**	**Dinner**
Visit 2	2 slices of medium white bread with butter and jam	1 cheese sandwich	1 can of Heinz chicken soup with vegetables
	1 cup of coffee/tea with milk and sugar	1 packet of crisps	2 slices of white bread
	1 pot of orange juice	1 piece of apple	1 slice of cake
	1 pot of fruit flavoured yogurt		
Visit 4	Porridge made with lactose free milk	2 slices of gluten free bread	1 packet of white microwavable rice
	1 cup of coffee/tea with lactose free milk and sugar	1 can of tuna	1 can of corned beef
	1 piece of banana	1 sachet of mayonnaise	1 can of tinned carrots
		1 packet of crisps	1 packet of oatcakes
		2 small satsumas	

Table 3-3: Nutrient composition of test meals in Visit 2 and Visit 4

Nutrient/serving	Visit 2 (Habitual diet)			Visit 4 (Low FODMAP Diet)		
	Breakfast	Lunch	Dinner	Breakfast	Lunch	Dinner
Energy (kcal)	439	518	752	429	605	920
Carbohydrate (g)	67.5	33.5	120	67.9	43.5	116.4
Protein (g)	11.1	11.3	22	14.3	44.4	32
Fat (g)	13.8	14.1	18.6	8.0	16.9	29.8
Fibre (g)	2.1	2.0	5.1	3.8	7.5	12.8

3.6.3.14 Visit 3

The third visit took place the day after Visit 2 at the 1.5T scanner of the SPMMRC and lasted up to 1 hour. Participants were instructed to fast from 8pm the night before except for a small amount of water permitted with essential medication.

During this visit, food diaries were collected before the procedure. Each participant had a breath sample taken using a GastroCHeck meter (Bedfont, UK), followed by a sequence of MRI scans lasting some 15-20 minutes per person.

Prior to the MRI scans participants were asked to change into the provided hospital examination gowns and reconfirm their MRI safety screening questionnaire. The MRI scan sequences measured colonic volume, colonic gas volume and other MRI parameters (T_1, T_2) that described physico-chemical characteristics of the bowel and its contents.

Participants were instructed on Visit 3 to start their diet after completing all procedures. They were given a low FODMAP breakfast (Table 3-1 and Table 3-2) and their study supplement. The first serving of the study supplement was taken together with breakfast at the SPMMRC, to ensure understanding of dosage and prevent under or over consumption.

At the end of the visit, participants were also given a 7-day food diary in which to record their diet during the intervention. This was to measure nutrient and FODMAPs intake, and to monitor diet compliance. In addition, they took away a package contaning low FODMAP food products, as described in Table 3-1 and Table 3-2; to supplement their diet.

From the eight day to the thirteenth day, participants adhered to the low FODMAP diet. A researcher from the study team contacted participants at least once during that time to discuss any issues arising and offer encouragement as required. Additionally, participants were also free to contact the study team regarding any issues that arose. As per MRI guidelines, they were reminded to abstain from alcohol for 48 hours before their next scan on the fourteenth day.

3.6.3.15 Visit 4

The fourth visit took place at the NDDC and lasted 15 - 20 minutes. This visit was similar to the second visit, with participants fasting until they attended the NDDC at 8.30am. They were instructed to continue recording their dietary intake in the food diary until their fifth visit.

During this visit, participants were provided with a low FODMAP breakfast and asked to swallow five transit markers. They continued to take their supplement for the rest of the day, with their last serving of supplement being the second serving the fourteenth day.

At the end of the visit participants took away a low FODMAP packed meal which consisted of lunch and dinner for consumption on that day (Table 3-1 and Table 3-2).

As described in the second visit, participants could snack on low FODMAP foods or beverages in addition to the main meals provided during this visit. They were instructed to continue their food diaries until the next morning, and to fast from 8pm the night before (except for a small amount of water permitted with any essential medication).

3.6.3.16 Visit 5

The fifth visit, on the fifteenth day, took place at the 1.5T scanner of the SPMMRC, lasting up to 1 hour. This visit was similar to the third visit. During this visit, participants returned their completed second food diary and low FODMAP booklets. They also brought in their supplement container, which was weighed to measure the amount remaining in order to assessing compliance.

Participants were subjected to the same procedures as at Visit 3: breath testing and MRI scanning. The completion of these procedures marked the end of the participant's involvement in the trial. They were offered a light snack and a drink at the end of the study day.

3.6.3.17 MRI Scan

During the third and fifth visit, participants had an MRI scan at the 1.5T scanner of the SPMMRC. Each sequence of MRI scans lasted 15-20 minutes per participant on each visit, in order to measure colonic volume and colonic gas volume.

3.6.4 Magnetic Resonance Imaging to Measure Colonic Morphology

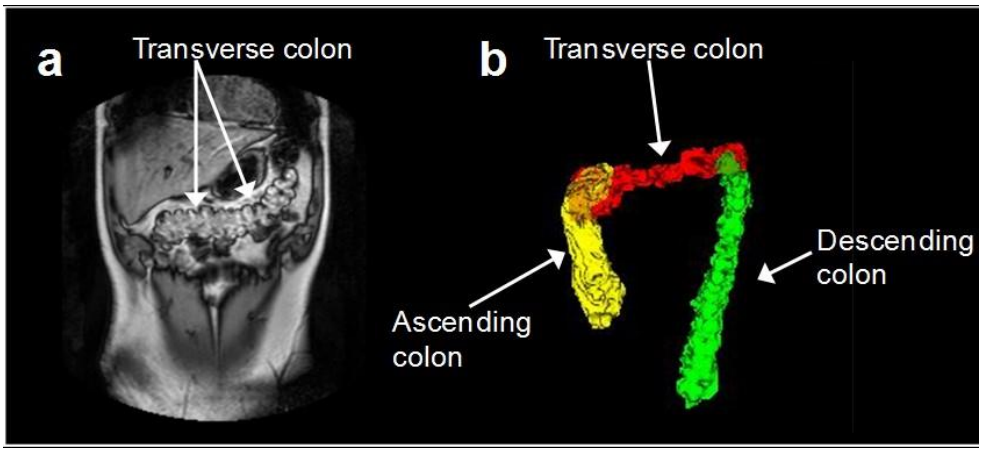
A recent pilot study in our department showed that adding 10g oligofructose (Major et al., 2014) to the diet increased colonic volume by 18% healthy volunteers. In this open label study, 16 healthy who consumed 10g of oligofructose a day (5g twice daily) for a week had an increase in colon volume and breath hydrogen. Therefore, in the present study, oligofructose was used with an increased dose of 7g twice daily to investigate similar effects.

3.6.5 Colonic Morphology

Calculation of colonic volume was made based on the previously described methods (Pritchard et al., 2014) using Analyze9^{TM} software (Mayo Foundation, Rochester MN, USA), where individual regional colon volumes were segmented manually from the coronal data on each image slice. Each colon region was identified within each coronal image slice, building a 3D representation of the morphology from which the volume of each region was measured as shown in Figure 3-3. Information from axial data was used to guide definition of the regions where anatomy was ambiguous.

Colonic volumes were measured by SP, a research fellow experienced in making such measurements who was blinded and not involved in the study.

Figure 3-3: Three-dimensional construction of colonic regions



This panel shows (a) one coronal 'dual FFE' MRI image of the transverse colon of a healthy volunteer taken at fasted baseline, and (b) a three-dimensional reconstruction of the different regions (ascending, transverse and descending) of the colon of another healthy volunteer. (Pritchard et al., 2014)

3.6.6 Whole Gut Transit Time (WGTT)

Whole gut transit time was measured following the Chaddock method (Chaddock et al., 2014). Transit was assessed and scored based on the scans performed 24 hours after marker ingestion, using a validated algorithm and equation. The previously documented scoring system divides the bowel into 8 sections (Figure 3-4), allowing each capsule to be scored according to its position in the bowel after 24 hours. A weighted average position score of the 5 pills (Figure 3-5) was determined by SP and CM for each volunteer, by looking at the images from each participant. The scores were then entered onto a spreadsheet, which calculated the whole gut transit time based on the scores of the 5 transit capsules and difference between these scores.

Figure 3-4:.Segmented colon scores

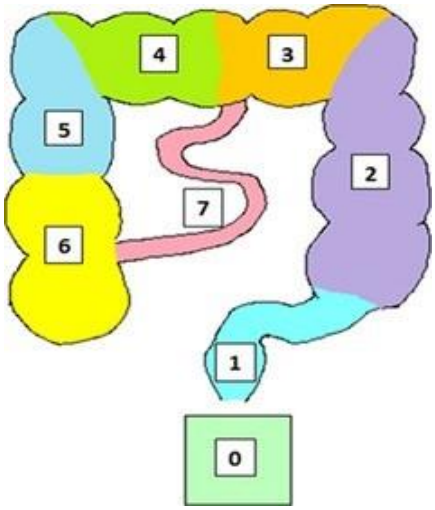
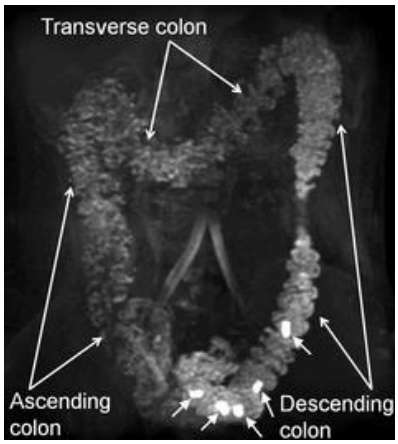


Figure of segmented colon used to score the MRI marker capsules at 24 hours, where 0 = not found (presumed to be excreted), 1 = sigmoid and rectum, 2 = escending colon, 3 = left transverse colon, 4 = right transverse colon, 5 = upper ascending colon, 6 = lower ascending colon, 7 = small bowel (Chaddock et al., 2014)

Figure 3-5: MRI image of transit capsules in the colon



This is a maximum intensity projection (MIP) of an MRI image showing five MRI marker capsules in the colon (indicated by close arrows). Images created using water-only images, acquired using the T1 weighted multi-echo FFE pulse sequence. Such images were used to locate the number and position of capsules remaining at 24h to calculate a whole gut transit time. (Chaddock et al., 2014)

3.6.7 Assessing energy, nutrient and FODMAP intakes

The methodology has been described in detail in Chapter 2. Total FODMAP intake was measured (by SK) by entering and analyzing 74 food diaries; 37 of

these were baseline dietary data recorded during habitual diet, with the remaining 37 containing intervention dietary data, which were assessed for compliance.

3.7 Outcome Measure

3.7.1 Primary endpoint

The primary endpoint was to measure changes in fasting colonic volume after one week of a low FODMAP diet, and supplementation with oligofructose or maltodextrin. The differences in the mean, within and between groups, were also measured.

3.7.2 Secondary endpoint

The secondary endpoints were set out to assess possible physiological explanations for differences between groups in the primary endpoint. These were:

- Changes in the whole gut transit time as determined by Weighted Average Position Score (WAPS) of MRI transit markers 24 hours after ingestion, after one week of dietary intervention.
- Change in fasting breath hydrogen concentration after one week of dietary intervention and supplementation, measured in parts per million.
- Changes in fasting breath methane concentration after one week of dietary intervention and supplementation, measured in parts per million.

- Changes in FODMAP intake after one week of dietary intervention and supplementation, measured as gram/day.

3.7.3 Planned Comparison

All outcome measures were based on differences between primary endpoints and secondary endpoints.

3.8 Statistical Analysis

The PERFeCT study (Major, 2016) found a mean 18% increase in colonic volume, following oligofructose supplementation, in addition to participant habitual diet. Using individual data from a previous 4-period crossover study of healthy volunteers (Murray et al., 2014), the within-individual coefficient of variation for fasting colon volume was calculated to be 17%.

Therefore, sample size for this study was calculated to provide 80% power for detecting a 15% greater increase in colonic volume with oligofructose compared to maltodextrin (α=0.05, 1-tailed). This produced a sample size of 36, randomised 1:1. Ethical permission was sought to enroll up to 45 participants to gather 36 complete data sets.

Continuous variables were tested for normality using Shapiro-Wilk test. Normally distributed data were analysed by parametric test, dependent t-tests or independent t-test and expressed as mean (SD). Non-normally distributed data were analysed using non-parametric analysis using Wilcoxon-signed rank and Mann-Whitney U tests and were expressed as median (IQR). Significance was reported where $p<0.005$ unless other indicated.

3.9 Results

3.9.1 Consort Diagram

The study consort diagram is shown in Figure 3-6. A total of 48 participants recruited and consented for the study. Three participants were excluded prior to enrolment as one met the ROME diagnostic criteria for IBS and the other two had recently been prescribed antibiotics. 45 participants were randomised and eight withdrew, five due to changes in their study schedule and the remaining three due to failure to attend Visit 2. The final sample size for the trial was 37 with 19 participants randomised to receive oligofructose and 18 randomised to receive maltodextrin.

3.9.2 Demographics and baseline data

Demographic data and baseline characteristics of participants in both groups are presented in Table 3-4. As participants were not matched for gender, there were more females in the maltodextrin group compared to the oligofructose group. There was no significant difference with regards to age, weight, height and body mass index between both groups. Two-thirds of participants were from White British or White European background.

One participant's colonic volume data had to be excluded from analysis due to corrupted data.

Figure 3-6: Consort Diagram of FOG study

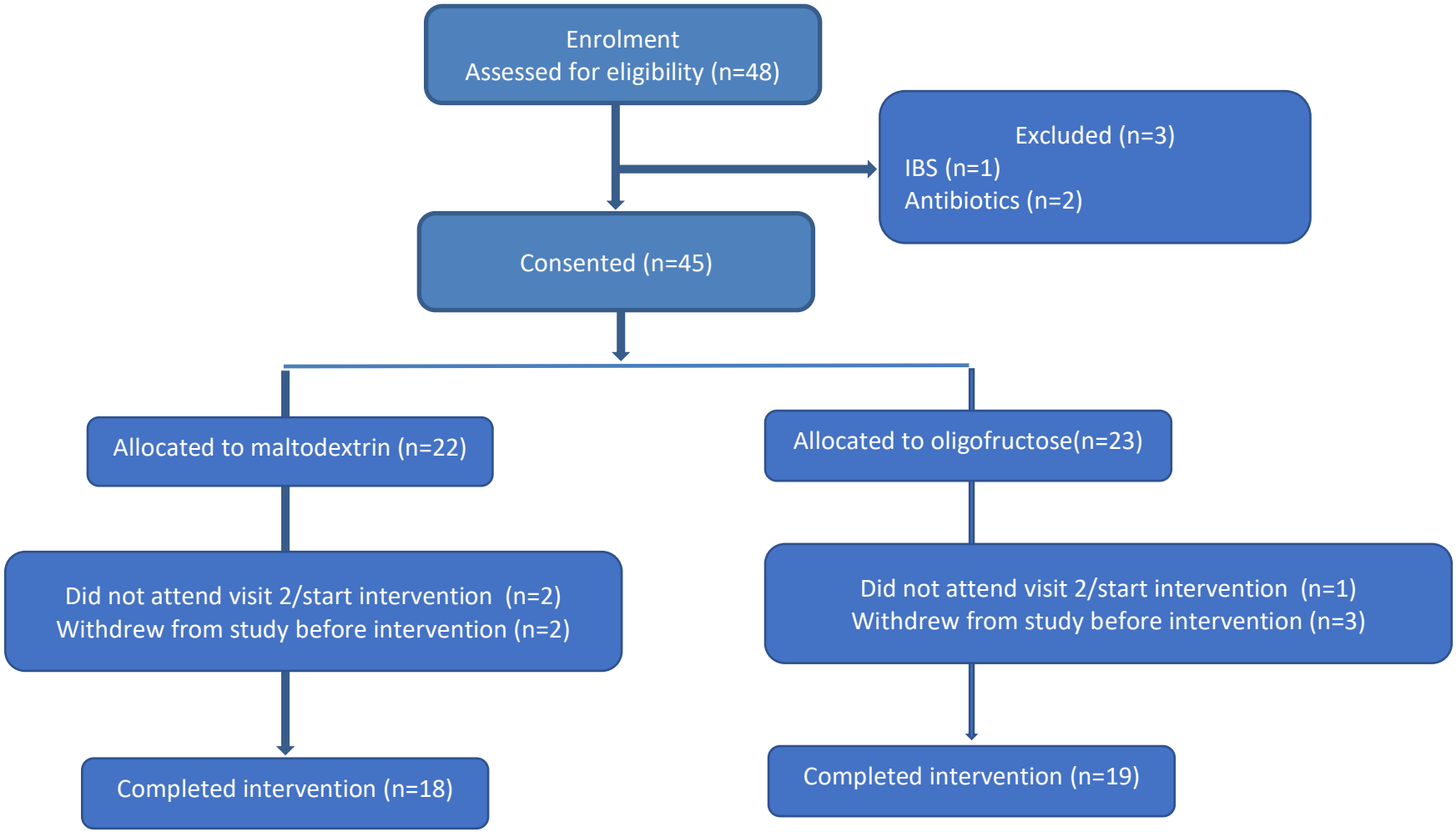


Table 3-4: Baseline demographic data for healthy volunteers in maltodextrin and oligofructose groups

Parameters	Maltodextrin Group n = 18	Oligofructose Group n = 19
Age, years median (IQR)	21 (20, 31)	21 (21, 32)
Females n (%)	14 (78)	10 (53)
Weight (kg) median (IQR)	64 (61-75)	70 (63-73)
Height (m) median (IQR)	1.7 (1.6-1.7)	1.7 (1.7-1.8)
Body Mass Index (kg/m2) median (IQR))	23 (22-25)	22 (21-24)
Ethnicity n (%)		
White British/European	14 (78)	14 (74)
Black British/Black	2 (11)	-
Chinese/Thai/Japanese	2 (11)	5 (26)
Current smoker n (%)	1 (7)	3 (20)
Baseline colonic volume (ml) mean (SD)	650.1 (179.1)	693 (150.5)
Baseline WAP (a.u.) mean (SD)	2.1 (1.4)	1.5 (0.7)
Breath Hydrogen (ppm) median (IQR)	13 (5, 26)	13 (3, 26)
Breath Methane (ppm) median (IQR)	4.5 (2, 45)	6 (3, 36)
FODMAP intake mean (SD)	17.4 (7.5)	18.8 (8)

3.9.3 Compliance to supplement

Calculation of compliance has been described in section 3.6.3.4. Overall, all participants demonstrated adequate compliance to supplement in both maltodextrin and oligofructose group. However, some of the participants in the oligofructose group consumed more than the recommended dosage which was 14g/d.

3.10 Primary Outcome Measure

3.10.1 Colonic Volume

The primary outcome measure was the difference in colonic volume between both arms calculated as percentage change. There was a significant increase in colonic volume from baseline in both groups. In the Maltodextrin group, mean increase was 90ml (650ml versus 741ml, p = 0.035). Meanwhile, the Oligofructose group had an increase of 110ml from baseline (693ml versus 803ml, p = 0.016). When converted to a percentage, maltodextrin indicated a mean increase of 15.5 % (95% CI 2.5-28.4 p = 0.035) and oligofructose 19.6% (95% CI 6.5-32.7, p = 0.016). There was no significant difference in percentage changes of colonic volumes in both groups (90ml versus 110 ml, p = 0.722) as shown in table 3.6. Table 3-5 shows individual changes and mean changes between groups for maltodextrin and oligofructose. Figure 3-7 shows individual changes for both maltodextrin and oligofructose and mean changes between maltodextrin and oligofructose. When segmented colon volumes were compared, the was a significant increase in ascending colon volume in the oligofructose group from with a reduction of 8mls (95.6) in the maltodextrin

group compared to an increase of 57ml (95.3) in the oligofructose group from baseline with p =0.05.

3.11 Secondary outcomes measures

3.11.1 Weighted Average Position Scores (WAPS)

There were no significant changes from baseline Weighted Average Position Scores in both groups. The maltodextrin group had a baseline score of 2.1 (1.4) and a follow up score of 2.0 (1.5) indicating that capsules were positioned in the descending colon. Meanwhile, the oligofructose group had a baseline score of 1.5 (0.7) and follow-up score of 1.3 (1.1), indicating that the capsules were in the sigmoid and rectum.

There were no significant changes in Weighted Average Position Scores from baseline in the maltodextrin group ($p<0.3$) and oligofructose group ($p<0.4$ shown in Table 3-5. There were also no significant changes from baseline within groups as shown in Table 3.6 with $p=0.718$.

3.12 Breath Test

3.12.1 Breath Hydrogen

There was a significant reduction in breath hydrogen within the maltodextrin group from baseline (13ppm versus 3.5ppm, $p = 0.01$). The oligofructose group, had an increase breath hydrogen from baseline but this was not significant (13ppm versus 23ppm, $p = 0.061$). There was a significant difference in breath hydrogen between the two groups at follow-up (maltodextrin: 3.5ppm versus oligofructose: 23ppm, $p = 0.001$). Table 3-4 and Figure 3-8 show individual

changes of breath hydrogen within groups and between groups. Table 3.6 show changes in breath hydrogen within groups. The maltodextrin group had a reduction in breath hydrogen with a median value of -7ppm (-25,-0.75) whereas the oligofructose group had an increase with a value of 10ppm

(-0.25,44.8). The differences between the changes from baseline within groups were significant with p=0.009.

3.12.2 Breath Methane

Breath methane levels were lower compared to breath hydrogen at baseline in both groups (5ppm versus 6ppm; p = 0.539).

There were no significant changes in breath methane from baseline when compared between and within groups. There were also no significant changes from baseline within groups as shown in Table 3.6. Figure 3-9 shows individual changes of breath methane within groups and between groups

Table 3-5: Mean colonic volume, breath hydrogen and breath methane at baseline and follow-up for maltodextrin and oligofructose groups

	Maltodextrin (MD) (n = 17)			Oligofructose (OF) (n = 19)			Baseline MD versus OF	Follow-up MD versus OF
	Baseline	Follow-up	p*	Baseline	Follow-up	p*	p**	p**
Colonic Volume Mean (SD)	650 (179)	741 (242)	0.035	693 (151)	803 (147)	0.016	0.288	0.096
Weighted Average Position Score (a.u) Mean (SD)	2.1 (1.4)	2.0 (1.4)	0.823	1.5 (0.7)	1.3 (1.1)	0.568	0.127	0.096
Breath Hydrogen Median (IQR)	13 (5-26)	3.5 (1-10)	0.010	13 (3-26)	23 (5-70)	0.061	0.648	0.001
Breath Methane Median (IQR)	4.5 (1.5-45)	5 (3-19)	0.943	6 (3-36)	4 (0-8)	0.041	0.593	0.244

*Paired t-test, baseline versus follow-up within groups for normally distributed data.

**Independent t-test, between groups, at baseline and follow-up.

Table 3-6 Changes in colon volume, weighted average position score, breath hydrogen hydrogen, breath methane and FODMAPs from baseline between groups

	Delta Maltodextrin (n=17)	Delta Oligofructose (n=19)	P**
Delta Colonic Volume Mean (SD)	90.43 (164.5)	110.3 (166.3)	0.7217
Delta Ascending Colonic Volume	-7.7 (95.6)	57.2 (95.3)	0.05
Delta Transverse Colonic Volume	39.5 (97.9)	44.3 (82.7)	0.8744
Delta Descending Colonic Volume	-23.4 (17.4)	26.1 (54.5)	0.9089
Weighted Average Position Score (a.u) Mean (SD)	0.044 (0.83)	0.179 (1.34)	0.718
Delta Breath Hydrogen Median (IQR)	-7 (-25,-0.75)	10 (-0.25,44.8)	0.009
Delta Breath Methane Median (IQR)	1 (-5.3,5)	-4 (-6,0)	0.411
Delta FODMAPs	-23.4 (17.4)	-22.4 (40.3)	0.921

**Independent t-test, between groups.

Figure 3-7: Individual data for fasting colonic volume at baseline (before), follow-up (after) and mean difference between groups

A: Individual fasting total colonic volume data for maltodextrin
B: Individual fasting total colonic volume data for oligofructose
C: Mean and SD fasting total colonic volume data for maltodextrin versus oligofructose

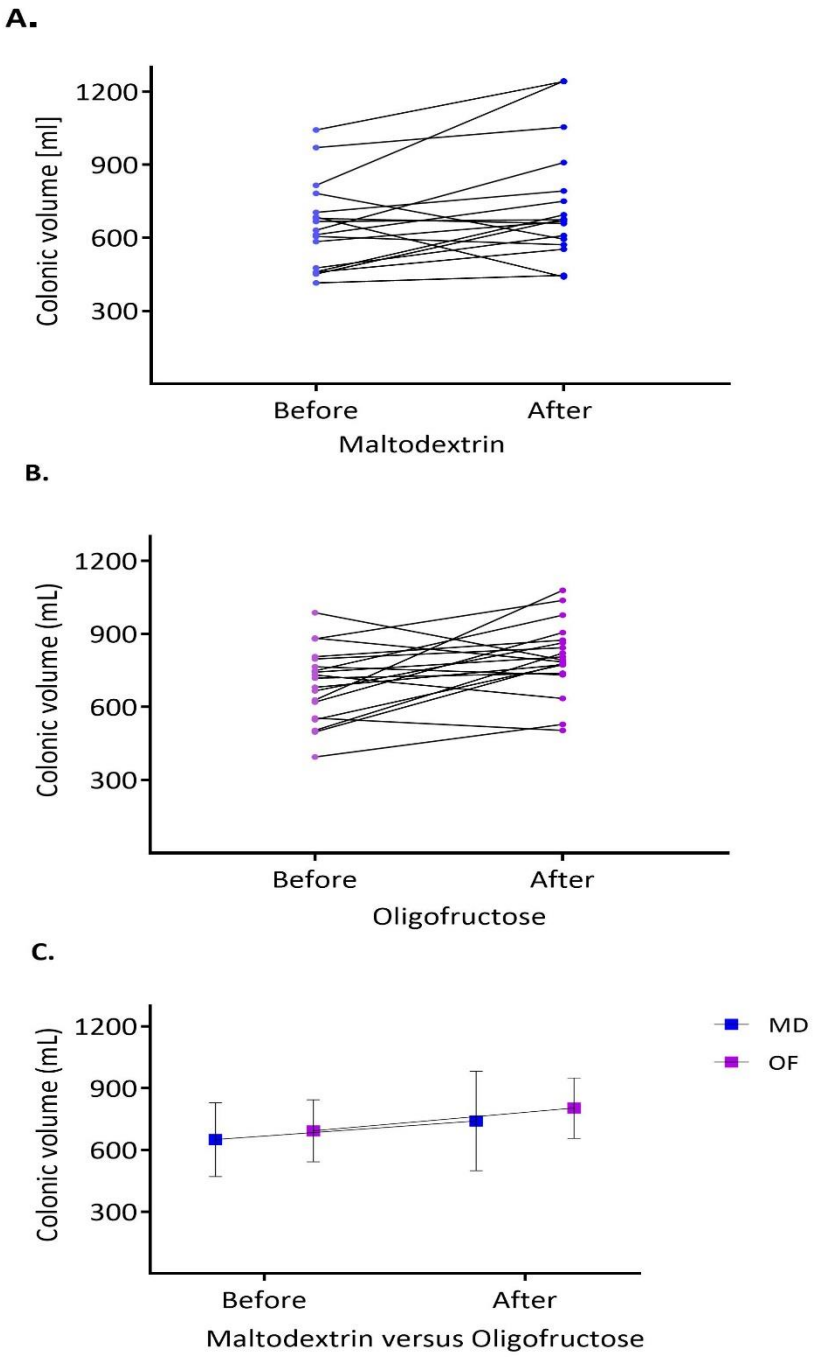


Figure 3-8: Fasting Breath Hydrogen concentration at baseline (before) and follow up (after) low FODMAPs dietary advice and supplementation.

A: Individual fasting breath hydrogen concentration data for maltodextrin
B: Individual fasting breath hydrogen concentration data for oligofructose
C: Median and IQR fasting breath hydrogen concentration data for maltodextrin versus oligofructose

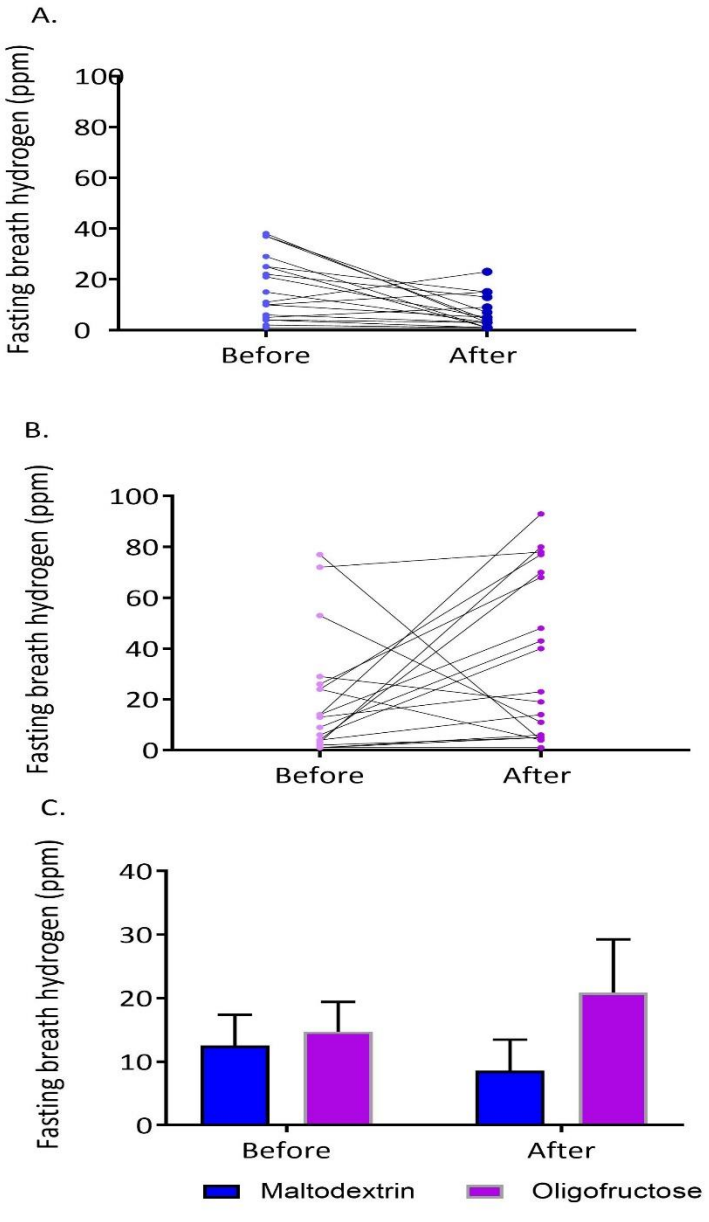
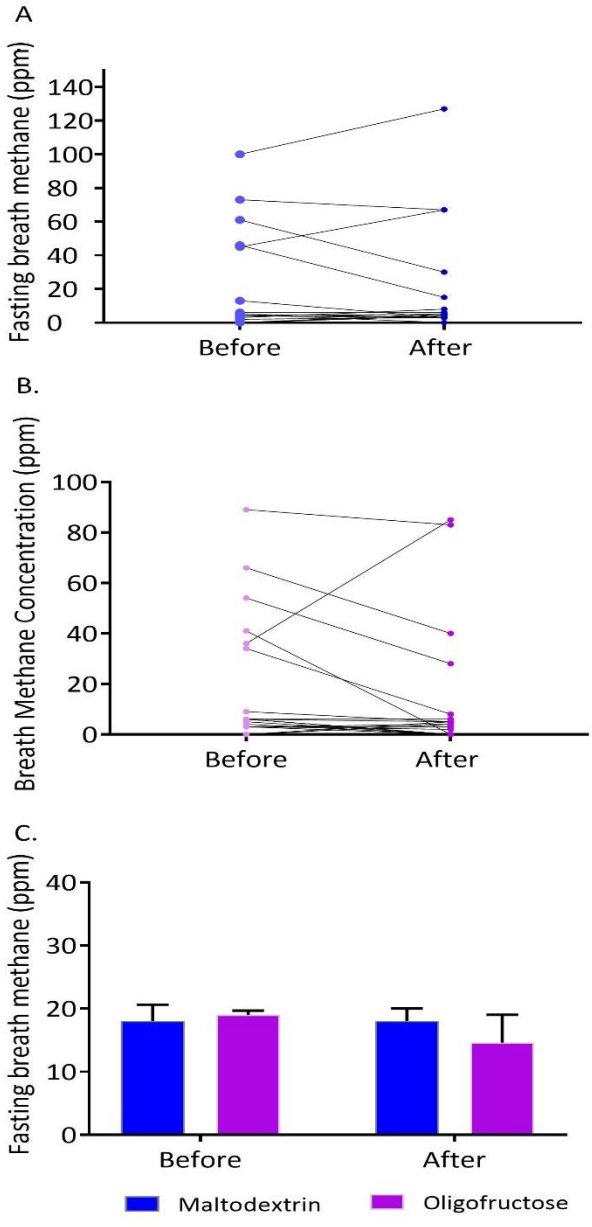


Figure 3-9: Fasting Breath Methane concentration at baseline (before) and follow up (after) low FODMAPs dietary advice and supplementation.

A: Individual fasting breath methane concentration data for maltodextrin
B: Individual fasting breath methane concentration data for oligofructose
C: Median and IQR fasting breath methane concentration maltodextrin versus oligofructose



3.12.3 Energy and macronutrient intake

There were no significant changes from baseline in energy, protein and fat and starch intakes in both groups (as shown in Table 3-7). However, both groups had a significant reduction in carbohydrate intake (Maltodextrin: 234g/d versus 189 p=0.009, Oligofructose:246g/d versus 200g/d p=0.023) as well as sugar intake (Maltodextrin:102g/day versus 76g/d, p=0.003, Oligofructose: 92g/d versus 63g/d, p=0.003). Although the maltodextrin supplementation provided 15g of carbohydrate during follow up it did not affect total carbohydrate intake

3.12.4 Fibre intake

Total fibre intake was significantly increased in the oligofructose group at follow up largely owing to the addition of oligofructose fibre through supplementation. (21g/d versus 32g/d, p=0.001). Therefore, there was a significant change in total fibre intake oligofructose group compared to the maltodextrin group from baseline (0.7g/d versus 10.9g/d, p=0.006).

Dietary fibre intake was from the diet of the participants and in the oligofructose group it was calculated by subtracting fibre from oligofructose supplementation from total fibre intake as described below.

Dietary fibre (oligofructose)= Total fibre intake – Fibre from oligofructose supplementation.

Therefore, there were no significant differences in dietary fibre intake between both groups from baseline (Maltodextrin:18.9g/d versus 19.6/d p=0.720, Oligofructose: 21g/d versus 19g/d p=0.463).

3.12.5 Micronutrient intake

There were no differences were noted for calcium and iron intake. Although not significant, the oligofructose group had at least 25g of iron intake at baseline and follow up compared to only less than 12g of iron intake in the maltodextrin group.

The definition of fibre in the UK has changed over the years, mainly due to changes in the methods of analysis described Chapter 1, section 1.12. Total fibre is measured using the AOAC method and includes NSP, non digestible oligosaccharides, resistant starch and polydextrose (Scientific Advisory Committee on Nutrition, 2015). However, not all products are analysed using the current method, which was adapted for ease of free trade. Therefore, some products may still be using the Englyst method to estimate total fibre, which potentially underestimates or overestimates fibre contents in foods.

3.12.6 Compliance to supplement

Mean supplement intakes in Maltodextrin and Oligofructose groups were (14.4g/d versus 16.4g/d) indicating high compliance to prescribed dosage which was 14g/d (section 3.8.3).

3.12.7 FODMAP intake

Total FODMAP intake (Table 3-8) was significantly reduced from baseline in both maltodextrin (17.7g/d. versus 1.7g/d, p=0.01) and oligofructose (18.9g/d versus 2.5g/d, p=0.01) groups. The highest individual FODMAP consumed at baseline was lactose in both groups (Maltodextrin: 9.9g/d versus Oligofructose: 10.6g/d).

Comparison between groups at baseline and follow-up showed no significant changes. Individual FODMAPs were significantly reduced from baseline to follow-up in both groups except for mannitol. There was a slight increase in both groups with the increase in oligofructose being significant (0.3g/d versus 0.9g/d, p=0.01). As both groups had decreased dietary FODMAP intake at follow-up, the only increase in FODMAP intake was from supplementation in the oligofructose group which amounted to 16.4g/d. As seen in Table 3.6, there was a reduction in FODMAPs from baseline with no significant changes between the groups, with p=0.921.

3.12.8 Comparison of energy adjusted nutrient intake in both males and females in both groups with age matched population data and dietary reference values

As seen in Table 3.8 and 3.9 energy adjusted protein and fat intake in both males and females both groups were higher than the population data as well as dietary reference values.

However, when DRV was compared with individual intakes at baseline, only one female from the maltodextrin group had low intake. When fat intakes were compared (n=1) female in the maltodextrin group had low intake followed by (n=4) males in the same group. Meanwhile (n=4) females and (n=6) males also had low carbohydrate intakes in the maltodextrin group. There were more females with low carbohydrate intakes in the oligofructose group at follow up (n=8) compared to the males (n=4). This was the reverse for carbohydrate intake whereby (n=4) females had lower intakes compared to (n=6) males. At

follow up, (n=1) females and (n=4) males had lower fat intake compared with the DRV in the maltodextrin group.

Carbohydrate intake in both males and females in both groups at baseline were higher than the population data. Intakes were low at follow up for population data. This is probably due to carbohydrate restriction as part of the low FODMAP diet. However, when compared with the DRV, carbohydrate intake was much lower at both baseline and follow up in both genders in both groups.

Although fibre intake was low in both male and female at in both groups at baseline, there was an increase in both female (19g/d to 30g/d) and males (24g/d to 36g/d) during follow up in the oligofructose group.

Table 3-7: Mean daily intakes of nutrients at baseline and follow-up of Maltodextrin and Oligofructose group (without supplementation)

	Maltodextrin (MD) (n=18)			Oligofructose (OF) (n=19)			Baseline MD versus OF	Follow-up MD versus OF
Macronutrients	Baseline	Follow-up	p*	Baseline	Follow-up	p*	p**	p**
Total Energy (kcal/d)	2044(474)	1904(648)	0.425	2224(539)	2068(744)	0.311	0.738	0.479
Energy from supplement		58.3(1.3)			31.5(5.2)			
Total energy – energy from supplement	2044(474)	1707(34.1)	0.001	2224(539)	1809(300)	0.010	0.738	0.334
Protein (g/d)	81.2(23.7)	91(41.7)	0.364	95.1(26)	99.3(46.8)	0.577	0.097	0.573
Fat (g/d)	83.4(24.6)	82(29.7)	0.852	90.6(29)	85.3(32)	0.499	0.423	0.750
Carbohydrate (g/d)	234(46.8)	189(63.5)	0.009	246(72.5)	200(85.8)	0.023	0.557	0.663
Carbohydrate from supplement		15(3)						
Starch (g/d)	123.7(38.4)	126(39.7)	0.747	150(56)	140(55)	0.427	0.104	0.409
Sugar (g/d)	102(36.5)	76(31.6)	0.003	92(31)	63(31)	0.003	0.351	0.196
Total Fibre (g/d)	18.9(5.5)	19.6(7.6)	0.720	21(7)	32(13)	0.001	0.305	0.001
Fibre from dietary intake (g/d)	18.9(5.5)	19.6(7.6)	0.886	21(7)	19.7(5.8)	0.463	0.305	0.740
Micronutrients								
Calcium (mg/d)	844.7(299)	756(362)	0.316	970(327)	869.6(426)	0.253	0.234	0.387
Iron (mg/d)	11.8(3.7)	10.8(5.6)	0.547	25.8(5.6)	24.5(45.5)	0.354	0.191	0.208

*Paired t-test, baseline versus follow-up within groups

**Independent t-test, between groups, at baseline and follow-up

Table 3-8: Mean Daily Dietary FODMAPs for both groups at baseline and follow-up

	Maltodextrin (MD) (n=18)			Oligofructose (OF) (n=19)			Baseline MD versus OF	Follow-up MD versus OF
	Baseline	Follow-up	p*	Baseline	Follow-up	p*	p**	p**
Total FODMAPs excluding OF supplement (g/d)	17.7(7.4)	1.7(1.6)	0.01	18.9(8.0)	2.5(1.6)	0.01	0.693	0.019
Fructans (g/d)	2.4(0.9)	0.3(0.5)	0.001	2.9(1.4)	0.2(0.2)	0.01	0.574	0.594
Supplement (g/d)	0	14.4(4.6)		0	16.4(2.4)			
GOS (g/d)	0.6(0.3)	0.1(0.3)	0.002	0.8(0.5)	0.2 (0.3)	0.01	0.254	0.498
Lactose (g/d)	9.9(6.8)	0.69(0.8)	0.01	10.6(6.0)	1.4(1.5)	0.01	0.584	0.094
Total fructose (g/d)	17.1(8.6)	11.1(7.0)	0.007	14.6(8.1)	10.6(7.8)	0.018	0.191	0.785
Excess fructose (g/d)	3.8(2.4)	0.4(0.3)	0.01	3.5(2.3)	0.5(0.3)	0.01	0.649	0.456
Sorbitol (g/d)	0.7(0.7)	0.4(0.6)	0.01	0.7(0.5)	0.9(0.9)	0.01	0.903	0.091
Mannitol (g/d)	0.2(0.2)	0.7 (0.1)	0.31	0.3(0.4)	0.9(0.2)	0.01	0.761	0.293

*Paired t-test, baseline versus follow-up within groups

**Independent t-test, between groups, at baseline and follow-up

Table 3-9 Energy adjusted fibre and nutrient intakes for both males and females from the maltodextrin group at baseline and follow-up

Parameter	**Female (MD) (n=14)**				**Male (MD) (n=4)**			
	Baseline	Follow-up	DRV	NDNS	Baseline	Follow-up	DRV	NDNS
Protein-RNI (g/day)	77 (21)	88 (30)	45	66.6 (20.8)	94 (24)	88 (18)	55.5	87.4 (29.5)
(%) protein energy	16	19		16	16	19		17
Fat (g)	79 (20)	85 (38)		62.4 (25.1)	98 (23)	81 (12)		76.6 (28.5)
% fat energy	36.5	40	33	34	40	39	34	33
Carbohydrate (g)	228 (28)	186 (53)	280-290	199 (66)	264.(47)	189 (27)	344-370	249 (78)
(%) carbohydrate energy	47	39	47	46	44	40	45	45
Fibre Individual minimum	18 (2.4)	19 (2.1)	30	20.7 (7.1)	22 (4)	20 (2.0)	30	17 (6.2)
Calcium-RNI (mg/day)	815.2 (321.2)	1017 (343)	700	746 (288)	948 (204)	791 (157)	700	897 (381)
Folate -RNI (µg/d)	235 (80.5)	252 (80.4)	200	253 (209)	215 (30)	249 (78)	200	300 (327)
Iron -RNI (mg/day)	11.7 (3.9)	28 (53)	8.7-14.8	9.3 (3.2)	12 (2.9)	15 (4.1)	8.7	11.6 (4.3)

DRV: Dietary Reference Value,

NDNS: National Diet and Nutrition Survey (combined 2008/9 ,2011/12, 2013/14); Energy and nutrient intake for males at baseline and follow-up compared with the UK population intakes and dietary reference values.

*Energy, macronutrient intakes taken from 19-64 year cohort

Table 3-10 Energy adjusted fibre and nutrient intakes for both males and females from the oligofructose group at baseline and follow-up

Parameter	**Female (OF) (n=11)**				**Male (OF) (n=8)**			
	Baseline	Follow-up	DRV	NDNS	Baseline	Follow-up	DRV	NDNS
Protein-RNI (g/day) (%) total energy intake	85 (11) 17	94 (41) 19	45	66.6 (20.8) 16	108 (21) 18	106 (42) 20	55.5	87.4 (29.5) 17
Fat (g) % total energy intake	78 (12) 36	81 (26) 37	33	62.4 (25.1) 34	107 (27) 37	92 (35) 39	34	76.6 (28.5) 33
Carbohydrate (g) (%) total energy intake	218 (28) 45	187 (64) 38	280-290 47	199 (66) 46	287 (70) 44	217 (89) 40	344-370 45	249 (78) 45
Fibre Individual minimum	19 (1.6)	30 (5)	30	20.7 (7.1)	24 (4.2)	36 (17)	30	17 (6.2)
Calcium-RNI (mg/day)	837 (245)	915 (326)	700	746 (288)	1152 (352)	767.2 (251)	700	897 (381)
Folate -RNI (µg/d)	269 (85)	273 (87.4)	200	253 (209)	300 (74)	232.4 (79)	200	300 (327)
Iron -RNI (mg/day)	32.6 (59.1)	14.2 (5.3)	8.7-14.8	9.3 (3.2)	16.5 (4)	11.2 (3.1)	8.7	11.6 (4.3)
Magnesium-RNI (mg/day)	320 (90)	323.3 (74.1)	270	238 (79)	354 (71.2)	276 (57)	300	302 (109)

DRV: Dietary Reference Value,

NDNS: National Diet and Nutrition Survey (combined 2008/9 ,2011/12, 2013/14); Energy and nutrient intake for males at baseline and follow-up compared with the UK population intakes and dietary reference values.

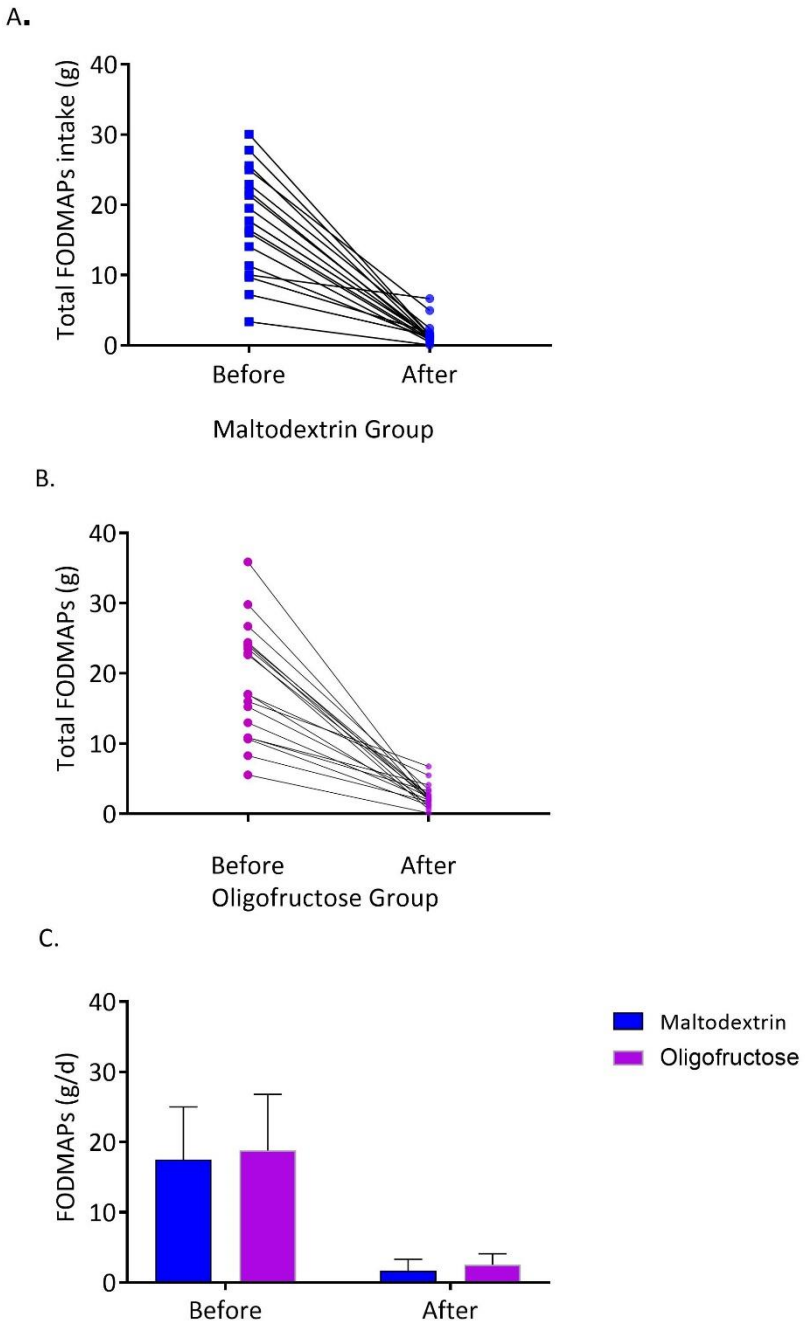
*Energy, macronutrient intakes taken from 19-64 year cohort

Table 3-11 Total FODMAP intake among male and female in both groups with and without oligofructose supplement

	Baseline (MD)			Follow-up(MD)			Baseline(OF)			Follow-up(OF)		
	Female	Male	p	Female	Male	p	Female	Male	P	Female	Male	p
Total FODMAPs(g/d) Mean (SD)	18(2.4)	17(0.8)	0.520	1.9(0.9)	1.7(0.2)	0.398	16(2.6)	23(6.2)	0.026	2.32(0.2)	3.0(0.7)	0.128
Total FODMAPs (g/d) With OF supplement Mean (SD)							16(2.6)	23(6.2)	0.026	16(0.2)	17(0.7)	0.128

Figure 3-10: Total FODMAPs at baseline (before) and follow-up (after) for maltodextrin and oligofructose (including supplement).

A: Individual total FODMAP intake for maltodextrin
B: Individual total FODMAP intake for oligofructose
C: Mean and SD total FODMAP intake for maltodextrin versus oligofructose



3.13 Discussion

The study hypothesis was that, there will be an increased fasting colonic volume in the oligofructose group after intervention, significantly greater than the increase in the maltodextrin group. The oligofructose group was expected to have an increased colonic volume compared to maltodextrin group due to supplementation. Both groups had a significant reduction in dietary FODMAP from baseline (Table 3-8 and Figure 3-10) except mannitol intake which increased in both groups. However, the mean increase was less than 1g/day and this was from food. Most studies using mannitol solutions have demonstrated an increase in luminal volume. In one CT study, participants who ingested a 3% mannitol (45g diluted in 1500mls) had an increase in luminal volume and showed sign of bowel distension (Prakashini, et al., 2019). The only effect in colonic volume was expected to come from supplementation in the oligofructose group However, this was not the case as both groups had a significant increase in colonic volume at follow up, with a percentage volume increase of 20% in oligofructose and 16% in maltodextrin thus disproving the original hypothesis.

3.13.1 Effect of dietary modification

The increase in colonic volume in the maltodextrin group is not from the supplement as maltodextrin is easily absorbed. The lack of difference between maltodextrin and oligofructose suggests that, despite its known prebiotic effects, oligofructose does not contribute to the increase in colonic volume observed. The rise in colonic volume in both groups could be due to increased

intake of modified starches (consumed in the low FODMAP diet), which act as resistant starch when heated.

Both groups adhered to the low FODMAP diet by reducing high FODMAPs content foods such as wheat-based cereals/grains, onion, garlic and milk. However, these were replaced with gluten-and-wheat-free products to substitute participants' main meal and snacks as recorded in their food diaries.

Gluten-free products are usually made from native modified starches such as tapioca, potato and corn starch (Horstmann, et al., 2017). Modification of these dried starches using heat treatment changes their structure which may affect digestibility (Gunaratne and Hoover, 2002). Thus they become resistant starches and due to their low digestibility, they end up being fermented in the colon. (Laurentin and Edwards, 2004),(Pereira and Leonal 2014),(Ferguson et al., 2000). Therefore, the rise of colonic volume in the maltodextrin group could be mainly due to increased intake of resistant starch (modified starches), resistant to α-amylase digestion, thus becoming a fermentable substrate for the gut microbiota (Gibson and Roberfroid, 1995), despite being on a low FODMAP diet.

3.13.2 Transit time

Having said that, the lack of effect of the oligofructose supplement may reflect the rapid fermentation of oligofructose within the ascending colon with conversion to SCFAs, whose absorption enhances water and sodium absorption, and so may have little effect on total colonic volume. By removing

gas-promoting substrates, the diet may remove one of the stimulants of colonic propulsion, slowing transit, which would tend to increase colonic volume.

In addition, the study did not control for gender or smoking, and it has been suggested that transit time differs between male and female and quicker in smokers compared to nonsmokers.

3.13.3 Variability in colon volumes

Colonic volume is quite variable, as shown in Table 3.5 and Table 3.8. This depends on several factors including tone, transit time, total faecal flow and the exact timing of defecation with respect to the MRI scans - since defecation reduces colonic volume by a third (Mego, et al., 2017).

A slowing of transit increases colonic volume if flow is constant. However, the results based on weighted average position score (WAPS) need to be interpreted with caution, as the validation study in healthy volunteers only showed a median of 0.8(0-1.6), so extrapolating beyond 24 hours may not be accurate (Chaddock et al., 2014).

The colonic volumes between genders were also quite variable, as shown in Table 3-12 and Table 3-13. There were only 4 male participants in the maltodextrin group compared to 8 in the oligofructose group. A linear regression showed 18 % variability, explaining difference related to gender in the Maltodextrin group p=0.05, 95% (-2.9 – 532.3).

Besides the primary outcome measure, about 16% of all variability in total FODMAP intake in the oligofructose group is also explained by a difference in

gender ratios, p=0.05, 95% (-2.9 -0.004). When adjusted for energy, there was a significant difference in macronutrient, fibre and total FODMAP intake at baseline between males and females in the oligofructose group as shown in Table 3.15.

Parameter	**Female (OF) (n=11)**				
	Baseline	Follow-up	DRV	NDNS	Baseline
Protein-RNI (g/day) (%) protein energy	85 (11) 17	94 (41) 19	45	66.6 (20.8) 16	108 (21) 18
Fat (g) % fat energy	78 (12) 36	81 (26) 37	33	62.4 (25.1) 34	107 (27) 37
Carbohydrate (g) (%) carbohydrate energy	218 (28) 45	187 (64) 38	280-290 47	199 (66) 46	287 (70) 44
Fibre Individual minimum	19 (1.6)	30 (5)	30	20.7 (7.1)	24 (4.2)
Calcium-RNI (mg/day)	837 (245)	915 (326)	700	746 (288)	1152 (352)
Folate -RNI (µg/d)	269 (85)	273 (87.4)	200	253 (209)	300 (74)
Iron -RNI (mg/day)	32.6 (59.1)	14.2 (5.3)	8.7-14.8	9.3 (3.2)	16.5 (4)
Magnesium-RNI (mg/day)	320 (90)	323.3 (74.1)	270	238 (79)	354 (71.2)

To date no MRI studies have shown the effect of gender on colonic volume or transit despite a difference in colon size in females and males. A colonoscopy study looked at 204 normal enema films of patients retrospectively in 104 females and 100 males. A detailed measurement of colon length including rectum, sigmoid, ascending, transverse and descending that was done from the prone of the film. The females had a significantly longer colon measured in median (155cm) compared to males (145 cm), p=0.005.(Saunders et al., 1996). Participants in this study were patients and the method of colon measurement may need validation. Therefore, this could not be used to interpret the results of the healthy volunteers in this study.

In summary based on the results of the primary and secondary outcome measures, it is worth considering the physical form/properties of foods

consumed by participants before and during the study as they may also have an effect on transit time (McIntyre, et al., 1997).

Perhaps a gender matched group of healthy volunteers versus patients would be more useful in planning future studies. Also, by controlling smoking and alcohol intake, the variability of colonic volume and intake between participants may be reduced.

Table 3-12: Primary outcome measures in males and females at baseline and follow-up between groups

	Baseline (MD)			Follow-up (MD)			Baseline (OF)			Follow-up (OF)		
	Female	Male	P	Female	Male	p	Female	Male	p	Female	Male	p
Colonic Volume (ml) Mean (SD)	575(221)	751(239)	0.251	630(244)	943(348)	0.170	699(181)	684(105)	0.818	797(177)	811(101)	0.824
Ascending Volume (ml) Mean (SD)	202(79)	228(35)	0.353	209(90)	265(41)	0.103	237(63)	253(72)	0.436	252(69)	301(71)	0.157
Transverse Volume (ml) Mean (SD)	227(89))	311(193)	0.454	265(128)	377(219)	0.393	296(99)	241(42)	0.118	333(98)	294(63)	0.310
Descending Volume (ml) Mean (SD)	148(82)	212(58)	0.125	158(70)	302(98)	0.005	172(65)	190(51)	0.502	198(59)	216(43)	0.453
WAPS (a.u) Mean (SD)	2.2(1.4)	1.7(1.6)	0.597	2.2(1.4)	1.6(1.7)	0.588	1.7(0.8)	1.3(0.6)	0.194	1.6(1.2)	1(0.8)	0.210
Breath Hydrogen (ppm) Median (IQR)	13(4-26)	18(7-35)	0.456	4.5(1-10)	5.5(1.5-12)	0.705	9(1-14)	28(4-67)	0.057	23(5-48)	44(12-76)	0.386
Breath Methane (ppm) Median (IQR)	4(0-21)	26(5-87)	0.061	5(3-14)	11(1.5-99)	0.452	6(3-54)	5.5(3-28)	0.647	5(2-40)	1.5(0-4.8)	0.071

Table 3-13: Changes in primary outcomes measures in males and females from baseline between groups

	Maltodextrin			Oligofructose		
	Female	Male	p	Female	Male	p
Delta CV (ml) Mean (SD)	56(149)	192(176)	0.227	98(190)	128(136)	0.694
Delta WAPS (a.u) Mean (SD)	0.04(0.9)	0.08(0.8)	0.937	0.1(1.6)	0.3(1.1)	0.786

Table 3-14: Energy adjusted nutrients among males and females at baseline and follow-up in maltodextrin group

Parameter	**Female (MD) (n=14)**				**Male (MD) (n=4)**			
	Baseline	Follow-up	DRV	NDNS	Baseline	Follow-up	DRV	NDNS
Protein-RNI (g/day)	77 (21)	88 (30)	45	66.6 (20.8)	94 (24)	88 (18)	55.5	87.4 (29.5)
(%) protein energy	16	19		16	16	19		17
Fat (g)	79 (20)	85 (38)		62.4 (25.1)	98 (23)	81 (12)		76.6 (28.5)
% fat energy	36.5	40	33	34	40	39	34	33
Carbohydrate (g)	228 (28)	186 (53)	280-290	199 (66)	264.(47)	189 (27)	344-370	249 (78)
(%) carbohydrate energy	47	39	47	46	44	40	45	45
Fibre Individual minimum	18 (2.4)	19 (2.1)	30	20.7 (7.1)	22 (4)	20 (2.0)	30	17 (6.2)
Calcium-RNI (mg/day)	815.2 (321.2)	1017 (343)	700	746 (288)	948 (204)	791 (157)	700	897 (381)
Folate -RNI (µg/d)	235 (80.5)	252 (80.4)	200	253 (209)	215 (30)	249 (78)	200	300 (327)
Iron -RNI (mg/day)	11.7 (3.9)	28 (53)	8.7-14.8	9.3 (3.2)	12 (2.9)	15 (4.1)	8.7	11.6 (4.3)
Magnesium-RNI (mg/day)	285 (93.9)	302 (96)	270	238 (79)	281 (45)	351 (124)	300	302 (109)

Table 3-15 Energy adjusted nutrients among males and females at baseline and follow-up in oligofructose group

Parameter	**Female (OF) (n=11)**				**Male (OF) (n=8)**			
	Baseline	Follow-up	DRV	NDNS	Baseline	Follow-up	DRV	NDNS
Protein-RNI (g/day) (%) protein energy	85 (11) 17	94 (41) 19	45	66.6 (20.8) 16	108 (21) 18	106 (42) 20	55.5	87.4 (29.5) 17
Fat (g) % fat energy	78 (12) 36	81 (26) 37	33	62.4 (25.1) 34	107 (27) 37	92 (35) 39	34	76.6 (28.5) 33
Carbohydrate (g) (%) carbohydrate energy	218 (28) 45	187 (64) 38	280-290 47	199 (66) 46	287 (70) 44	217 (89) 40	344-370 45	249 (78) 45
Fibre Individual minimum	19 (1.6)	30 (5)	30	20.7 (7.1)	24 (4.2)	36 (17)	30	17 (6.2)
Calcium-RNI (mg/day)	837 (245)	915 (326)	700	746 (288)	1152 (352)	767.2 (251)	700	897 (381)
Folate -RNI (µg/d)	269 (85)	273 (87.4)	200	253 (209)	300 (74)	232.4 (79)	200	300 (327)
Iron -RNI (mg/day)	32.6 (59.1)	14.2 (5.3)	8.7-14.8	9.3 (3.2)	16.5 (4)	11.2 (3.1)	8.7	11.6 (4.3)
Magnesium-RNI (mg/day)	320 (90)	323.3 (74.1)	270	238 (79)	354 (71.2)	276 (57)	300	302 (109)

Table 3.12 Total FODMAP intake among male and female in both groups with and without oligofructose supplement

	Baseline (MD)			Follow-up(MD)			Baseline(OF)			Follow-up(OF)		
	Female	Male	p	Female	Male	p	Female	Male	P	Female	Male	p
Total FODMAPs(g/d) Mean(SD)	18(2.4)	17(0.8)	0.520	1.9(0.9)	1.7(0.2)	0.398	16(2.6)	23(6.2)	0.026	2.32(0.2)	3.0(0.7)	0.128
Total FODMAPs (g/d) With OF supplement Mean (SD)							16(2.6)	23(6.2)	0.026	16(0.2)	17(0.7)	0.128

3.14 Conclusion

In conclusion, both groups showed a rise in colonic volume. This may reflect the effect of maltodextrin and oligofructose supplementation together with a slowing in transit in both groups. The wide intra and inter-individual differences and imbalance of gender may have reduced the power of the study to detect a difference due to oligofructose. There were several other factors which may have had an impact on colonic volume such as colonic water and colonic gas. However, this is a first randomised controlled trial using MRI to assess a mechanistic effect of a low FODMAP diet. The principal findings of this study showed an increase in colonic volume, due to both maltodextrin and oligofructose supplementation which was unexpected which requires further investigation.

CHAPTER 4 EFFECT OF DIFFERENT PHYSICAL FORMS OF FERMENTABLE CARBOHYDRATES (FODMAPS) ON GASTROINTESTINAL VOLUME, APPETITE AND SYMPTOMS IN HEALTHY VOLUNTEERS. (THE APPLE STUDY)

4.1 Background

“Eat an apple on going to bed and you’ll keep the doctor from earning his bread” is an old Welsh proverb (Philips ,1866).This phrase has changed over time, now more commonly expressed as, “An apple a day keeps the doctor away”(Achin L., 2013),(Wright E., 2010). Apples are a popular fruit in the UK diet, with its antioxidant properties having been investigated in relation to the prevention of cancer, asthma, cardiovascular diseases and Alzheimer’s disease (Hyson A., 2011). In addition, prebiotic properties of apples have also been explored in relation to CVD risk reduction (Ravn-Haren et al., 2013) and their effect on gut microbiota (Koutsos et al., 2017),(Masumoto et al., 2016), Koutsos, et al., 2015).

Apples are also high in FODMAPs, containing both sorbitol and free fructose (Muir et al., 2009); increased consumption of FODMAPs has been indicated in symptom severity among IBS patients. Mechanistic studies have shown that a 40g dose of inulin induces distension in patients, due to colon hypersensitivity (Major et al., 2016). Meanwhile, in healthy volunteers, the same amount of inulin distends the colon and 40g of fructose increases small bowel water content with no severe symptoms (Murray et al., 2014). Findings from both studies confirmed FODMAPs osmotic effects on luminal volume and a fermentative effect in the colon. However, in order to observe and measure such an effect, this large dose of ingested FODMAPs was not representative of routine dietary FODMAP intake of neither healthy volunteers nor patients. The increase in small bowel water content was also possibly due to quick gastric emptying and intestinal secretion. In another study, our group confirmed that

mannitol (a polyol) emptied into the small bowel much quicker than glucose. Initially, a decease in postprandial small bowel water was observed, possibly due to rapid nutrient-driven fluid absorption; however, this was followed by an increase in small bowel water content as a result of mannitol stimulating intestinal secretion (Luca Marciani et al., 2010).

Therefore, a reduction in FODMAP intake is advocated in IBS patients, in order to reduce luminal volume and gas to minimise gastrointestinal symptoms such as osmotic diarrhea, bloating and pain (Barrett and Gibson, 2012). However, improvement following dietary restriction has been shown in only 75% of patients (Shepherd et al., 2008). As the diet is restrictive, compliance is an issue with most patients (Hill et al., 2017). Additionally, a three-to-four week dietary restriction of FODMAPs alters gut microbiota, as well as reducing the prebiotic effect of FODMAPs (Halmos et al., 2015),(Staudacher et al., 2012).

Consequently, it is of great interest to assess how different physical forms of high FODMAP foods are handled by the gut in order to understand if their strict elimination is at all necessary in patients. Previous studies had shown that altering the physical form of apples also changed satiety response (Flood-Obaggy. and Rolls ., 2009),(Haber et al., 1977), but the underlying mechanism was still unclear. This encouraged us to study apples, primarily because of their high FODMAP content and variety of test meal forms (i.e. whole apple, apple juice and apple puree) which could be easily produced besides being part of normal diets.

Therefore, this study was designed to look at the effect of different forms of apples on gastrointestinal volumes, symptoms and appetite.

4.2 Aims

The study reported here addressed the questions below:

i. Do differing physical forms of fermentable carbohydrates such as solid, liquid and puree affect gastrointestinal volumes, satiety and appetite?

ii. If so, what are the differences in these effects?

The aim of this study was to define the underlying mechanisms whereby the physical form of FODMAPs induce differing effects on gastrointestinal responses, satiety and symptoms in healthy volunteers.

4.3 Objectives

To compare: gastrointestinal volumes and gases using MRI techniques; and satiety and symptoms induced by different physical forms of apple when measured over time over time.

4.4 Hypotheses

The primary hypothesis was that the faster emptying of liquid (apple juice) compared with solid (whole apple) or puree (pureed apple) would lead to a greater increase in small bowel water content (SBWC) following test meal ingestion.

The secondary hypothesis was that the faster delivery of fermentable carbohydrates to the small intestine, predicted to occur with pure juice, would cause greater water secretion and accelerated transit to the colon. This would

reduce the time taken for absorption and increase the amount of carbohydrate (available for fermentation by hydrogen-producing colonic microbiota) entering the colon. Thus, an increased concentration of breath hydrogen (from baseline) will be evident in participants who ingest liquid, as compared with participants who ingest puree or solid.

4.5 Methods

4.5.1 Study Design

This study was an open label, three-way crossover, randomised, controlled trial in healthy volunteers. It was approved by the Research Ethics Committee of the School of Medicine at the University of Nottingham (A13102015 SoM NDDC SPMMRC). The study has been prospectively registered on clinical trials.gov (NCT03714464).

4.6 Participants

Healthy participants were recruited using advertisements across Nottingham University campuses, via social media and through the departmental database. This database contains volunteers who have expressed an interest to participate in studies and had given consent to be contacted. Prospective participants were screened for eligibility (during their initial visit. The study was fully explained, with an information sheet given to each participant.

Participants were recruited between March and July 2016, the last participant completing the study in August 2016

Table 4-1 Inclusion and exclusion criteria

Inclusion Criteria
Aged 18 or older
Able to give informed consent
Exclusion Criteria
Aversion to test product
Unable to eat whole fruit due to poor dentition or other reasons
History declared by the candidate of pre-existing gastrointestinal disorder that may affect bowel function
A positive diagnosis of IBS based on the ROME III criteria (described below in Methods, section 4.73)
Reported history of previous resection of the oesophagus, stomach or intestine (excluding appendix)
Intestinal stoma
Any medical condition potentially compromising participation in the study e.g. diabetes mellitus, respiratory disease limiting ability to lie in the scanner
Contraindications for MRI scanning i.e. metallic implants, pacemakers, history of metallic foreign body in the eye(s) and penetrating eye injury.
Unable to limit alcohol intake to ≤ 35 units/week and ≤ 8 units per day during trial, as well as avoid alcohol the day before each study day
For the duration of the study, unable to stop drugs known to alter GI motility including mebeverine, opiates, monoamine oxidase inhibitors, phenothiazines, benzodiazepines, calcium channel antagonists (selective serotonin reuptake inhibitors and low dose tricyclic antidepressants will be recorded but will not be an exclusion criteria)
Inability to lie flat or exceed the scanner limits of weight > 120kg
Poor understanding of English language
Participation in night shift work the week prior to the study day. Night work is defined as working between midnight and 6.00am
Alteration in habitual diet in the last 6 months
Anyone who in the opinion of the investigator is unlikely to be able to comply with the protocol e.g. cognitive dysfunction, or chaotic lifestyle related to substance abuse etc.

4.7 Study Visits

Each participant attended four study visits, with a minimum of 1 week between each visit. Participants had their breath hydrogen measured and had an MRI scan at baseline, after their test meal then at 45-minute intervals for a total of

5 hours. Questionnaires, using 100mm visual-analogue scales, were also completed at each time point, measuring satiety and gastrointestinal symptoms.

4.8 Test meals

All three test meals provided in the trial comprised of Pink Lady apples, sourced from a supermarket chain (JS Sainsbury Limited UK). The meals consisted of whole apples (solid), apple puree (puree) and apple juice (liquid).

The 3 test meals were isocaloric with 178kcal per meal.

4.8.1 Solid Test Meal A (whole apple)

Each test meal consisted of 350 g of whole apple (bought on test day), cut and cored in a sterile environment by the research investigator.

4.8.1.1 Preparation of Test Meal B (apple puree) and Test Meal C (apple juice)

Both test meals were prepared in a hygienic food preparation processing area at Campden BRI Ltd (Chipping Campden, UK), an organisation renowned for their quality control. This facility is accredited for their technical services in accordance with recognised International Standard ISO(IEC) by the United Kingdom Accreditation Service (UKAS). Services include food processing, analysis and testing, chemistry and biochemistry, product development and numerous other services pertaining to food. Preparation of both test meals was undertaken by EB, LM and SK supervised by Campden BRI staff in accordance with documented food processing standards protocol.

4.8.2 Puree Test Meal B (Apple Puree)

Approximately 40 kg of apples were washed in a sieve under a spray of ambient mains water, to remove extraneous matter and foreign bodies; damaged and rotten apples were also discarded. The apples were drained for two minutes, cored and cut, before being immersed in an ascorbic acid solution to prevent oxidization. Subsequently, 26.4 kg of apple pieces were placed into a steam pan with 13.2 kg of added water. The apples were then steamed for 20 minutes, their final weight being 22.8 kg. Finally, the steamed apples were pureed in an industrial cutter (Mado bowl cutter, MSM 769 SL, SUPRA Industrial Cutter 220, GmbH, Germany). A total of 48 x 400 g cans of apple puree were produced (pH 3.38)

4.8.3 Liquid Test Meal C (apple juice)

Approximately 40 kg of apples were washed in a sieve under a spray of ambient mains water, to remove extraneous matter and foreign bodies; damaged and rotten apples were also discarded. The apples were drained for two minutes, cored, then immersed in an ascorbic acid solution to prevent oxidization. They were milled and pressed using a Bertuzzi fruit press (Bertuzzi Fruit Processing, Srl, Italy). The resultant juice was collected in a sterilised container under the fruit press, with the pulp contained within a clean pressing cloth. This process was repeated until 85% of the apples were juiced. Packaging was done in a sterile room, utilising lacquered fruit cans. Cans were labelled using a permanent marker. Juice was then poured into the cans using the gilling table, which was adjusted to give the correct headspace of approximately 10mm. The

cans were vacuum sealed. A total of 48 x 400g cans of apple juice were produced (pH 3.34).

Test meals B and C were then pasteurised in a tank of water at 100°C for 10 minutes. Then they were cooled for 15 minutes in a tank of chlorinated mains water to obtain an equilibrium internal temperature of 38 to 41C. The cans were then left to dry before being stored in a dry ambient can store.

4.8.4 Nutrient composition of test meals

The nutrient composition analysis of the test meals was carried out by Campden BRI Ltd, Gloucester, UK. Total carbohydrate was measured by calculation, using TES-AC-335 code by extracting moisture, ash, fat and protein. Available carbohydrate was measured by calculation using the same code, by extracting fibre from total Carbohydrate. Table 4.2 shows the analysis of the test meals per 100g of edible portion.

Each test meal described in Table 4.2 was standardised for energy and volume; water was given to ensure the final volume of each test meal was 600ml in total. The rate of ingestion was not standardised to reflect real life scenario.

4.8.5 Questionnaires

During their initial visit (visit 1), after granting their consent participants were screened using the ROME III criteria, a diagnostic questionnaire. The questionnaire ("Appendix B : Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders," 2010) asked if participants suffered from recurrent abdominal pain or discomfort in the last three months, with onset being at least six months previously. Female participants had an option to respond as to

whether or not this was related to menstrual cycles. Those who answered yes to the first question, but no to the second, had then to choose if their symptoms were associated with:

a) improvement in defecation; and/or

b) onset associated with a change in frequency of stool, and/or

c) onset associated with change in form/appearance of stool

Participants who either answered no to question one, or yes to question two, were deemed eligible and enrolled into the study; those who did not were excluded.

4.8.6 Satiety Questionnaire

A zero to 100 mm visual analogue scale (VAS), using a validated method (De Graaf, 1993) was used to record hunger, satiety, fullness, prospective consumption and desire to eat, in order to subjectively measure the motivation to eat. At each end, the scale (depicted below each question) included descriptor words to indicate extremes. For example, the first question (related to hunger) had a range of responses from “I am not hungry at all”, to “I have never been more hungry” (Blundell et al., 2010, Flint et al., 2000).

4.8.7 Symptom questionnaire

This questionnaire also assessed subjective gastrointestinal symptoms using a VAS scale. The response scale used was the same as that of the Satiety questionnaire. Participants were asked if they experienced symptoms such as gas/flatulence, bloating, abdominal pain and diarrhoea. Responses were

recorded on the scale using a vertical line to indicate symptoms ranging from "Not at all" to "As bad as it could be" (Francis, et al.,1997).

4.9 Procedure and interventions

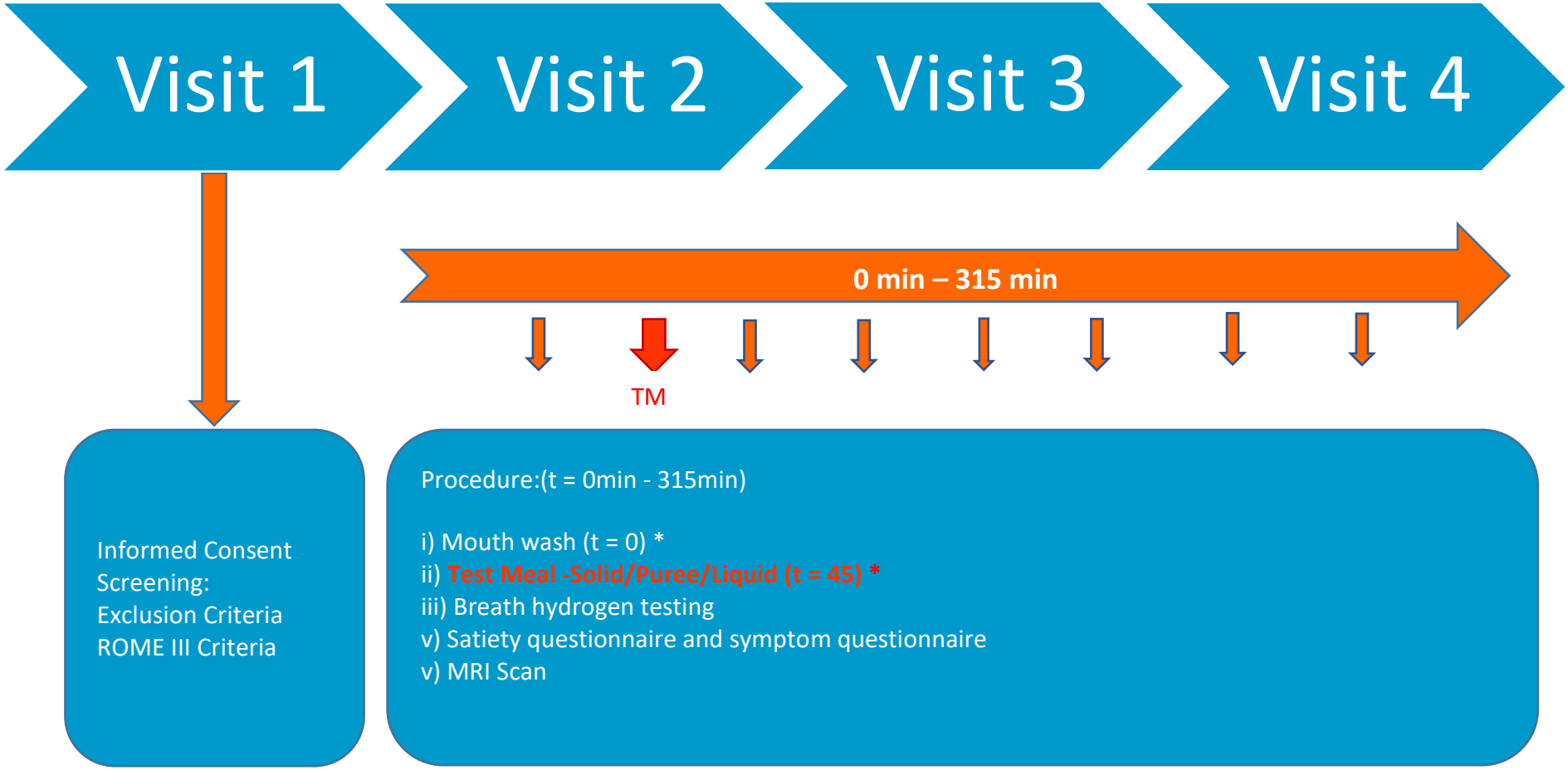
Interventions took place at the 1.5T Sir Peter Mansfield Imaging Centre (SPMIC), School of Physics and Astronomy, University Park Campus, Nottingham, as detailed in Figure 4.1

Table 4-2 Nutrient and FODMAP composition of test meals

Nutrient	WA	AP	AJ
Weight (g)/meal	350	384	338
Volume (ml)/meal	427	376	340
Total Energy (kcal)/meal	178	178	178
Carbohydrate (g)/100g	16	13.4	13.5
Fructose (g)/100g	14.4	11.3	13.5
Glucose (g)/100g	1.5	1.8	1.4
Sucrose (g)/100g	6.0	2.4	5.0
Sugar (g)/100g	11.4	10	12.9
Free Fructose (g)/100g	3.1	4.0	5.1
Sorbitol (g)/100g	0.6	0.32	0.4
Fibre (g)/100g	1.9	2.1	0.5
Total FODMAPs (g)/meal	13.0	16.6	18.6

WA: Whole Apple; AP: Apple Puree; AJ: Apple Juice

Figure 4-1: Trial regimen for the Apple Study



4.9.1 Visit 1 (Consent) – Week -1

Visit 1 took place at the NDDC, lasting 20-30 minutes. Following consent, participants were screened against eligibility criteria. Only eligible candidates were enrolled into the study. An MRI safety screening questionnaire was also completed as part of this process. Additionally, a record of height, weight, current medication, medical conditions and smoking history was also recorded.

Prior to Visit 2, participants were asked to abstain from alcohol from 8 am the day before (24 hours before the MRI scan).

4.9.2 Visit 2, 3 and 4 – Week 2, 3 and 4

Participants arrived around 8.30 am, having fasted from 10.00 pm the previous evening. They were permitted small amounts of water with medication but not after 7 am. Upon arrival, participants were greeted by the research investigator and introduced to the scanner operator for completion of MRI safety screening processes.

Participants were then given disposable scrub garments to wear, with visit number and MRI scan sequencing being identified using sticky labels. The study clock was set to 9.00 am, after which participants completed their baseline fasting breath test using a portable hand-held breath H2 meter (Gastro+ Gastrolyzer, Bedfond Scientific, Kent UK), followed by a baseline fasting MRI scan.

Prior to the baseline breath test participants were asked to rinse their mouths using standard chlorhexidine, non-alcoholic mouthwash (Corsodyl, GlaxoSmithKline, UK) to help avoid fermentation of the test meal in the mouth,

which could affect the breath test results. They were then given a symptom and satiety questionnaire to complete, followed by the initial baseline scan.

Within 25 minutes of the baseline breath test, participants received test meal (A, B or C), for consumption within 20 minutes.

This was followed by a second breath test, completion of the Symptom and Satiety questionnaires and an MRI scan. The same sequence of intervention was carried out every 45 minutes until 270 minutes postprandial.

At the end of the scans, participants were offered a light packed lunch and then discharged.

4.9.3 MRI Protocol

Based on our validated MRI protocol, images were acquired using a whole-body 1.5T scanner (Achieva, Philips Medical System, Best, The Netherlands). Volunteers had to be in a supine position (head first) in the scanner, a 16-element SENSE (Philips Medical System) variant. A torso coil was wrapped around the abdomen. Participants were required to fast overnight (from 10 pm the night before) for each study day, with only a small cup of water (50 ml) being permitted on waking on the morning of the study.

4.9.3.1 Small bowel water content

This is a method validated by our MRI group used to measure freely-mobile water in the small bowel (SBWC), (Hoad et al 2007). The sequence in this imaging technique produced lighter, high-intensity signals from the liquid regions of the body, and dark signals for other tissues and organs. A coronal single-shot turbo spin echo sequence was used to acquire 24 image 'slices' with a single expiration breath hold of 24 s (TR / TE = 8,000 / 320 ms, 512 × 512 reconstructed matrix, voxel size 0.78 × 0.78 × 7 mm ^{3}). Reproducibility analysis was done two data set of participants for selected time points by SK. 70% of the measured values were the same for inter observer meanwhile 75% of the values measured were the same for intra observer.

4.9.3.2 Gastric Emptying

Gastric emptying was measured using a balanced gradient echo sequence which acquired 50 contiguous axial slices under 1 expiration breath hold of 16.5 s (TR / TE = 2.98 / 1.49 ms, flip angle 80 ° ,256 × 256 reconstructed matrix, reconstructed in-plane resolution 1.56 × 1.56 × 5 mm ^{3}, SENSE 2.0). The imaging sequence was able to yield good contrast between stomach content and surrounding organs. Similar to small bowel water content, both inter observer (between SK and JA) and intra observer analysis (SK) was done in datasets of two participants. There were 17% difference in inter observer values.

4.9.3.3 Colonic Volume and Gas

Colonic gas was determined using a coronal dual-echo gradient echo sequence that acquired 24 contiguous slices (1.76×1.76×7 mm ^{3}) with one expiration breath hold of 15 s (TR / TE1 / TE2 = 157 / 2.30 / 4.60 ms, 256 × 256

reconstructed matrix, voxel size 1.76 × 1.76 × 7 mm ^{3}). This sequence allowed simultaneous collection of both in-phase and out-of-phase images, where voxels containing both water and fat are either bright or dark respectively, hence edges of different organs had black outlining which afforded ready identification of the intestinal outline. Each period of imaging lasted between 5 and 10 minutes, after which participants repaired to their lounge.

Depending on sequence, each image set was acquired with an expiration breath-hold of between 13 and 24 seconds, monitored using a respiratory belt. Participants spent approximately 10 minutes inside the magnet (including set-up) at each time point, the intervening time being spent relaxing in the adjacent volunteer lounge. They were advised to sit upright, in order to minimize any posture effect on digestion of test meals. MRI sessions for each participant lasted for a total of 5 hours 15 minutes, with 45 minutes interval between each scan. Reproducibility analysis for interobserver and interobserver was done by expert SP and this was less than 15% difference for both values.

4.9.4 Randomisation and Blinding

All participants completed the intervention in this three-way crossover design. The order of the test meal was allocated by study co-investigator Dr Pritchard. Symptom, satiety and breath hydrogen data were entered into the study database after each study day. Analysis of MRI data was undertaken 'blind' of test meals, breath hydrogen, symptoms and satiety results. A single experienced operator carried out the analysis of gastric emptying (JA) and

colonic volume (SP) to reduce inter-observer variability. The small bowel water analysis was undertaken by SK, who also ran the study.

4.9.5 Data Analysis

Gastric volumes were measured by JA using commercial software (IDL, Research Systems, Boulder, CO). This was done using a mouse to manually trace on each image slice the region surrounding both the meal and around the gas depicted within the stomach. These regions across all the slices gave a total sum of meal and gas volume, corresponding with total gastric volume.

The SBWC was measured by SK using in-house software previously validated as published (Hoad et al, 2007).

Gastric emptying was determined by measuring half emptying time (T_{50}) and half emptying rate (T_{Rate50}) using a previously described and validated equation (Kwiatek et al, 2009) utilising MATLAB version 2016b (The MathWorks Inc, United States).

Colonic volume and colonic gas were measured by SP using Analyze 9 software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN.). The measurements followed a similar protocol to gastric volume, whereby regions of interest were manually traced on each image slice around the ascending, transverse, and descending segments of the colon as previously reported (Pritchard et al., 2014).

Gastric emptying is usually rapid and completed in 135 min as previously shown (Marciani et al., 2010). Therefore, total observation time for all variables in this study was divided into an early phase (0-135min) and late phase (135-270min). Gastric emptying has an effect on SBWC during the early phase, while pancreatic and biliary secretions together with absorption and rate of transit to the colon, influence small bowel volume during the late phase.

4.10 Outcome Measure

4.10.1 Primary endpoint:

The primary endpoint was to compare the postprandial AUC of SBWC between the 3 different test meals.

4.10.2 Secondary endpoints:

Secondary endpoints included: differences between test meals from baseline in:

- Gastric volume (ml)
- Colonic volume (ml) and colonic gas in arbitrary units (a.u.)
- Breath hydrogen (H_2) excretion in parts per million (ppm)
- Satiety scores using a visual analogue scale; 0-100mm at each time point.
- Symptom intensity scores using a visual analogue scale; 0- 100 at each time point

4.10.3 Sample size and statistical analysis

There are no comparable studies on which to base a power calculation; however, a previous study (Luca Marciani et al., 2010) showed that SBWC AUC 135-270 minutes was 34.8(21)ml after mannitol ingestion (n=18 healthy volunteers). Thus it was calculated that with $\alpha = 0.05$ and a power of 80%, it would be possible to detect a 42% change in SBWC between groups using 18 participants.

The crossover design of the study enabled each participant to serve as their own control for the duration of the study.

Statistical analysis was carried out using Graph Pad Prism for Windows, Version 7.0 (Graph Pad Software, La Jolla California USA). Data was assessed for normality using Shapiro Wilk test. Normally distributed endpoints were analysed using parametric methods and reported as mean (±SEM). Non-normal data was analysed using non-parametric methods and expressed as median (interquartile range). The study design enabled a two-way repeated measures analysis of variance (RM-ANOVA) to assess the significance of differences.

Post-hoc analysis was only carried out when ANOVA was significant for multiple paired comparison using Tukey's post-hoc test. Significance for all evaluated measures was interpreted at $P<0.05$.

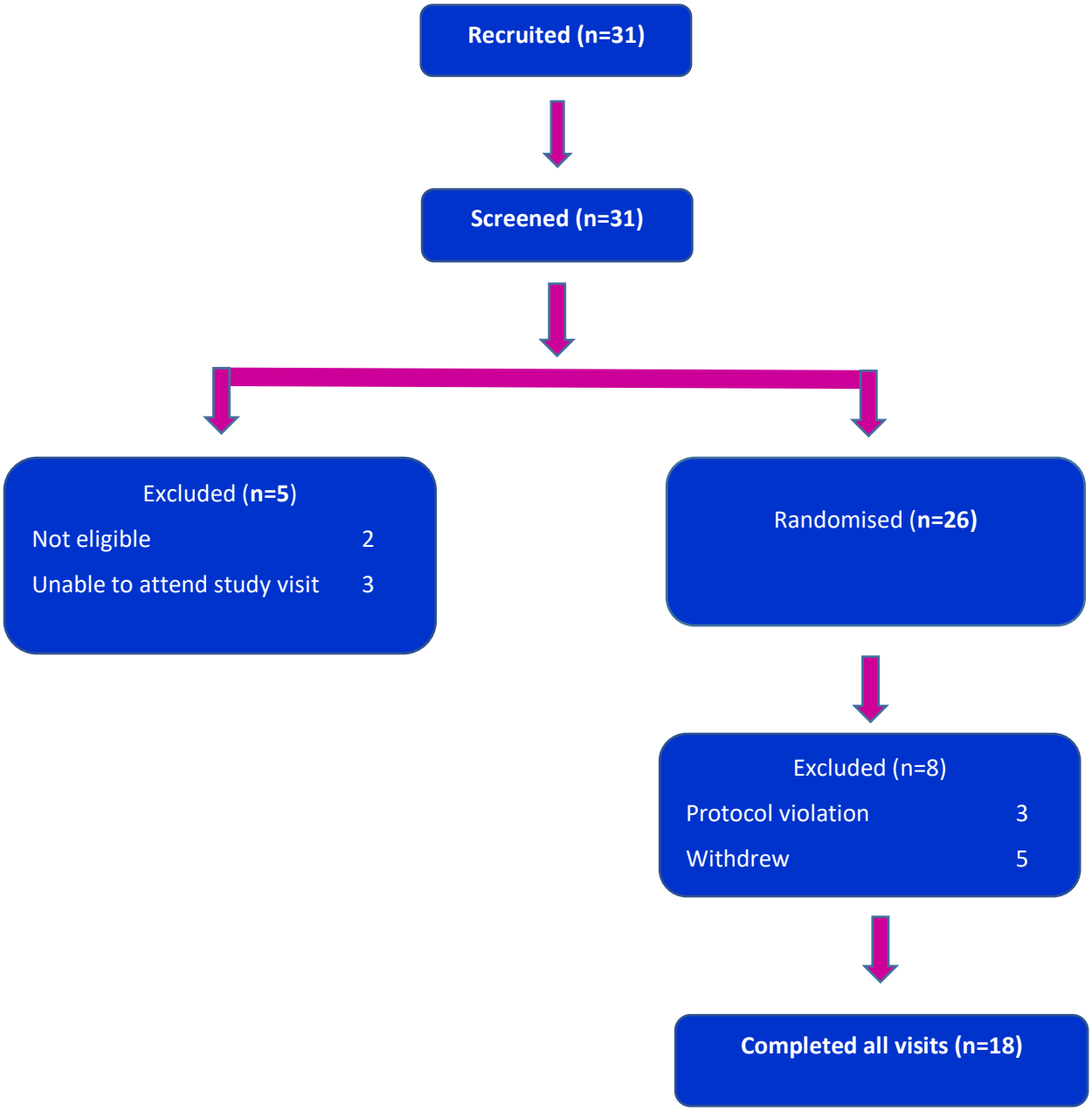
Mean and standard error will be used to compare means between groups. SE is used. Based on the study objectives it is more appropriate to use standard error, as the objective of the study was to compare the mean between groups.

4.10.4 Consort Diagram

A total of 31 participants were screened and recruited for this study. Of these, 5 participants were excluded during the screening phase. 2 became ineligible due to IBS symptoms, with the remainder unable to commit to all four study visits. Of the 26 participants who were randomised, 8 were withdrawn from the study after visit 1. A total of 3 participants were unable to finish the test meal (protocol violation), 5 withdrawing from the study when unable to commit further attendance. In summary, 18 participants attended all study visits to complete the study and were subsequently included in the analysis.

4.11 Results

Figure 4-2: Consort Diagram of Apple study on the effect of different physical form of carbohydrates on gastrointestinal volume, gas, appetite and symptoms responses



4.11.1 Baseline demographics and characteristic of participants

Baseline characteristics are presented in table 4.3 below. Most of the participants were female (78%), non-smokers (87%), with the largest ethnic group being white Caucasians (39%). This is a relevant population, similar to the IBS population who are currently being advised on low FODMAP diets in our present clinical setting.

Table 4-3 Baseline Characteristics of Participants

Variable	
Age	25(4)
Female n (%)	14(78)
Male n (%)	4 (22)
Weight	62.3(13)
BMI	22.7(3.5)
Smoker n (%)	2(13)
Non-smoker n (%(	16(87)
Ethnicity n (%)	
White British/European	7(39)
Black British/Afro-Caribbean	1(6)
Chinese	3(17)
Hispanic	5(28)
Arab	1(5)
Others (Turkic)	1(5)
Vegetarian n (%)	4(22)

Values are expressed as mean (SD) or number (%)

4.11.2 Outcome measures

In order to link gastric emptying, small bowel water content and colon volume the results will be presented in the above order.

4.11.2.1 Gastric Emptying

There was a rapid decrease in gastric volume in all three groups (Figure 4.4) between 45-135min after ingestion of the test meal. The reduction in volume as calculated using two-way ANOVA was significant in all three groups ($p<0.001$). Post-hoc test showed a significant difference between WA versus AP ($p=0.001$), WA versus AJ ($p<0.0001$),) and AP versus AJ ($p=0.0166$).

The was also a significant difference in AUC between all three test meals ($p<0.0001$). Post-hoc test showed a significant difference between WA versus AP ($p<0.0001$) and WA versus AJ ($p<0.0001$) and AP versus AJ ($p=0.0202$).

Gastric emptying time and rate was also compared between groups (see Figure 4.6). The half-emptying time for WA was 64.1min (3.3), followed by AP which was 41.2min (2.8) and AJ 38.4min (2.9). A one-way ANOVA showed significant differences in half-gastric emptying time between groups ($p<0.001$). Post-hoc tests showed significant difference in gastric emptying time between WA versus AP ($p<0.001$) and WA versus AJ ($p<0.001$) but not between AP versus AJ ($p=0.7612$).

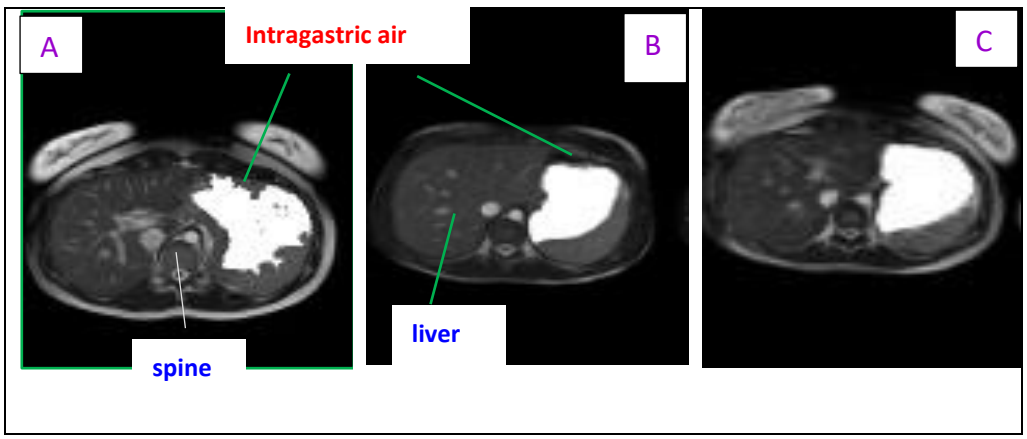
Gastric emptying rates were the lowest for WA: 3ml/min (0.2) compared to AP: 4.2ml/min (5.7) and AJ: 4.5ml/min (6.2). A one-way ANOVA showed significant difference in gastric emptying rates between all test meals ($p<0.0001$). Post-

hoc test showed significant difference between WA versus AP (p=0.0001) and WA versus AJ (p=0.00031) but not between AP versus AJ (0.6722).

Total gastric content is the total sum of gastric volume and gastric air. This volume was higher for WA [171.9ml (63)], as compared to AP [147.8 ml (59.5)] and AJ [133.6 ml (55.3)]. Differences were significant between all three test meals with p=0.0006. Post-hoc tests showed significant difference between WA versus AJ (p=0.004) as well as between WA versus AP (p=0.0367). Difference between AP versus AJ was not significant. (See F).

AUC for total gastric content for all three test meals was also significant, with p=0.005. Post-hoc test showed a significant difference between WA [8.5 l/min (2.3)] versus AP [7.2 l/min (2.3), p=0.0322] and WA versus AJ [6.5 l/min (2.2), p=0.0004]. There were no significant differences between AP versus AJ (0.2860).

Figure 4-3: MRI image of stomach content with test meal A, B, and C.

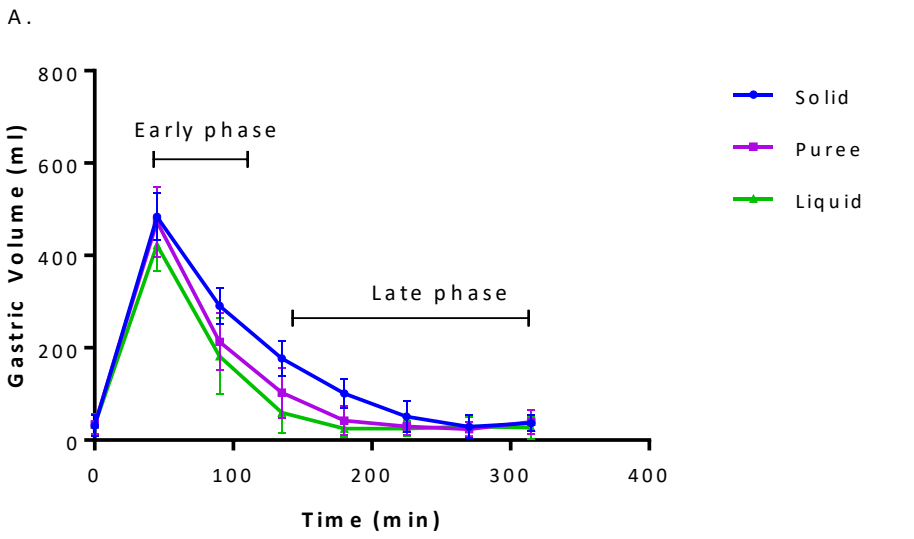


Images were acquired from three different volunteers at different time points. Image A is whole apple with intragastric air and appears to be in semi solid form. Image B is apple puree with more liquid and a small amount of solid present with intragastric air. Image C is apple juice which is in liquid form.

Figure 4-4: Gastric volume over time

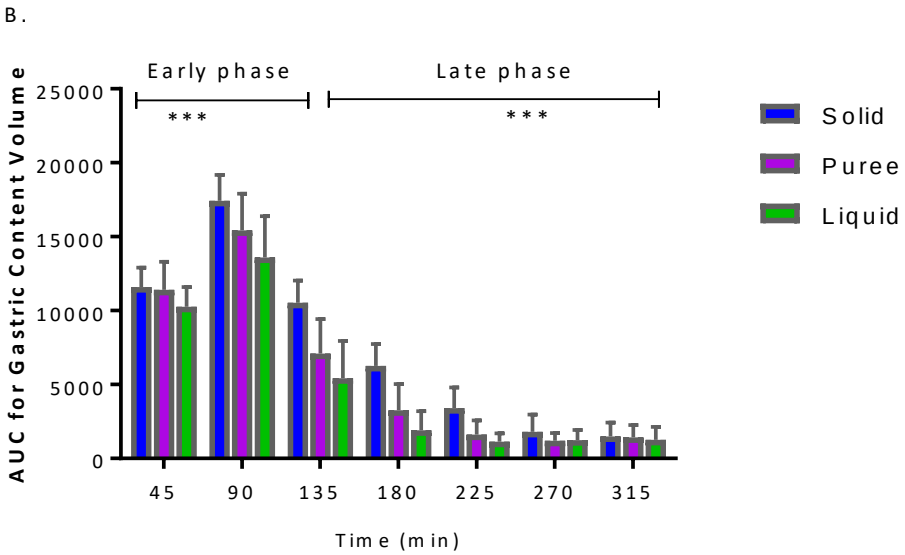
A: Mean gastric volume from T=0-315 min for all test meals

B: Mean AUC for gastric volume content from T=0-315 min for all test meals



Early phase - T=45min to T=135min

Late phase - T=135min to T=270min



*** p<0.001 between groups from in early phase and late phase

Early phase - T=45min to T=135min

Late phase - T=135min to T=270min

Figure 4-5: Total gastric content over time

A: Mean total gastric volume from T=0-315 min for all test meals

B: Mean AUC for total gastric volume content from T=0-315 min for all test meals

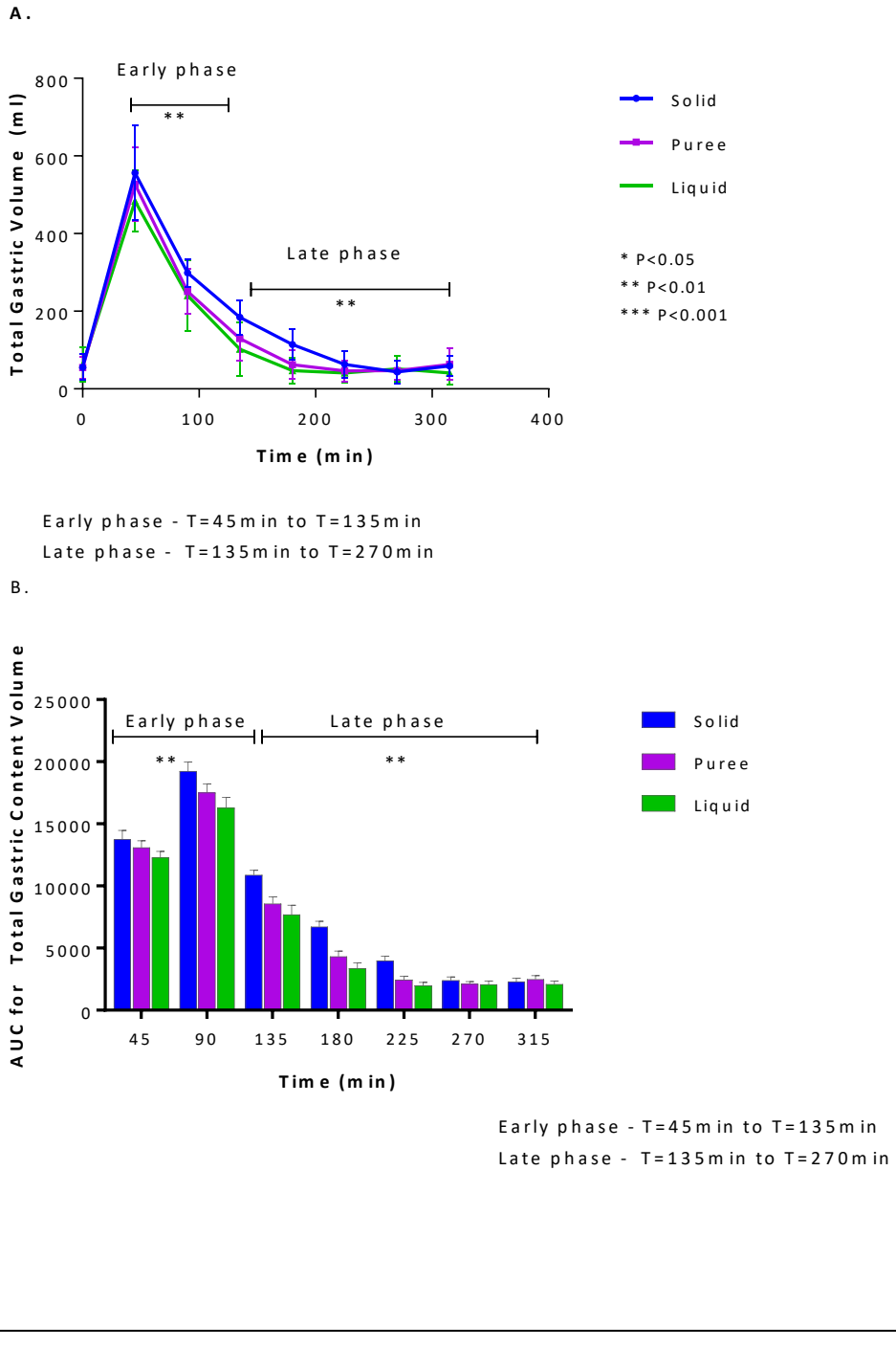
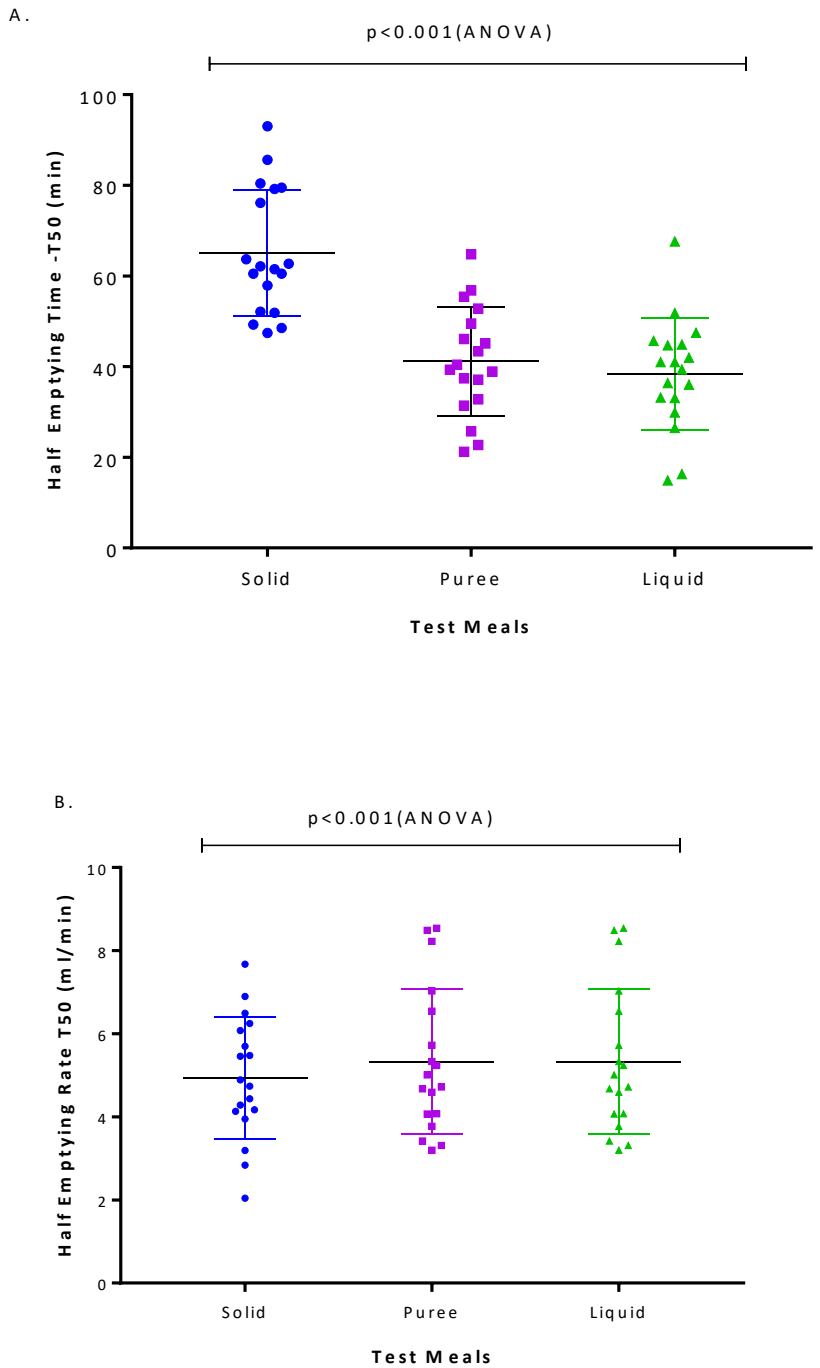


Figure 4-6: Half gastric emptying time and rate for all test meals over time

A: Half Emptying time (T50) for all test meals

B: Half Emptying rate (T50) for all test meals



4.11.3 Primary outcome measure

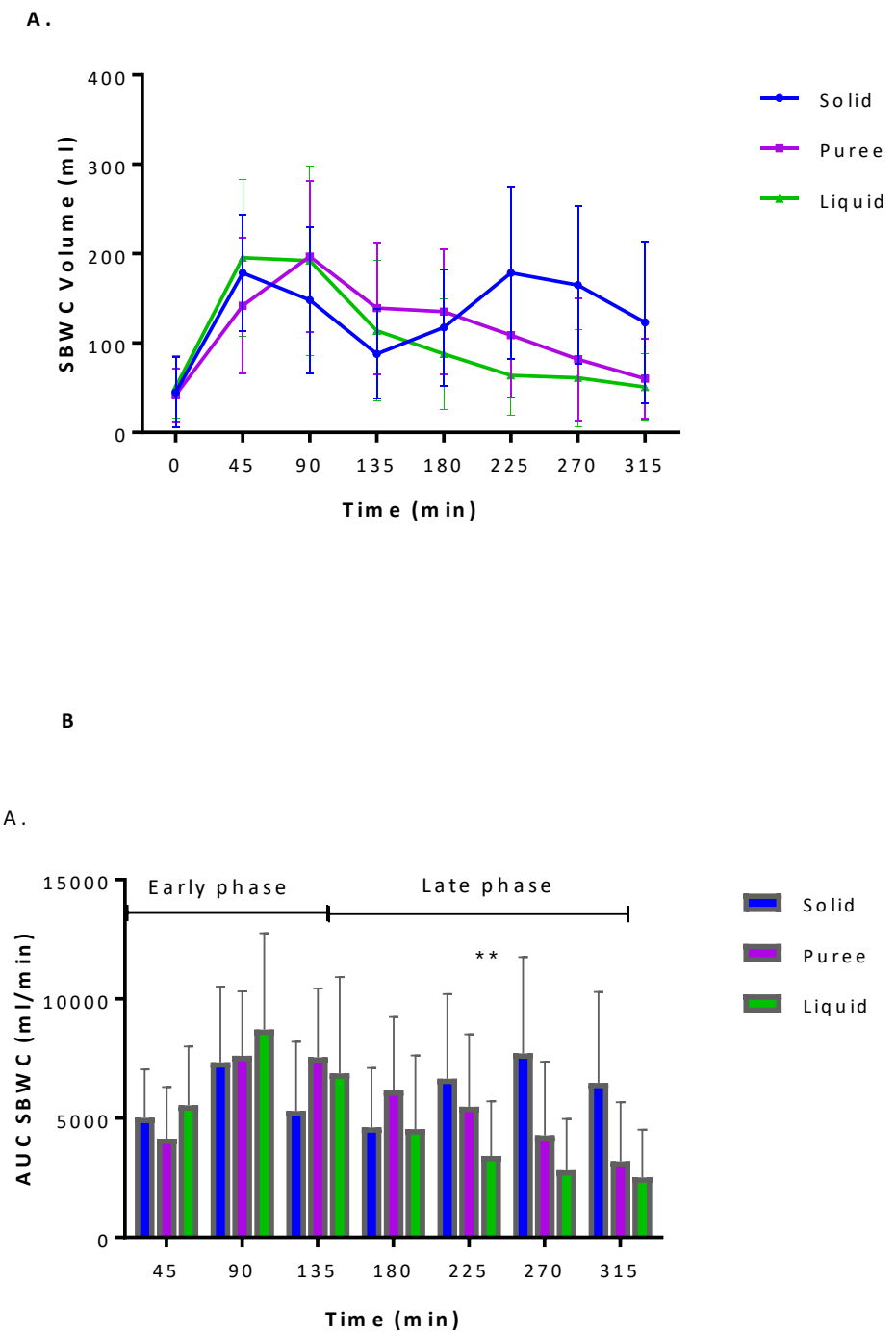
Figure 4.7A shows SBWC for all three test meals over time during the early phase and late phase. There was a steady rise in SBWC post feeding, followed by a decrease at 135 min in all groups. WA had a 51% reduction in volume during the early phase compared to other meals. Interestingly, during the late phase at 225 min, the WA meal showed a biphasic response with a second peak, whereas the SBWC volumes for both the AP and AJ continued to decline.

The primary outcome measure for this study was the AUC for small bowel water content (SBWC) over time following meal ingestion. There were no differences in SBWC AUC in the early phase between groups from 0-135 min, ($p=0.457$). However, during the late phase from 135 – 315 min the differences were significant, based on AUC calculated using two-way ANOVA ($p=0.0081$). Post-hoc analysis with Tukey's multiple comparisons test showed a significant difference between WA versus AJ ($p=0.0057$).

Figure 4-7: SBWC volume and AUC from baseline in test meals over time

A : Mean small bowel water content from T=0-315min for all test meals

B : Mean AUC for small bowel water content from T=0-315 min for all test meals



4.11.3.1 Colonic Volume

Colonic volumes were segmented into ascending colon volumes, transverse colon volumes and descending colon volumes as shown in Figure 4.8. There were no significant differences between both segmented colonic volumes and AUC for all segmented colon volumes. However, both ascending colon and transverse colon had an increase in volume from baseline at T=270 min and T=315 min respectively.

Ascending colon volumes increased by 23.5% from baseline in WA, and by 24.3% in AP, but decreased by 3.5% in AJ. 45 minutes later, there were similar increases in transverse colon volumes at T=315 min with a 29.3% increase in WA, with an 18.6% increase in AP as well as a modest increase of 5.5% in AJ. When compared to descending colon volume, the increase from baseline was noted at T=270 min, in which only a 3.9% increase in WA, 11.7% increase in AP and a 4 % decrease in AJ were recorded.

Colonic volume analyses were performed on a data set of 16 participants, as 2 participants could not be segmented. This was due to poor quality images, the result of excessive movement during scanning.

As seen in Figure 4.9 during the early phase, colonic volumes at 135 minutes for WA showed a sharp decline, whereas that of AP showed a slight increase and that of AJ remained unchanged. However, during the late phase, both WA and AP seen increases in colonic volume, compared to AJ which continued to decline. The difference in mean volumes were not significant between the test

meals during early phase, with p=0.9847, similarly in the late phase with p=0.4739.

The differences in AUC colonic volume were also insignificant between all test meals in the early phase at p=0.9978, as well as during the late phase with p=0.4562.

4.11.3.2 Colonic Gas

The differences in colonic gas volume was observed to be insignificant between all three groups in both the early phase and late phase, with p=0.3007 and p=0.1365. AUC for colonic gas were also insignificant for all test meals, with p=0.3250 during the phase and p=0.1346 during the late phase.

4.11.3.3 Breath Hydrogen

As recorded data was not normally distributed, non-parametric analysis was carried out. Differences in breath hydrogen concentration for all test meals were not significant in the early phase, recording p=0.9708, and p =0.693 in the late phase. Difference in AUC for breath hydrogen concentration for all test meals was also not significant in both early and late phases, with values of p=0.2244 and p=0.772 respectively.

Figure 4-8 Mean segmented colonic volume for all test meals over time

A. Mean ascending colonic volume from T=0-315 min for all test meals

B. Mean transverse colonic volume from T=0-315 min for all test meals

C. Mean descending colonic volume from T=0-315 min for all test meals

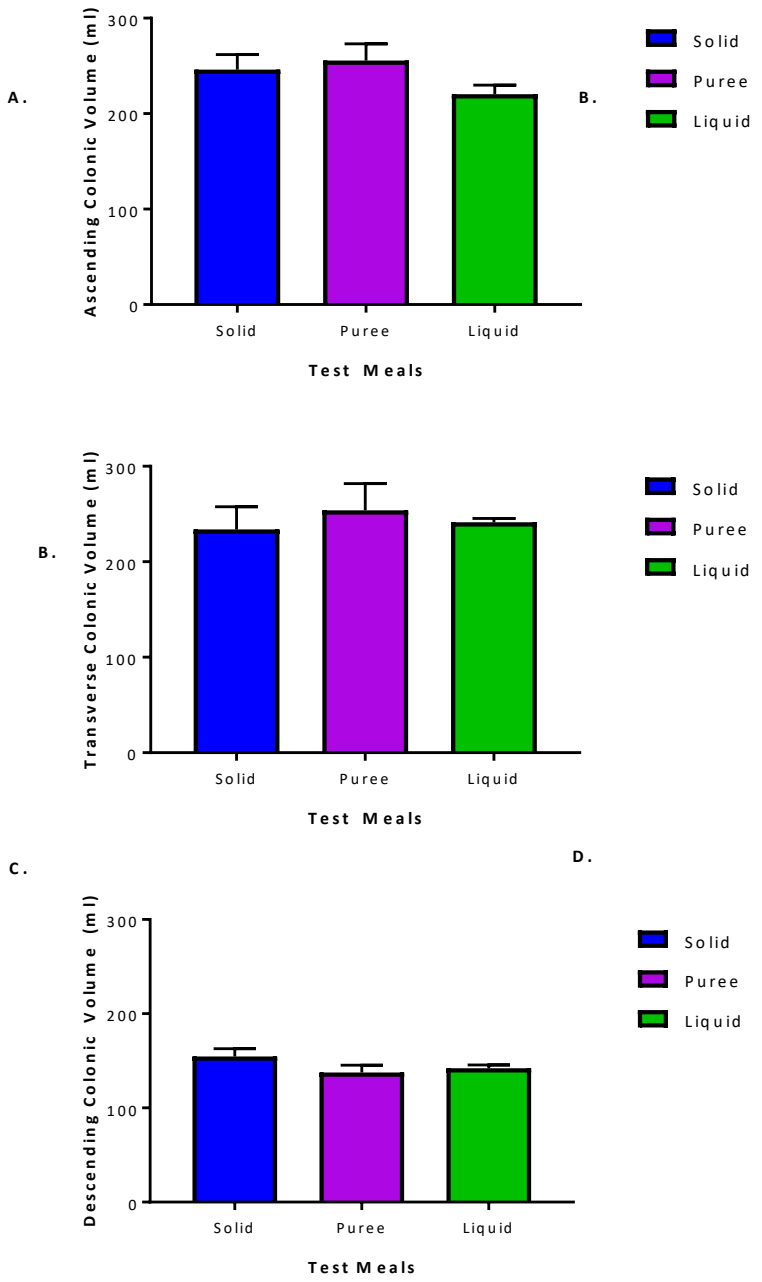


Figure 4-9: Colonic volume and AUC over time for all test meals

A: Mean total colonic volume from T=0-315 min for all test meals

B: Mean total gas volume from T=0-135 min for all test meals

C: Median breath hydrogen from T=135-315 min for all test meals

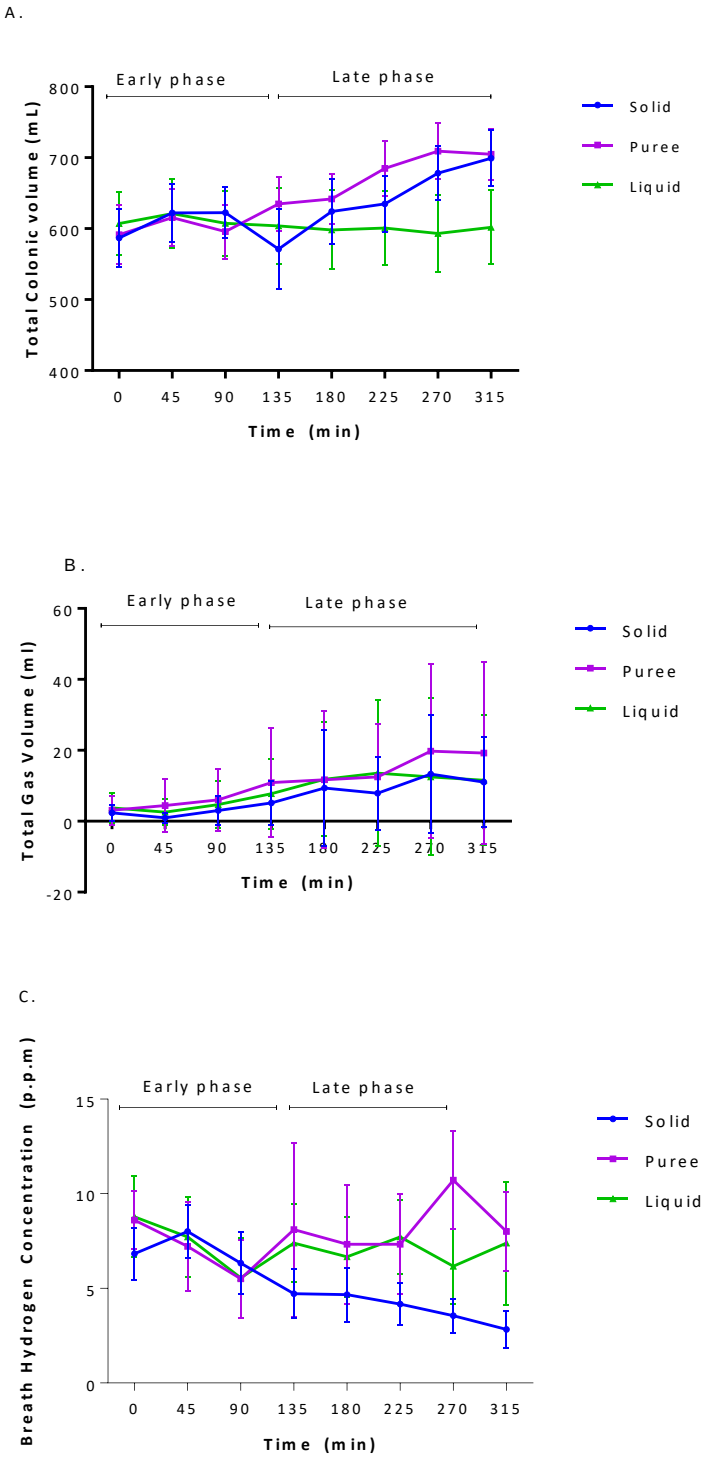


Table 4-4 Mean AUC for small bowel water content, total colonic volume, colonic gas and breath hydrogen in early phase and late phase mean (SEM)

	Solid	Puree	Liquid	P value
Early Phase (0-135 min)				
Small bowel water content	5.9 (0.7)	6.4 (1.2)	7 (0.9)	0.464
Total colonic volume (n=16)	14.5 (2.5)	13.1 (2.6)	12.1 (2.5)	0.9859
Colonic gas (n =16)	0.2 (0.03)	0.4 (0.08)	0.4 (0.06)	0.3700
Breath hydrogen	0.3 (0.2,0.3)	0.3 (0.3,0.4)	0.3 (0.3,0.4)	0.693
Late Phase (135-270 min)				
Small bowel water content	6.3 (0.6)	5.9 (0.7)	4.4 (0.9)	0.0081 *
Total colonic volume (n=16)	84.8 (22.3)	90 (19.5)	80.9 (29)	0.9972
Colonic gas (n=16)	0.6 (0.07)	0.9 (0.09)	0.8 (0.04)	0.7486
Breath hydrogen	0.2 (0.1,0.2)	0.4 (0.3, 0.4)	0.3 (0.3,0.4)	0.772

All results are expressed in Mean (SEM) except breath hydrogen which is in Median (IQR)ANOVA was performed for all parameters except breath hydrogen. Kruskal Wallis test was performed

4.11.4 Appetite Scores

Participants appetite ratings on hunger, desire to eat and prospective consumption increased steadily over time in the late phase (see Figure 4.11). The reverse was observed for satiety and fullness scores which declined steadily. There were no differences between groups in AUC for appetite scores, except for the fullness score in the early phase with $p<0.001$.

4.11.5 Symptom scores

Similarly, there were no differences between groups in AUC symptom scores (Figure 4.12).

Figure 4.11: Mean change in appetite scores for all test meals over time

A: Hunger scores from T=0-315 min for all test meals

B: Fullness scores from T=0-315 min for all test meals

C: Satiety scores from T=0-315 min for all test meals

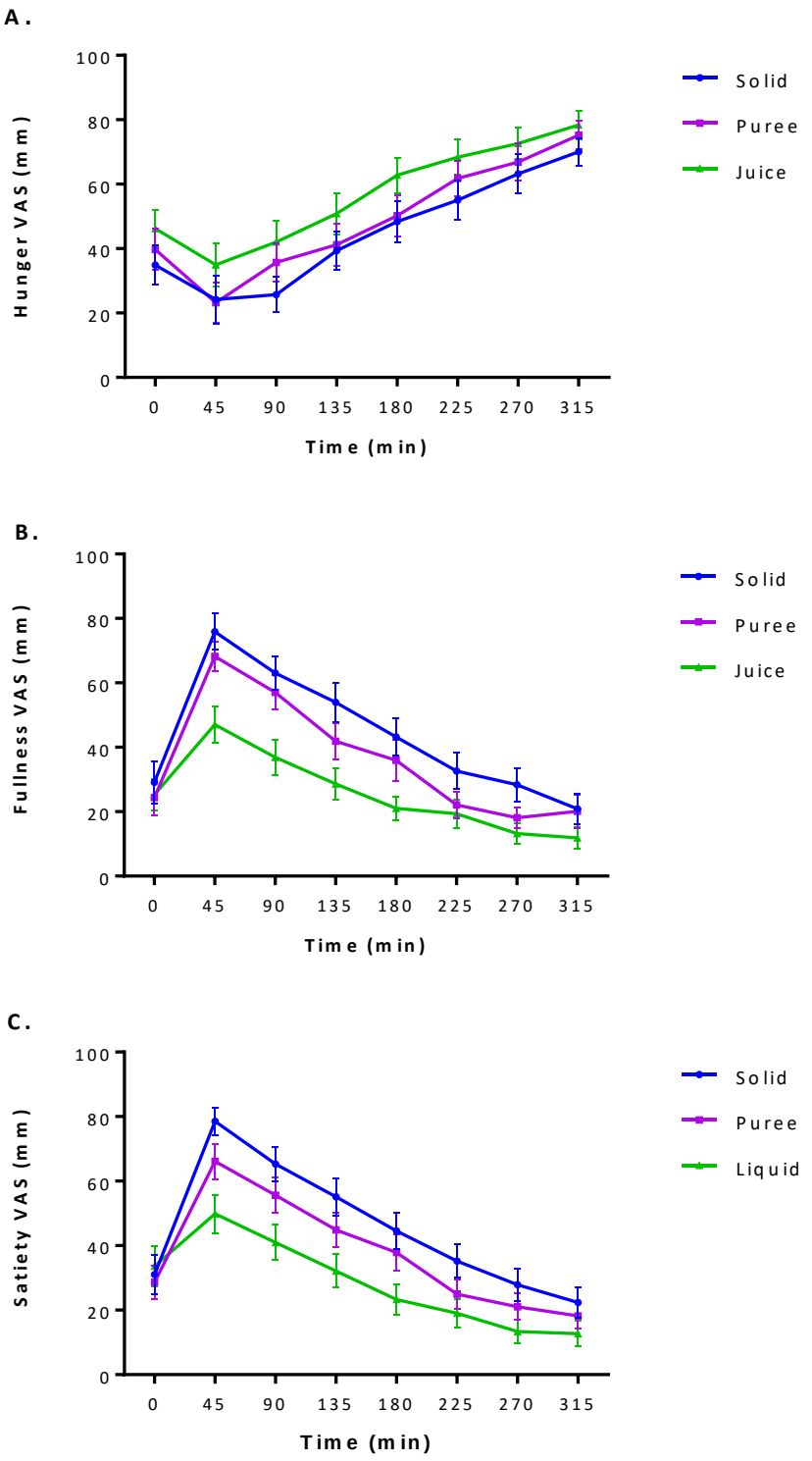
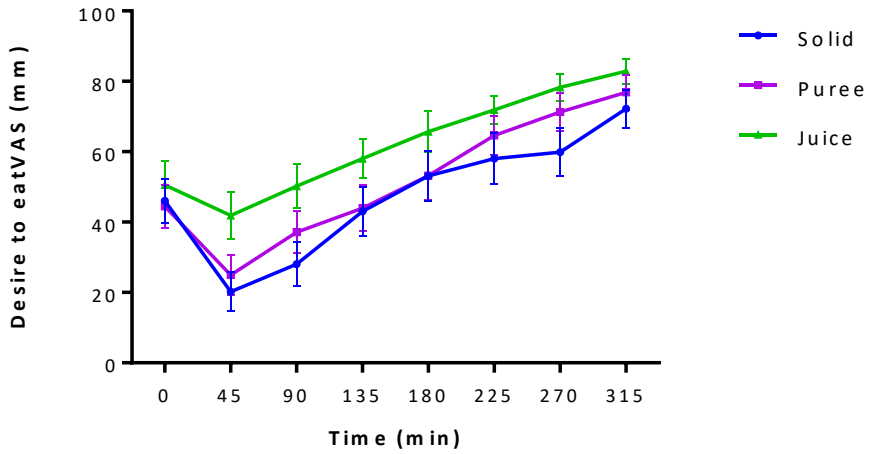


Figure 4-11: Mean change in appetite scores for all test meals over time

D: Desire to eat scores from T=0-315 min for all test meals

E: Prospective consumption scores from T=0-315 min for all test meals

D.



E.

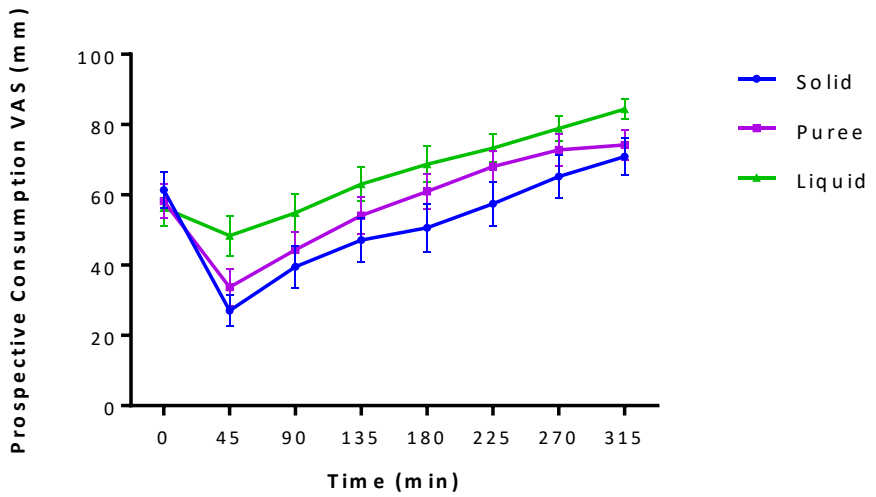


Figure 4-12 Symptom scores for all test meals over time

A. Symptom scores for from T=0-315 min for whole apple

B. Symptom scores from T=0-315 min for apple puree

C. Symptom scores from T=0-315 min for apple juice

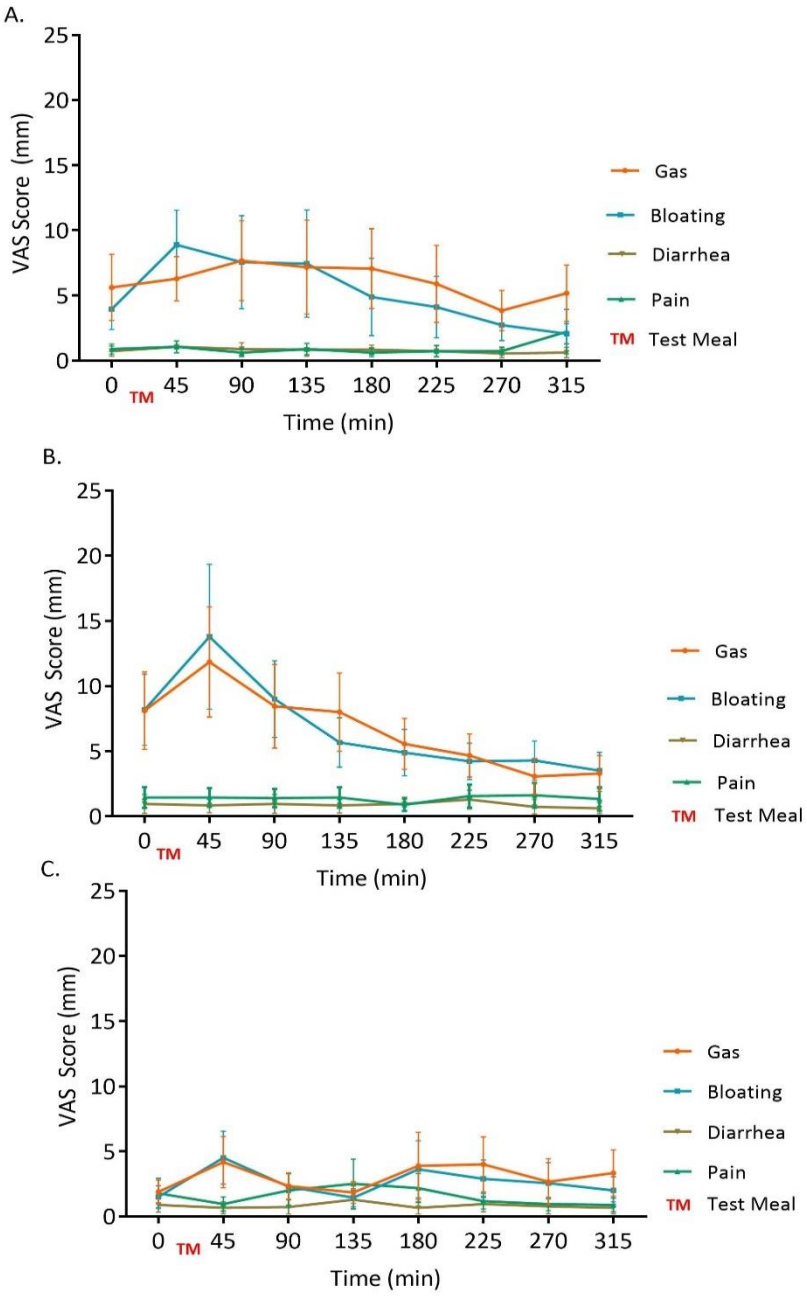


Table 4-54-6 AUC of appetite and symptom scores. Mean (SEM) in early and late phase

	Solid	Puree	Liquid	ANOVA (P value)
Early Phase (45-135 min)				
Appetite scores				
Hunger	1.3(0.9)	1.5(0.1)	1.9(0.1)	0.924
Fullness	2.7(0.2)	2.4(0.2)	0.04(1.1)	0.001**
Satiety	2.8(0.2)	2.4(0.2)	1.9(0.1)	0.069
Desire to eat	1.4(0.2)	1.6(0.1)	2.2(0.1)	0.300
Prospective Consumption	1.8(0.2)	2(0.1)	2.4(0.1)	0.126
Symptom scores				
Gas	0.3(0.02)	0.4(0.03)	0.1(0.02)	0.757
Bloating	0.3(0.2)	0.4(0.06)	0.1(0.04)	0.371
Pain	0.04(0.003)	0.06(0.0004)	0.8(0.01)	0.309
Diarrhoea	0.04(0.001)	0.04(0.0001)	0.04(0.004)	0.543
Late Phase (135-270 min)				
Appetite scores				
Hunger	2.5(0.2)	2.7(0.2)	3(0.2)	0.764
Fullness	1.6(0.2)	1.2(0.2)	0.8(0.1)	0.176
Satiety	1.6(0.2)	1.3(0.2)	0.9(0.1)	0.258
Desire to eat	2.6(0.2)	2.8(0.2)	3.2(0.2)	0.441
Prospective Consumption	2.6(0.2)	3(0.2)	3.3(0.2)	0.824
Symptom scores				
Gas	0.3(0.03)	0.2(0.04)	0.1(0.01)	0.413
Bloating	0.2(0.04)	0.2(0.01)	0.1(0.009)	0.529
Pain	0.04(0.009)	0.06(0.004)	0.07(0.01)	0.230
Diarrhoea	0.03(0.003)	0.04(0.005)	0.04(0.002)	0.789

4.12 Discussion

This study was designed to measure the effect of different physical properties of apples on gastrointestinal responses, appetite and symptoms.

4.12.1 Gastric emptying

The physical property of a meal has been shown to influence gastric emptying. Once solids enter the stomach, in large particles, they are blended by antral grinding (Houghton et al., 1988) to produce a homogenous dispersion which potentially becomes more viscous than water (Marciani et al., 2000). Separation of solids and liquids in a mixed liquid/solid meal within the stomach allows for a faster overall gastric emptying, as the liquid is “decanted” over remaining solids with a faster early fall in gastric volume. However, when the same liquid and solid components are homogenized to form a soup, gastric emptying is delayed due to the abolishment of this sieving effect (Marciani et al., 2012).

The test meals in this study comprised of whole apples (solid), puree (semi-solid) and apple juice (liquid) with the juice being the quickest test meal to be emptied from the stomach, followed by puree and whole apple.

It has been demonstrated that solids take a longer time to empty and they have a liner and lag phase contributing to a biphasic pattern of emptying. In a manometry study, subjects were fed 100 g beef and liver meal and liquid which was either 200 mls of normal saline or 25% of dextrose. It was observed that the liquid component emptied after a short lag period, but the 25% dextrose had a delay in emptying compared to saline. It was suggested that the solids

were initially in the proximal stomach reservoir area and was then redistributed to distal stomach during the emptying of liquid from the stomach. In addition, the liquid phase was retained in the distal stomach as there were no mechanical digestion needed. It was also concluded that the proximal stomach is important in controlling gastric emptying of solids whereas the distal stomach is important in emptying of liquids. (Collins et al., 1991). In another scintigraphy study participants were fed a solid test meal with liquids. The physical form of the solid component was observed to influence the lag phase. The solid test meal in this study were similar in size, calorie and macronutrients. However, gastric emptying in in these meals was later corrected by equilibrium and it emptied with constant rate producing a biphasic phase. In addition, the 300 ml water given with the meal emptied first, followed by the solid particles (Siegel et al., 1988). The test meals in this study was given together with water, therefore this would have been emptied first for the whole apple and puree test meal as well as the juice.

In addition, solids used in this study had a significantly higher half emptying time (T50) $p<0.001$, confirming that solid apple emptied slower when compared to puree and liquid (Figure 1.6). The physical form of masticated solids, being part liquid and part solid, is likely to have much greater overall resistance to flow within the stomach which most likely explains the slower emptying. Further studies have shown that increasing meal viscosity slows gastric emptying (Marciani et al., 2001). However, measuring the viscosity of the partially-masticated apple would be complex, requiring specialised equipment that was unavailable, but we can assume that this viscosity would

be higher than that of puree. The faster emptying of nutrients into the duodenum from the juice and puree meals would be expected to activate negative feedback, reducing observed differences, but evidently not enough to completely abolish the effect. Although viscosity was not measured in this study, the puree test meal appeared to be viscous and the solid would have had similar properties upon mechanical digestion. Even though viscosity may not have influenced gastric emptying, it may have had an effect on fullness. An MRI study on varying viscosity and nutrient content showed that fullness, correlated linearly with total gastric volume, was higher for high viscosity meals compared to low viscosity meals.

Nutrient meals also contributed to large antral volumes (Marciani et al., 2001). This was observed in this study there was significant difference between AUC of fullness between 45 to 135 minutes between the test meals. The area under the curve was higher for both whole apples and puree compared to juice seen in Table 4.6. Although the stomach had emptied in the last phase between 270-315 minutes, no significant differences were observed in fullness. In another study, on in-vitro digestion, it was observed that high viscosity beverages had significant effect on satiety (Logan, et al., 2015). There were no significant differences in AUC of satiety between all test meals in both the early phase or the late phase. However, whole apples had higher VAS scores and AUC volume followed by puree and juice.

Cooking also modifies physical and chemical properties of food. The puree test meal was made by boiling in water cored apples to soften them, before being

pureed in a mixer. This cooking process may have indirectly affected gastric emptying rate, with the puree being softer, thus reducing antral grinding requirements (Lillo et al., 2002). The effect of heat treatment (through boiling) would have facilitated digestion of the starch molecules in puree (Lee, 2005), by exposing sites of enzymatic action. Whether this significantly altered the puree behaviour is a matter that would require a further controlled study in which this factor was varied.

It has also been demonstrated in this study that the physical form of apples does influence gastric emptying. However, there are other factors to consider such as meal composition, nutrient content, motility and gender (Leiper, 2015). The meal composition and nutrient was controlled for this study, with the exception of fibre due to processing techniques; motility was not an objective. As this was a crossover study, participants were also their own controls, and due to the sample size, gender could not be controlled for.

When the stomach is emptying, digested particles in a form known as chyme is delivered to the duodenum. This is through a bidirectional movement of water and electrolytes across intestinal lumen (Leiper, 2015). In order to maintain the absorptive capacity of the small intestine, the receptors regulating gastric emptying (found in the duodenum and ileum) delay gastric emptying (Camilleri, 2006).

4.12.2 Small bowel water content

The hypothesis being tested in this study was that the liquid form of apples will significantly increase small bowel water content (SBWC) in participants, in

comparison to solids or puree. However, no evidence of such increment was found over time.

The postprandial SBWC as shown in Figure 4.3, initially decreases for all test meals, due to rapid nutrient-driven fluid absorption (Marciani et al., 2010). However, in the later phase of the study, only the solids increased SBWC significantly compared to both puree and liquid. The highest increase in SBWC volume was from solids, observed at 225 min with a 75% increase from baseline. This resulted in the triphasic pattern in SBWC for the solids due to the fall in volume immediately after feeding, followed by an increase in volume at 45 minutes and a fall at the early phase at 135 minutes and an increase in volume during the late phase at 225 minutes. As expected, the increase of SBWC of solids at 45 minutes post feeding did increase satiety as shown in Figure 4.11C, but this was not observed during the second peak in late phase at 225mins despite similar AUC for the SBWC volume as the early stage.

This pattern has been observed in the bran study (Marciani et al., 2010), whereby there was an initial fall in volume post feeding, followed by a rise in volume to a plateau and subsequent reduction. Gastric content was half emptied between 90 minutes to 135 minutes but there was a reduction at 135 mins in small bowel water for all three test meals. Although there was a rise in volume for whole apples over time, this was not observed for puree and juice. A possible explanation could be that the first increase in SBWC after solids, puree and juice was due to ingestion of the test meal, whereby the liquid was first emptied into the small bowel - as all test meals were given with water.

Despite the gastric volume emptied at 135 minutes for all test meals, there was still a fall in SWBC at the same time. This was probably due to the fluids in the terminal ileum emptying into the ascending colon. AUC for colon volume showed an increase in the late phase from 135 minutes to 270 minutes, compared to the early phase, confirming the rapid emptying of SBWC into the colon.

The second peak in SBWC was observed in solids similar to the peak in the Marciani et al., bran study. (Marciani et al., 2010) and not for the other two test meals. This could be due to the indigestible solids in the apple meal that may have trapped fluid in the terminal ileum and delayed entry into the colon, compared to the other two test meals. This can be seen in Table 4.4. which shows the SBWC for all test meals over time.

It was expected that the juice would increase SBWC, but it rapidly emptied into the colon compared to the other two test meals. When colon volumes were observed, only the juice had a decrease in volume over time, suggesting reabsorption of water from the juice and clearance from the colon possibly due to an osmotic effect.

The test meal consisted of fructose and glucose, absorbed via Glut 2 or Glut 5 transporter, while sorbitol was through passive diffusion. It was expected that these fermentable carbohydrates would cause similar effects in SBWC as those seen in previous mechanistic studies. This was not observed because the fermentable sugar content was lower than what was ingested in these mechanistic studies. It was not possible to provide 40g of fructose or 10g of

sorbitol in the form of apples. This could not have been consumed by the participants in the study due to large number of apples involved. The amount of carbohydrate that was given was based on volunteer ability to finish the test meals (for all test meals).

4.12.3 Colonic Volume

Colonic volume, colonic gas and breath hydrogen changes were compared between groups from baseline. The AUC of breath hydrogen was the highest for puree, followed by that of liquid but much lower in solid. Possible mechanisms include the rapid absorption of liquid compared to solids, thus reducing the time available for absorption, or reduced absorption of carbohydrate from the puree owing to its high viscosity. However, the differences between the groups were not significant.

As participants were healthy volunteers with normal colons, there were no significant differences between groups in the symptom scores. Normal colons are able to relax and accommodate large gas loads without causing symptoms, as compared to patients with gastrointestinal functional disorders (Malagelada and Valle, 2003).

4.12.4 Appetite scores

Comparison of appetite score AUC (Table 4-6) showed that there was significant difference between groups in fullness scores (p=0.001). Post-hoc test showed the difference between solid vs liquid as being significant (p-0.0255). Meanwhile, there were no differences between the other scores. However, satiety and fullness scores were slightly higher in the solid group

when compared to the other two groups, whereas hunger, desire to eat and prospective food consumption scores were higher in the liquid group. This was also the same in the late phase.

This difference persisted long after the stomach had emptied, therefore it is deduced that this was due to influences from lower down the gut, especially the colon. Fermentation products, including short chain fatty acids generated by FODMAPs in apples, are likely to signal to various central receptors which may be important in appetite regulation (Sleeth et al., 2010). The lower satiety experienced after juice may reflect the increase in digestibility, induced by the processing involved in preparing the puree and juice, reducing the load of undigestible FODMAPs entering the colon for these two meals.

One possible explanation is that the increase in colonic volume and colonic gas in the late phase contributed to the feeling of fullness and satiety. This could be a fermentative effect of the test meals. Our studies were acute, but a recent study in mice has raised the possibility that chronic ingestion of fermentable carbohydrate potentially increased PYY cell density associated with reduction in food intake (Brooks et al., 2017).

During the digestion process in the stomach, gut hormones are secreted from enteroendocrine cells. One of such hormones is incretin which consists glucagon-like peptide-1 (GLP1) which enhances postprandial insulin. secretion. It has been demonstrated that GLP-1 triggers satiation and elevated GLP-1 has been shown to have glucose lowering effect (Kim and Egan, 2008).

However, one study looked at whether fructose intake could stimulate GLP-1 secretion and its effect on appetite. In a single blind randomised study, 8 males were given 75g fructose, or 75g glucose followed by 75g fructose later. GLP-1 as well as venous glucose and insulin were then measured. Appetite scores were also measured using VAS questionnaires. The study showed that ingestion of glucose significantly increased plasma glucose, insulin and GLP-1. Although fructose also stimulates GLP-1 and insulin, the response is less compared to 75g glucose. However, neither GLP-1 nor insulin played a major role in regulating satiety. (Kong et al., 1999). However, the fructose content of the test meals in this study was only between 11g to 14g and it is unsure if the test meals could have stimulated this response. Based on this evidence, perhaps it is useful to measure gut hormones in feeding studies particularly when assessing appetites.

Although this study has provided new data, it did have its limitations. Firstly, the expense of the scanning restricted participant numbers and hence the power to detect small differences. Thus, while we could show an effect on gastric emptying, the effects on the colon were smaller and would need larger numbers to confirm significance of observed changes.

The second factor which complicates the conclusions is, as a result of processing, the reduced fibre content in the liquid meal. Although all test meals were matched for calories and volume and comprised of 13-19g of total FODMAPs they were not matched for fibre due to processing technique. The apple juice was cold pressed, therefore the final product contained only 1.7g

of fibre compared to 9.4g in the solids and 8.1 in the puree. This may have also been a factor in the acceleration of juice into the colon from the small bowel. If a meal marker had been included, it would have been possible to measure gut transit which could be an important intermediate variable. Such markers are under development in University of Nottingham and could be used in future studies.

4.13 Conclusion

Based upon the results of this study, it can be concluded that altering the physical form of a meal has an effect on small bowel water content and gastric emptying. However, there are still some unanswered questions as to whether they may effect colonic volumes and influence gastrointestinal symptoms in patients with IBS. This would require more sizeable studies with greater numbers of both patients and healthy volunteers, in order to compare the effect and overcome the inherent variability of these measures.

CHAPTER 5 A PILOT RANDOMISED CONTROLLED TRIAL COMPARING THE EFFECTS OF LOW FODMAP DIET VERSUS HABITUAL DIET, ON COLONIC VOLUME AND SYMPTOMS IN IBS PATIENTS. (CRAIGS STUDY)

5.1 Background

The low FODMAP diet has gained worldwide recognition as a dietary therapy for patients with IBS and Functional Gastrointestinal Disorders (FGID). How the diet alters luminal content remains unclear.

Over the past decade, the efficacy of the low FODMAP diet in IBS patients, (Staudacher et al., 2011),(Barrett et al., 2010),(Shepherd et al., 2008), lead to its recommendation as a second line dietary therapy for patients in the UK (McKenzie et al., 2016), in addition to its adoption as part of the NICE guidelines in 2015 (IBS in Adults: Diagnosis and Management [CG61], 2008). A recent randomised placebo controlled trial showed that IBS patients who followed a low FODMAP diet for four weeks had significant reductions in symptom scores, compared to patients on a sham diet (Staudacher et al., 2017). This coincides with the author's first findings, whereby a reduction in gastrointestinal symptoms such as abdominal pain, bloating and diarrhoea was observed in 76% of patients on low FODMAP diet, compared to those who followed standard dietary advice (Staudacher et al., 2011). Meanwhile, the second findings reported similar results, with 68% of patients experiencing a reduction in symptoms compared to 23% of patients on a habitual diet (Staudacher et al., 2017). Patients are advised, by a dietitian trained in FODMAPs processes, to follow the low FODMAP diet for 4-6 weeks. However, symptom changes have been reported within 14 days of diet commencement (Halmos et al., 2014), suggesting that 2 weeks of a low FODMAP diet may be adequate to control symptoms and therefore increase compliance rate.

Mechanistic studies by our group have demonstrated that fructose exerts an osmotic effect, increasing small bowel water content; additionally, with both patients and healthy controls, inulin increases colonic gas and breath hydrogen levels due to rapid fermentation (Major et al., 2016),(Murray et al., 2014). However, both studies were looking only at the individual effect of fructose and inulin FODMAPs. The FOG study (see Chapter 3) is the first study to investigate the effects of a high FODMAP versus low FODMAP diet on the colonic volume of healthy volunteers. The volunteers followed a low FODMAP diet for one week, before being randomised to receive either a daily 14g dose of oligofructose, a prebiotic supplementation (high FODMAP), or the same amount of maltodextrin [a simple sugar] (low FODMAP). Both groups had an increase in colonic volume from baseline (Oligofructose 19.6%, versus Maltodextrin 15.5%), despite the low FODMAP diet not being expected to exert an effect on the colonic volume. The high FODMAP diet induced fermentation which raised concentration of breath hydrogen, in addition to increasing the level of bifidobacteria due to the prebiotic effect of oligofructose (Sloan et al., 2016). The results of the FOG study confirm previous findings whereby a change in colonic environment, particularly fermentation (Ong et al., 2010), and gut microbiota (Halmos et al., 2016) is influenced by altering FODMAP intake. However, symptoms were not assessed in this study and the association between low FODMAP diet and colonic volume could not be determined.

As such, the CRAIGS study was aimed at addressing uncertainties of previous studies. The primary objective of the study was to assess and compare changes of fasting colonic volume in IBS patients who have been randomised to follow

either a low FODMAP or habitual diet. Based on previous findings, the hypothesis of the study is that patients on a low FODMAP diet of two weeks duration will have reductions in colonic volume and breath hydrogen, along with global symptom improvement compared to patients on habitual diets.

5.2 Aim

To assess whether a low FODMAP diet versus Habitual diet alters fasting colonic volume in IBS patients.

5.3 Objectives

Primary Objective

- To measure (by MRI) fasting colonic volume at baseline and follow-up in the low FODMAP group versus habitual diet group by comparing between groups changes from baseline.

Secondary objective

- To measure changes in colonic fermentation by assessing fasting breath hydrogen using a breath hydrogen meter.
- To compare the proportion of IBS patients reporting a clinically important improvement in symptoms following a low FODMAP diet, versus their habitual diet.

5.4 Hypotheses

The null hypothesis is that there is no difference in fasting colonic volume from baseline between those IBS patients on a low FODMAP diet, and those on a habitual diet.

The alternative hypothesis is that there will a decrease in fasting colonic volume from baseline in those following a low FODMAP diet, compared to those in the habitual diet group.

5.5 Methods

5.5.1 Subjects: Primary Care Research Network

Under the care of their General Practitioners (GPs), IBS patients who met the eligibility criteria were invited to participate in this study through the Primary Care Research Network (PCRN). The investigator (SK) was supported by the PCRN co-ordinator, who identified and approached GPs within the Nottinghamshire area who were willing for their practices to act as Patient identification centres (PICs) for the study.

A total of four practices expressed an interest to participate, after which they were given a list of inclusion and exclusion criteria (see Table 5-1) to facilitate records searches to help identify potential patients. This search was conducted by either the GP or the Practice Manager, to estimate the number of patients eligible to participate in the study. As this was a basic search, not all eligibility criteria were assessed. Therefore, all patients were re-screened by the investigator during their first visit

On completion of the searches, practices accordingly advised the PCRN co-ordinator after which a meeting was held between the co-ordinator, investigator and Practice Manager/GP from the selected sites in order to explain the study in detail. The screening process was also explained in detail, with all practices agreeing to do a secondary search. They were given site files

containing the study protocol, questionnaires and ethical approvals from REC, R&D (NUH) and R&D (Nottinghamshire Healthcare NHS), to be kept at the PIC sites. Additionally, they were given a soft copy by email of all the documents, including a template letter for the GP. This document served as an invitation letter from the GP to their eligible patients. Within four to eight weeks of the meeting, the investigator was contacted via email by the practices to advise of the final number of patients that were suitable as potential participants.

5.5.1.1 Study Pack

Study packs for each practice were prepared by the investigator, based on the final number of eligible patients. Each of these packs contained:

i) Patient information sheet (PIS) for the study

ii) Reply slip with three options: the first indicating patients were happy to be contacted by the investigator to explain more about the study, the second option being 'maybe' and the third indicating 'no interest' in the study; at the bottom of each slip there was space for patient comments.

iii) A postage-paid envelope to mail the reply slip back to the investigator.

iv) A white A4 unsealed stamped & addressed envelope (2nd class large) which contained item numbers (i) to number (iii).

The study packs were then delivered to the PIC sites by the investigator, unsealed to enable addition of the invitation letter by the Practice Manager. The study pack was then sealed by the practice and posted to the patients. A total of 550 packs were despatched from PIC sites.

5.5.2 Recruitment from secondary care and other sites

Besides the PCRN network, recruitment was also done within Nottingham University Hospitals (NUH) and campuses. A4-size study posters were displayed in public viewing areas and corridors of the above locations, including libraries and faculties. Concurrently, social media such as Twitter and Facebook were also utilised to advertise the study to the general public.

Those who expressed an interest in participation subsequently contacted the investigator by phone or email. Respondents were then emailed a copy of the PIS as well as being contacted by the investigator for a detailed explanation of the study, and to commence the screening process. Eligible patients were invited to attend their first visit.

All patients were recruited between January 2016 and February 2017, with the last patient completing the study in July 2017.

5.5.3 Study Design

This was a parallel group, randomised controlled trial at the BRU and SPMIC trial sites. All patients were randomised to one of the two parallel treatment arms. The study was approved by REC East Midlands, Nottingham 2, reference number 15/EM/0468 and was prospectively registered on clinical trials.gov (NCT03729622).

1. Low FODMAP (Immediate low FODMAP dietary advice)

2. Habitual diet (Delayed low FODMAP dietary advice)

Table 5-1 Screening Criteria

Inclusion Criteria
Aged 18 and above
Able to give informed consent
Previous diagnosis of IBS by medical practitioner
Meeting ROME III criteria for IBS
Investigations showing negative tissue transglutaminase (tTG) IgA antibodies
Full blood count does not indicate anaemia
Exclusion Criteria
Limited understanding of English
Pregnancy declared by the participant
History declared by the candidate of pre-existing gastrointestinal disorder, including but not limited to: Inflammatory Bowel Disease, Coeliac Disease, Pancreatitis, Gallstone Disease (biliary colic, cholecystitis), Diverticulitis, cancer of the gastrointestinal tract, reported history of previous resection of any part of the gastrointestinal tract other than appendix or gallbladder, intestinal stoma
Red flag symptoms such as rectal bleeding, family history of bowel or ovarian cancer, a change in bowel habit to looser and/or more frequent stools persisting for more than six weeks in a person aged over 60 years
Habitual use of opiate analgesics likely to alter bowel function e.g. morphine. Use of antibiotics in the preceding four weeks other than for treatment of index infection
Any condition where the candidate is likely to require a course of antibiotics in the next 3 months e.g. severe chronic respiratory disease, recurrent urinary tract infection, lower limb ulceration
Inability to complete the symptom questionnaires, e.g. cognitive dysfunction limiting memory and understanding
Anyone who in the opinion of the investigator is unlikely to be able to comply with the protocol, e.g. chaotic lifestyle related to substance abuse
Dietary practice incompatible with safe implementation of the trial diet e.g. Veganism
Medical reasons for pre-existing controlled diet that cannot be safely randomised e.g. end-stage kidney disease, diabetes
Patients taking prebiotic/probiotic supplements

5.5.4 Expected duration of participant participation

Patients took part in the study for approximately 12-14 weeks.

5.5.5 Removal of patients from therapy or assessments

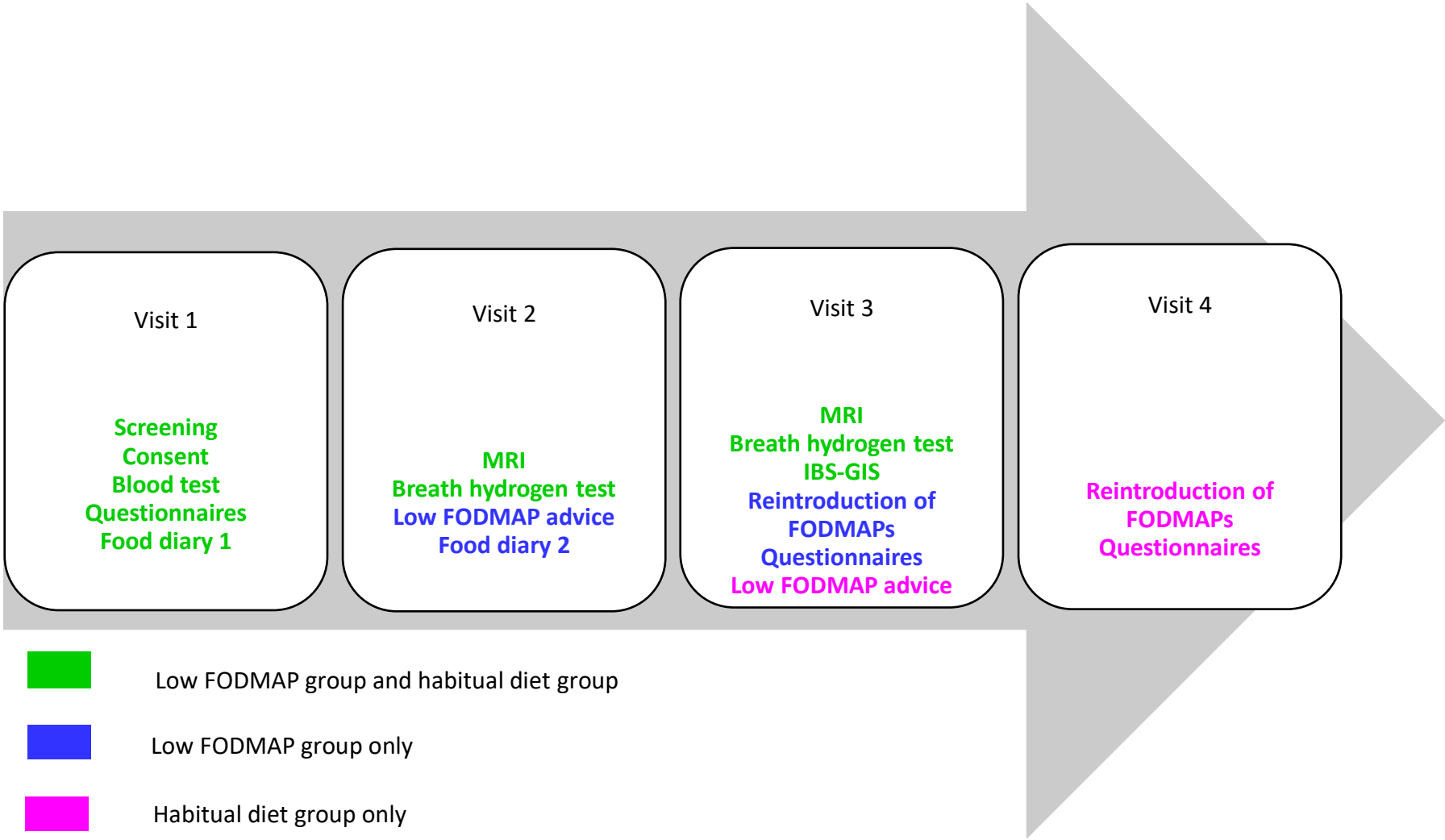
Patients were free to withdraw, or were withdrawn, from the trial for the following reasons:

1) Safety reasons (including pregnancy)

2) Failure of participant to adhere to protocol requirements

3) Loss of follow-up

Figure: 5-1 Schematic diagram of study process and intervention



5.5.6 Contact with patients identified by PIC sites

Prior to Visit 1, the investigator contacted by phone or email those patients who expressed an interest in study participation. Patients were then given a detailed explanation of the study whilst being concurrently screened. Those who could not be reached by email or phone were contacted via text message to schedule a suitable time for the investigator to contact the patients. Patients who were eligible were invited to attend their first visit at the QMC.

5.5.7 Detailed Descriptions of Study Schedule and Visits

5.5.7.1 Study Visit 1 (V1) – Screening, consent and baseline questionnaire

During this visit the investigator explained the study in detail, gained consent and screened patients to assess their eligibility. Patients were met at the Biomedical Research Unit by the investigator. Once consent was obtained, eligibility was assessed using inclusion criteria, a screening questionnaire based on the ROME III criteria ('Appendix B: Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders', 2010) and blood results. The screening criteria (except blood results) have been explained in detail in Chapter 3.

It was also explained to patients that they were free to withdraw from the study at any time.

Blood Test

Blood samples were taken by the research nurse or trained health care assistant from all patients who had not been previously screened for coeliac disease (CD). These samples were for the IgA tissue Transglutaminase Antibody test (tTG) and Full Blood Count (FBC). A negative result indicated no presence of coealic disease (CD) antibodies meanwhile full blood count is done to exclude other diagnosis.

Patients who confirmed previous screening as above then consented for their lab results to be viewed online by the investigator or research assistant. Those who had not been tested in these respects were requested to provide blood samples; all agreed.

All blood samples taken were labelled, sealed and delivered to the pathology lab by the investigator. Patients with negative tTG and normal haemoglobin were deemed eligible and were invited to attend visit. All patients subjected to the blood test returned negative results for tTG, and FBC found within normal range, therefore were eligible to participate in the study.

Questionnaires

Once enrolled, participant height and weight data were recorded into the case report form. They were given five questionnaires to complete and a food diary with guide to take away, as listed below:

1) Part One of the IBS Symptom Severity Scale (IBS-SSS)

This is a validated questionnaire to assess symptom severity in IBS patients using VAS (Francis, et al., 1997). There were five items in the questionnaire with a total score of 500. Four of these were VAS assessing abdominal pain, bloating/ distension, disturbance of bowel habit and impact on life and the fifth was proportion of days with pain. Patients marked the VAS using a cross, based on their symptoms in the last 10 days. The severity was categorised based on their total scores. Scores of 75 to 175 were mild cases, 175 to 300 were moderate, more than 300 were severe and less than <75 were in remission.

2) The EQ-5D-5L and EQ-VAS

This is a validated tool to measure patient quality of life in five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression (Herdman et al., 2011). Each dimension has five levels: no problems, slight problems, moderate problems, severe problems and extreme problems/inability. The scores for each level ranged from zero (no problems) to four (extreme problems) with a maximum score of 20. The questionnaire was combined with a vertical 100mm VAS to assess global quality of life (EQ-VAS). Patients had to tick a box based on their current level of QoL for all five dimensions and mark x on the VAS.

3) Hospital Anxiety and Depression Scale (HADS)

This is a validated self-assessment scale to detect depression and anxiety amongst patients (Zigmond and Snaith, 1983). A total of 14 statements were returned, with seven assessing 'anxiety' and the other seven assessing 'depression' based on how the patients had felt in the past week. The statements were both positive and negative; each of them had a maximum score of 3, giving a total score of 21 for each scale (anxiety/depression). A score between 0 and 7 was categorised as normal, 8-10 as borderline abnormal (borderline case) and 11-12 as abnormal (case).

4). Dietary Trigger Questionnaire

This questionnaire was developed by SK to assess the most common self-reported food triggers in IBS patients. Additionally, the questionnaire also collected information on eating habit changes influenced by symptoms or other factors, such as individual experience and dietary advice given by either a dietitian or other medical practitioners (as well as external sources of information such as books, internet and social media). The content of the questionnaire was developed based on recent studies within the UK (Hayes et al., 2014), (Williams, et al., 2011), and Europe (Böhn et al., 2013). It was piloted among patients and healthy volunteers within the School of Medicine. The first two pages of the questionnaire were divided into food groups: cereals/grains/breakfast cereals, vegetables, fruits, animal products, pulses/legumes/nuts and others, milk and milk products, beverages and others. Patients were asked to identify any food and beverages which triggered

their symptoms in each food group. They were also asked about changes in eating habits over the last 12 months, factors which influenced dietary alterations, any medical tests undertaken and demographic data such as education level, occupation and household income (optional).

5) MRI Safety Screening Questionnaire

This has been explained in detail in Chapter 3. Patients without contra-indications were scheduled to have 10-minute MRI scans twice during the duration of the study. Their first baseline scan was during Visit 2 for both groups, with the follow-up scan during Visit 3. Patients who chose not to be scanned due to claustrophobia were still included in the study as the MRI scan was optional. After completing the questionnaires, patients were given instructions by the investigator (a registered dietitian) on how to complete a 7-day food diary (see Chapter 2.5.3.4).

At the end of the visit, patients took away a 7-day food diary complete with guidance. The food diary and guide were based on based on the European Prospective Investigation into Cancer and Nutrition (EPIC) study diary (Bingham et al., 1997) and the guide was based on based on the National Diet and Nutrition Survey (NDNS) diary which includes food photographs for estimating food portions (Public Health England & Food Standards Agency, 2012).

Patients where then requested to either post their completed food diary, or to submit it on Visit 2. Those who did not post the diary or schedule their next visit were contacted 2 weeks after Visit 1 to remind them to post the diary. Patients who failed to do so were not enrolled in the study.

5.5.7.2 Study Visit 2 (V2) – MRI Scan, Breath test and Dietary Advice

MRI scan

The MRI scan measured colonic volumes at two time points and lasted about 10 minutes per scan. The first scan on Visit 2 was at baseline and the final scan was on Visit 3 for both groups. Visit 2 took place at the GE 1.5T scanner of the SPMIC on A floor of the School of Medicine, University of Nottingham and was within 1-4 weeks after Visit 1. Most patients returned within a week for Visit 2. However, some returned a few weeks later due to awaited blood test results, work commitments or vacation.

Patients were met by SK and escorted to the MRI unit, before being signed in in accordance with safety procedures. When SK was unavailable, the health care assistant's help was sought to escort the patients to A Floor. A few days before the scan, patients were contacted by SK to remind them about their appointment and also advised to follow the fasting protocol as described in chapter 3 and chapter 4. As the MRI was optional, patients who due to anxiety did not wish to have a scan were still included in the study. Patients who were scheduled to start the intervention diet during the Christmas break were advised to postpone their start date until after the holidays.

Breath test

After the MRI scans, patients were escorted back to the BRU by SK or health care assistant to undertake the breath hydrogen test using either Gastro4check, or if unavailable, a portable hand-held breath H2 meter Gastro+ Gastrolyzer. (Both machines were from Bedfond Scientific, Kent UK). A fasting

breath test was performed with a healthy volunteer, producing identical readings, indicating that the replacement machine readings were accurate. A level >10ppm indicated colonic fermentation (Kumar et al., 2010),(Eisenmann et al., 2008).

Patients who did not have an MRI scan were also required to fast before attending Visit 2 for their breath test.

Low FODMAP dietary advice

Patients in the low FODMAP group stayed on after their MRI and breath test to receive their dietary advice from SK. This session lasted 45-60 minutes. Using written materials described in Chapter 3, patients were given low FODMAP diet advice and instructed to follow this for 2 weeks. It was recommended that they start the diet at least one day after Visit 2 in order to better plan their meals.

Food diary

Patients in the low FODMAP group were given a second food diary to complete during the second week of their low FODMAP diet, for submission on Visit 3. In the weeks between Visit 2 and Visit 3, patients in the Low FODMAP group were contacted by email or telephone to discuss any issues arising from the diet. Arrangements for Visit 3 were also confirmed at this time. Patients in the habitual diet group who served as controls were given the food diary after their third visit and contacted between Visit 3 and Visit 4.

Study Visit 3 (V3) – MRI scan, Breath test, low FODMAP diet (Group 2) and **Reintroduction Diet (Group1)**

In the weeks prior to Visit 3, patients were contacted by phone, email or letter to remind them to fill in their food diary (low FODMAP group) and to fast for Visit 3 (both groups). Visit 3 took place from 2-4 weeks after Visit 2, or 2 weeks after patients completed the low FODMAP diet, in order to accommodate scanner availability.

Patients from both groups completed their final MRI scan and breath test before receiving dietary advice from SK. Prior to this advice, patients in both groups completed a validated IBS-GIS questionnaire used in IBS patients (Gordon et al., 2003), which facilitates measurement on the global improvement scale following a low FODMAPs diet. There were two questions in this questionnaire, the first addressing bowel symptoms over the past 2 weeks, with seven Likert scales ranging from substantially worse to substantially improved. The second question, for which they had to choose a yes/no response, assessed if over the past 2 weeks patients had had adequate relief from their symptoms. Those with substantial or moderately improved symptoms were categorised as responders; those without, as non-responders.

Patients in the low FODMAP group also completed EQ-5D-5L, EQ-VAS and IBSS questionnaires for the second time to assess improvement in symptoms, post dietary advice. After this, patients in the low FODMAP group were given advice on reintroducing FODMAPs back into their diet using a standard challenge protocol (based on the booklet 'Reintroduction of FODMAPs' developed by

Kings College). Patients in the habitual group were given advice on low FODMAPs diet as described in Visit 2. The advice on low FODMAP diet given to this group was at the end of the study as it was not part of the main study. Dietary advice was given to ensure all patients received low FODMAP advice.

5.5.7.3 Visit 4 - Reintroduction Diet (Group 2)

This was an additional visit only for patients in the habitual diet group. As part of the protocol, they received dietary advice to reintroduce low FODMAP diet during this visit and excluded the MRI scan and breath test. Patients were given the same dietary advice and booklet. They returned their second food diary, which was given to assess compliance, and completed both IBS-SSS and QoL questionnaires.

5.5.7.4 Compliance

The response rate to an elimination diet is usually between 15-71% among IBS patients (Niec et al., 1998); there is no specific method to assess compliance to a low FODMAP diet for IBS patients. A previous Australian study looked at dietary compliance by assessing the proportion of days where patients reported adherence to the recommended diet. (Halmos et al., 2014). Meanwhile, a UK study used questionnaires measuring symptom severity and quality of life during pre and post intervention in order to assess compliance (Staudacher et al., 2011).

In this study, dietary compliance was assessed objectively. Dietary data was analysed and compared between both groups at baseline and follow-up; those with a high dietary FODMAP intake were considered to be non-compliant and

excluded from the final analysis. Additionally, the second food diary also served as a method to assess the consistency of dietary advice delivered by the investigator to both groups. A food diary was deemed valid if the recorded calorie intake was at least 80% of the participant's resting energy expenditure on a minimum of 4 days during the dietary data recording period.

5.5.7.5 Randomisation

Patients were randomised by investigator SK to two blocks, to receive either a low FODMAP diet or habitual diet, by using a software from www.randomization.com. Both investigator and patients were not blinded in this investigation.

5.5.7.6 MRI Protocol

Patients were scanned on a research-dedicated 1.5T GE MRI scanner, using a parallel imaging SENSE 16-element torso coil. A range of MRI sequences were used. Colonic volumes and gas were measured and calculated by SP, an experienced physicist, using the method by Pritchard et al. (Pritchard et al., 2014). Individual regional colon volumes were manually segmented from the coronal data on each image slice using Analyze9^{TM} software (Mayo Foundation, Rochester MN, USA). Each colon region was identified within each coronal image slice, building a 3D representation of the morphology, from which the volume of each region was measured. Information from axial data was used to guide definition of the regions where anatomy was ambiguous.

5.6 Outcome measure

The primary endpoint was the difference between baseline and follow-up in fasting colonic volume changes, as assessed by MRI between IBS patients on a low FODMAP diet, compared with those on a habitual diet. The secondary endpoints were established to assess possible physiological explanations for differences within and between groups in the primary endpoint. These were:

i) Changes from baseline in fasting breath hydrogen concentration (measured in parts per million) in both groups, and the difference in changes between both groups.

ii) Proportion of patients in the low FODMAP group with adequate symptom control, following a low FODMAP diet for 2 weeks

5.6.1 Exclusion from study

Patients who did not adhere to their randomised diet, or commenced on antibiotics over the study period, were in protocol violation and thus excluded from the study.

5.6.2 Planned Comparison

All outcome measures were based on differences between primary endpoints and secondary endpoints. Due to time constraints, analysis for the thesis was carried out before completion of the study.

5.6.3 Energy, nutrient and FODMAP intakes

Food diary data was entered into a nutritional analysis programme, Dietplan P3 (Forestfield Software Limited version 6.70.75) (see Chapter 2.5.3.5 for details). Energy, nutrient and FODMAP intakes were generated by the investigator, SK.

5.7 Statistical Analysis

The statistical analysis was undertaken with statistical support from the School of Medicine using SPSS and GraphPad software, on University of Nottingham computers with server back-up. Where parameters are described as proportions, such as the global improvement score questionnaire which assessed adequate relief of symptoms, they were analysed by Chi-squared test. Participants who reported substantially or moderately improved symptoms, over the two weeks following the low FODMAP diet, were categorised as responders; those who reported slight improvement, no changes or worsening symptoms were categorised as non-responders (Gordon et al., 2003). Numerical parameters were tested for normality using the Shapiro-Wilk method and if appropriate analysed by parametric methods; otherwise by non-parametric methods. No interim analyses were planned.

5.7.1 Sample size and justification

Data from a previous 4-period crossover study of healthy volunteers found a within-individual coefficient of variation for fasting colonic volume of 17% (Giles Major et al., 2016). A sample size of 40, randomised 1:1, would give 80% power to detect a 30% increase in the change in volume between treatment

arms (α = 0.05, 1-tailed). This change is commonly used to assess the minimally important difference in colonic parameters, such as transit with 30% change being seen, for example in recent trials of drugs effective in treating constipation (Simren, 2011). To allow for 10% dropout, recruitment was aimed at 22 patients into each arm, i.e. 44 patients, to gather 40 complete data sets.

5.8 Results

5.8.1 Data analysis

The study was completed in August 2017. Analysis of data for this thesis includes data collected up until 27 participants completed the study. Of these, only 17 patients were included in the analysis of colonic volume as the MRI images of four patients were distorted; the remaining 6 were not scanned owing to patient preference. All 27 patients were included in the follow-up symptom scores and compliance to low FODMAPs diet.

5.8.2 Consort Diagram

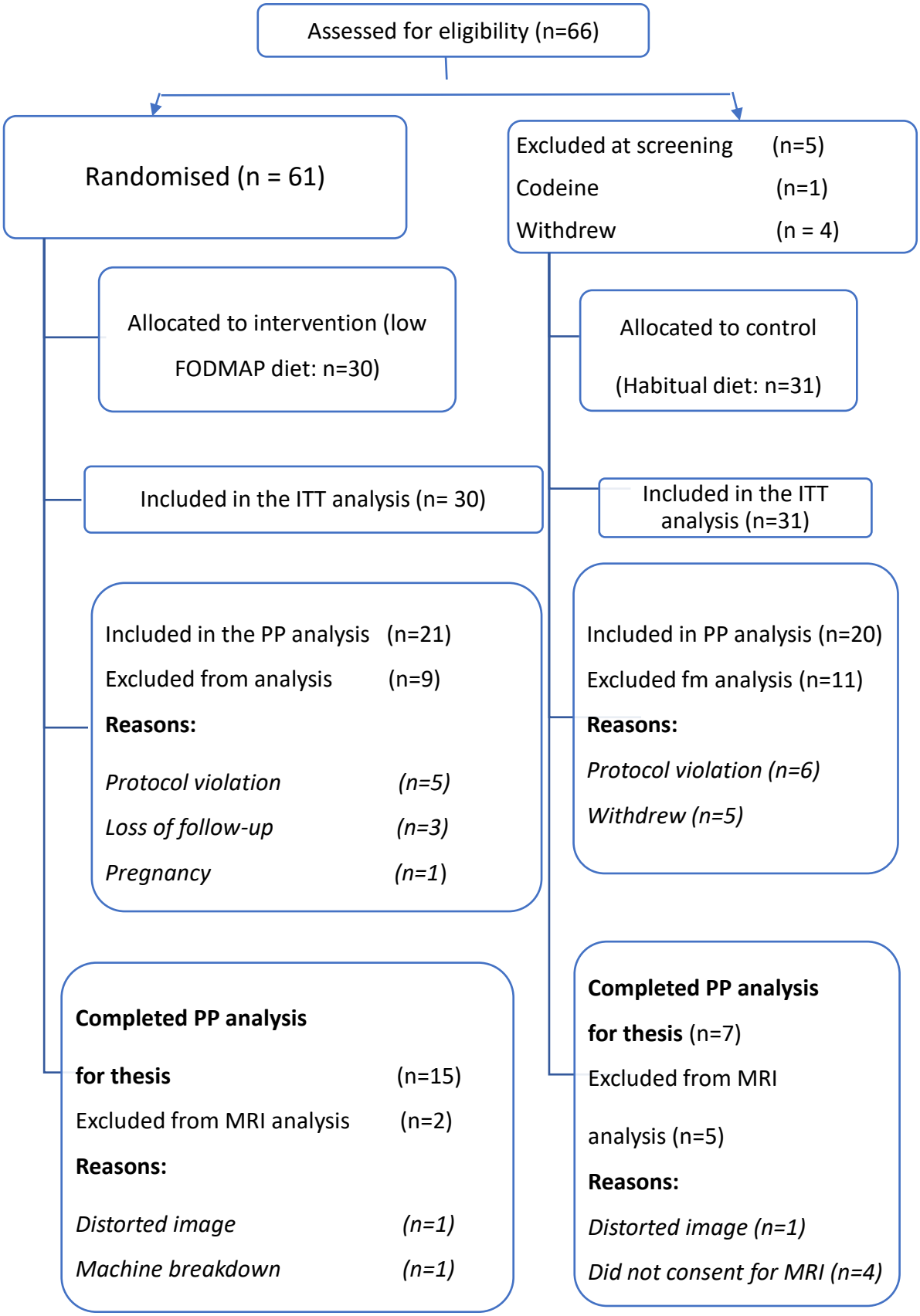
A total of 66 patients were assessed for eligibility as shown in the consort diagram (Figure 5-2). Five of these were not enrolled; one did not meet the inclusion criteria (due to use of codeine based medication); the remaining four were unable to attend the scheduled visits due to work commitments.

After both visits, 20 patients were excluded from the study. Nine of these were from the intervention group. Of these, six were due to protocol violation (3 commenced on antibiotics, 2 did not return food diaries and one restricted their diet prior to intervention); 2 were withdrawn due to loss of follow-up, and one became pregnant. 11 patients were excluded from the control group

during both visits. Six were due to loss of follow-up; the remaining five withdrew from the study, deeming the diet too restrictive. A total of 41 patients were randomised to intervention (n=21) and control (n=20), and included in the intention-to-treat (ITT) population with predefined endpoints (change in colonic volume from baseline, improvement in symptom scores and response to global symptom question).

Only 32 patients will be included as per protocol analysis for colonic volume, as five of them were not scanned owing to patient preference; the remaining four returned distorted images due to excessive movement. However, all 41 patients were included in the analysis of follow-up symptoms, breath hydrogen and nutrient analysis.

Figure 5-2: Consort Diagram for the CRAIGS study



5.8.3 Demographics

Table 5-2 describes the demographics and characteristics of the patients that were studied. Patients in both groups were predominantly females, aged between 38 to 43 years with less than 10% of them being current smokers. Patients were not matched for age or gender in this study, but overall, both groups appeared to be well-matched with no differences between groups for age, sex, BMI and smoking. The only difference was for vegetarianism, with 2/15 (13%) on a vegetarian diet in the Low FODMAP group.

Using ROME III criteria, patients were classified as IBS-D, IBS-C or IBS-M. More than half of the patients were categorised as IBS-M, with 53% (8/15) in the low FODMAP group, and 75% (9/12) in the habitual diet group. Less than one third of patients were categorised as having IBS-D and IBS-C. However, coincidentally, there were slightly more patients with IBS-D in the low FODMAP group, at 20% (3/15), compared to only 8% (1/12) in the Habitual diet group. This was also similar for IBS-C, with 27% (4/15) of patients from the low FODMAP group compared to 17% (2/12) in the habitual diet group. There were no significant differences between the groups in baseline colonic volume. There were no differences between the groups in median breath hydrogen concentration {low FODMAP group 5ppm (3, 9) versus habitual diet group 5ppm (3,7) p=0.447}.

HADS were divided into anxiety and depression categories. Approximately two thirds of patients in the low FODMAP group had normal anxiety scores, 67% (10/15), the remaining patients returning borderline scores 33% (5/15). In the habitual diet group, however, there were more patients with borderline

anxiety scores 42% (5/12), and one sixth, 17% (2/12), of patients had high scores.

Depression scores were low in both groups, with less than one third of patients having only borderline score. Of these, 7% (1/15) were from the low FODMAP group, and 25% (3/12) were from the habitual diet group.

Table 5-2: Baseline demographics and clinical characteristic of patients from low FODMAP group versus habitual diet group.

Parameters	Low FODMAP diet (n=15)	Habitual diet (n=12)
Age, mean (SD)	38 (15)	43 (19)
Females n (%)	12 (80)	11 (92)
BMI, mean (sd)	25.2 (4.6)	24.4 (4.2)
Ethnicity n (%)		
White British/European	12 (80)	11 (83)
Black British/Black	2 (13)	1 (8)
South Asian	-	1 (8)
Chinese	1 (7)	-
Vegetarian n (%)	2 (13)	-
Current smoker n (%)	1 (7)	1 (8)
IBS subtypes (n%)		
IBS-Diarrhea	20 (3)	8 (1)
IBS-Constipation	27 (4)	17 (2)
IBS-Mixed	53 (8)	75 (9)
Baseline Colonic Volume (95%CI)	592.4 (441,740)	478.2 (295,662)
Breath Hydrogen (ppm) median (range)	5 (3,9)	5 (3,7)
Hospital Anxiety and Depression score (HADS)		
Anxiety n (%)		
Normal	10 (67)	5 (42)
Borderline	5 (33)	5 (42)
Case		2 (17)
Depression n (%)		
Normal	14 (93)	9 (75)
Borderline	1 (7)	3 (25)

5.8.4 Compliance

Compliance to dietary advice has been explained in section 5.5.7.4. Analysis of dietary intake (Table 5-3) showed that both groups in the ITT group adhered to the low FODMAP diet.

5.8.5 Adverse event

The total number of reported adverse events was very small. Only two patients reported worsened IBS symptoms throughout the duration of the RCT, both from the Low FODMAP group.

5.8.6 Primary outcome measure

The primary outcome measure was the difference in colonic volume between both patient groups, calculated as percentage change as shown in Table 5.3. Both groups had an increase in colonic volume from baseline as shown in Table 5.3; however, only the Habitual diet group increase was significant [478.2ml (147.6) to 589.6 (193.4), p = 0.021]. When the segmented colon volumes were compared, the habitual diet group had a higher increase in volumes from baseline. There was a 50% increase in descending colon volume, as well as a 27% increase in transverse colon, and a modest 8 % increase in the descending colon. As shown in Table 5.6, changes in segmented colon volumes were compared between both groups. All segmented colon had an increase in volume in the habitual diet group compared to the low FODMAP group which had a reduction in both ascending colon volume and transverse colon volume. There was an increase in transverse colon volume for the Habitual diet group;

41.2 ml (40.2) whereas there was a reduction of 30.8ml (142.5) in the low FODMAP group. However, these changes between groups were not significant.

In comparison, the low FODMAP group had a 7.3% decrease in transverse volume from baseline, and a 13.2% increase in descending colon from baseline.

The percentage change in colonic volume from baseline was not significant between groups [low FODMAP: 12% (34ml) (95%CI (73, 140) versus habitual diet group: 23% (114ml) 95%CI (28, 194), p=0.195).

When changes within groups from baseline were compared, both groups had a reduction in low FODMAP intake as expected. The low FODMAP group had a reduction of 9.3g (7.7) compared to the Habitual diet group with a reduction of 13.3g (5.5) but these differences were not significant.

5.8.7 Secondary outcome measure

5.8.7.1 Changes in breath hydrogen concentration

Both groups had low basal breath hydrogen concentration, less than 10ppm as shown in Table 5.3. At follow-up, there was only a slight decrease in median breath hydrogen concentration in the low FODMAP group [5ppm (3, 9) to 4ppm (3, 7), p=0.360)], with a slight increase in the habitual diet group [5ppm (3, 7) to 6ppm (4, 9), p=0.556]. However, these differences were not significant. The differences between both groups at follow-up were also not significant [4ppm (3, 7) versus 6ppm (4, 9), p=0.110] as were as the mean changes from baseline with low FODMAP group [-1 (-5, 1) p=0.360) versus habitual diet group [-0.5(-2.8, 1) p=0.556].

5.8.7.2 Proportion of patients with improved symptoms (IBS-GIS score)

As shown in Figure 5-3, there were more responders in the low FODMAP group (12/15) compared to the habitual diet group (2/12); the differences were significantly different, calculated using Fisher's exact test (p=0.0018). A large proportion of responders in the low FODMAP group reported slight improvement in symptoms (7/15), followed by 4/15 who reported moderate improvement with only 1/15 reporting a substantial improvement in symptoms. Meanwhile, in the habitual diet group, both responders (2/12) reported only a slight change in symptoms. However, among the non-responders, one sixth of patients (2/12) had no changes in their symptoms, while only 1/12 reported substantially worse symptoms. One third (4/12), and one quarter of patients (3/12), had moderately worse or slightly worse symptoms respectively.

In terms of adequate symptom relief, around 73% (11/15) of patients from the low FODMAP group reported adequate symptom relief. However, only 17% of patients in the habitual diet group reported this, with the difference between both groups being significant (p=0.0063).

Table 5-3: Colonic Volume, Segmented Colonic Volume and Breath Hydrogen concentration between low FODMAP group and Habitual diet group

	Low FODMAP Diet (n=15)			Habitual Diet (n=12)			Low FODMAP diet versus Habitual diet at follow up
	Baseline	Follow-up (Visit 3)	p*	Baseline	Follow-up (Visit 4)	p*	p**
Colonic Volume Mean (SD)	592.4(244.8)	626.2(227.8)	0.502	478.2(147.6)	589.6(193.4)	0.021	0.342
Ascending Colonic Volume	204.4(63.8)	205.9(77.3)	0.933	203(96.5)	220(71.3)	0.481	0.592
Transverse Colonic Volume	249.8(154.9)	231.6(133.7)	0.612	174.6(63.7)	222(69.2)	0.063	0.186
Descending Colonic Volume	138.2(65.1)	150.4(70.4)	0.464	100.6(35.5)	147(127)	0.333	0.225
Delta Colonic Volume	33.8(176.2)			111.4(67.1)			0.195
Breath Hydrogen Median (IQR)	5(3,9)	4(3,7)	0.360	5(3,7)	6(4,9)	0.556	0.748

The low FODMAP group returned their second food diary at visit 3. The habitual diet group returned their food diary at visit 4.

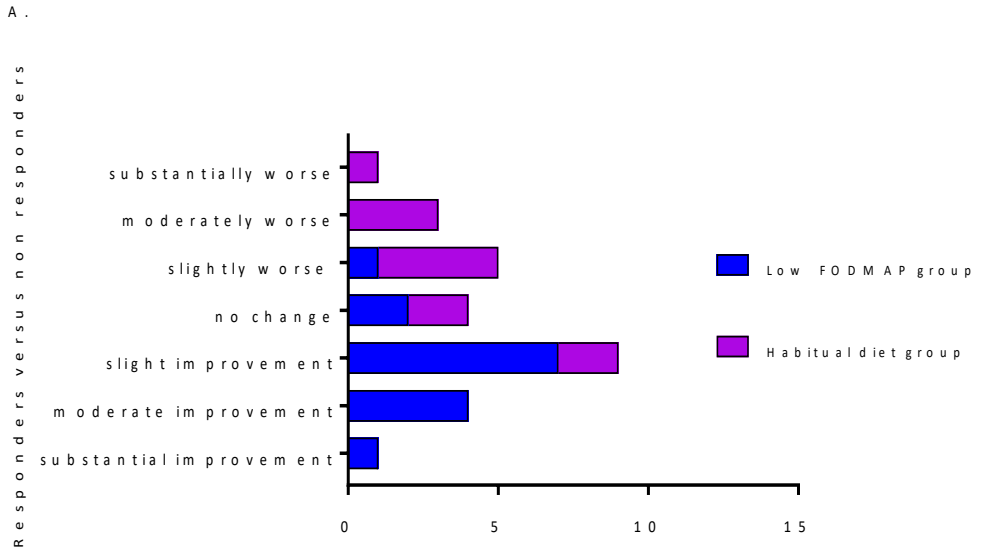
Table 5-2 Comparison of changes between low FODMAP diet group and Habitual diet group

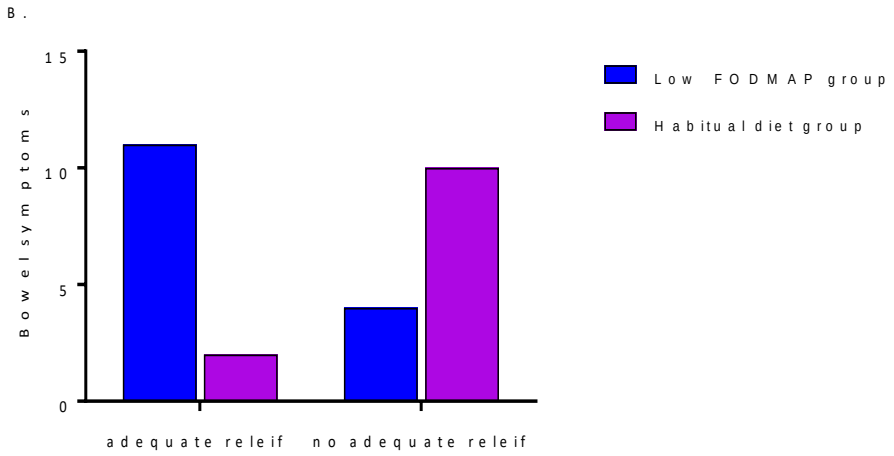
	Low FODMAP Diet (n=15)	Habitual Diet (n=12)	
	Delta LFD	Delta Habitual diet	p*
Delta Colonic Volume Mean (SD)	33.8	111.4	0.136
Delta Ascending Colonic Volume	-5.1 (57)	34.7 (62)	0.193
Delta Transverse Colonic Volume	-30.8 (142.5)	41.2 (40.2)	0.246
Delta Descending Colonic Volume	3.7 (55.1)	43.3 (84.8)	0.250
Delta Breath Hydrogen Median (IQR)	-1 (-5,1)	-0.5 (-2.8,1)	0.516
Delta FODMAPs	-9.3 (7.7)	-13.308 (5.5)	0.123

Figure 5-3: Response to IBS-GIS questionnaire

A: Responders versus non-responders to symptoms for both groups

B: Adequate relief of symptoms in both groups





5.8.8 Energy and Nutrient intakes

Only the habitual diet group had a significant reduction in energy and fat intake from [1754kcal (447) to 1467kcal (458.4), p=0.003], and 70g (20) to 55.2g (18.3) with p=0.015 respectively, as shown in Table 5-4. However, carbohydrate, starch, sugar and sodium were significantly reduced in both groups. Carbohydrate in the low FODMAP group was reduced from 220.6g (51.4) to 180.5g (32.7) with p=0.010. Meanwhile, in the habitual diet group carbohydrate was reduced from 209g (54) to 158g (42.3) with p=0.001. Both starch and sugar in the low FODMAP group were decreased from 125.1g (30) to 106.7g (25.5), p=0.010 and 87.7g (31.3) to 62.4g (22.5), p=0.011 respectively. In the habitual diet these were reduced from 123 (44.1) to 95g (24); p=0.014 and 73.9g (19.8) to 55.1g (25.7), p=0.003 respectively. There were no significant reductions in other micronutrient intakes.

5.8.9 FODMAP intake

As shown in Table 5-5, in both groups from baseline there were significant reductions in total FODMAP intake. The low FODMAP group had a reduction from 12.2g (7.7) to 2.9g (1.9), p=0.001, whereas the habitual diet group had a decrease from 16.5g (6.1) to 3.2g (1.9), p=0.001. Both groups were compared after they had completed their low FODMAP diet. However, a significant reduction in individual FODMAP intake of both groups was only observed for fructan, free fructose, mannitol and lactose. As shown in Table 5.6 changes between the low FODMAP group and Habitual diet group from baseline was not significant with -9.3(7.7) versus -13.3 (5.5),p=0.123.

5.8.10 Nutritional adequacy during low FODMAP diet.

Nutrient intakes of females (Table 5-6) and males (Table5-7) in both groups were compared to dietary reference values (DRVs)(Public Health England, 2016b), and national population intakes in age and gender-matched individuals in the UK (NDNS)(Public Health England and Food Standards Agency, 2014), in order to assess nutritional adequacy during the intervention phase when FODMAPs were restricted.

Female patients in low FODMAP and habitual diet groups had higher intakes of energy, protein, fat and carbohydrate compared to population intakes as shown in Table 5.6. However, energy intake in both groups {low FODMAP diet: 1726 (232) versus habitual diet: 1796(276.4)}, were much lower than estimated average intake, which was between 2079kcal – 2175kcal. However, in both groups there was little difference in the energy percentage contributed by carbohydrate intake, compared to population intake and dietary reference value. Calcium, iron and folate intake were also higher than DRVs among females.

Meanwhile, male patients in the low FODMAP group had lower intakes of energy protein and fat, compared to population intake and DRVs as shown in Table 5.7. In comparison, the reverse was observed in patient in the habitual diet group (n=1), who had a higher energy and macronutrient intake. Micronutrient intake among males in both groups was still higher than population intake and DRVs, despite differences compared to population intake.

When patients baseline intake was compared with the DRV, (n=9) female patients did not meet their EAR requirement in both the low FODMAP group and Habitual diet group, whereas (n=1) male did not meet the EAR in the low FODMAP group. Two female patients did not meet the protein requirement from the low FODMAP group. All patients met the DRV for fat in both groups. However, there were (n=7) female patients that did not meet the DRV for carbohydrate in both groups.

During the intervention, (n=10) female patients did not meet their EAR in the low FODMAP group and all female patients in the habitual diet group did not meet theirs either (n=11). Only one female patient in the low FODMAP group that did not meet her protein RNI. Meanwhile (n=3) female patients and (n=2) male patients from the low FODMAP group did not meet their DRV for fat. In the habitual diet group (n=2) female and one male did not meet the requirements. About (n=8) female and (n=2) male patients from the low FODMAP group did not meet the DRV for carbohydrate. This was similar in the habitual diet group with (n=9) female and (n=1) male patients not meeting their DRV as well.

Table 5-4: Mean daily Energy and Nutrient intakes at baseline and follow-up in Low FODMAP and Habitual diet group

	Low FODMAP diet (n=15)			Habitual diet (n=12)		
	Baseline	Follow-up	P*	Baseline	Follow-up	p*
Energy (kcal/d)	1847.5(310)	1623.7(370.5)	0.053	1756.4(446.7)	1467(458.4)	0.003
Protein (g/d)	71.4(15.7)	74.2(20.9)	0.587	75.3(22.4)	68.4(24.8)	0.123
Fat (g/d)	74(14.8)	66.6(24.9)	0.252	70(20)	55.2(18.3)	0.015
Carbohydrate (g/d)	220.6(51.4)	180.5(32.7)	0.010	209(54)	158(42.3)	0.001
Starch (g/d)	125.1(30)	106.7(25.5)	0.010	123(44.1)	95(24)	0.014
Sugar (g/d)	87.7(31.3)	62.4(22.5)	0.011	73.9(19.8)	55.1(25.7)	0.003
Fructose	13.2(7.3)	9.6(2.6)	0.057	14.8(8.5)	8.7(4.5)	0.034
Fibre (g/d)	17.5(4.9)	17.4(4.2)	0.965	19.8(8.2)	14.5(3.2)	0.026
Calcium (mg/d)	771.4(232)	735.9(296.4)	0.665	859.2(283)	627.9(292.5)	0.170
Folate (µg/d)	255.3(85)	245(74.3)	0.587	253.6(86.5)	238.2(47.9)	0.223
Iron (mg/d)	14.5(5.1)	12.7(5.2)	0.148	12.8(5.2)	9.6(3.6)	0.070
Magnesium (mg/d)	331(97.1)	301.9(77.7)	0.334	406.6(94.8)	254.8(63.6)	0.102

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Table 5-5 Mean daily FODMAP intake at baseline and follow-up in low FODMAP group and Habitual diet group

	Low FODMAP diet			Habitual Diet		
	Baseline	Follow-up	P	Baseline	Follow-up	P
Total FODMAPs	12.2(7.7)	2.9(1.9)	0.001	16.5(6.1)	3.2(1.9)	0.001
Fructan	2.5(1.3)	0.3(0.3)	0.001	2.6(1.4)	0.4(0.3)	0.001
GOS	0.5(0.3)	0.2(0.3)	0.001	0.6(0.6)	0.2(0.3)	0.056
Free fructose	2.3(2.8)	0.5(0.3)	0.029	1.9(1.2)	0.7(0.5)	0.008
Mannitol	0.2(0.2)	0.01(0.02)	0.001	0.2(0.3)	0.04(0.05)	0.079
Sorbitol	0.4(0.5)	0.05(0.04)	0.033	0.3(0.4)	0.1(0.1)	0.092
Lactose	6.3(4.8)	1.8(1.9)	0.002	11.0(5.9)	1.8(1.8)	0.001

Table 5-6 Energy and nutrient intakes of females and males from habitual diet group and low FODMAP group at baseline compared with NDNS data and DRV

Parameter	**Female**				**Male**			
	Habitual diet (n=11)	Low FODMAP (n=11)	DRV	NDNS	Habitual diet (n=1)	Low FODMAP (n=3)	DRV	NDNS
Energy (kcal) EAR (kcal/d)	1726 (322)	1796 (276.4)	2103-2175	1641 (504)	2847	2053 (416.7)	2581-2772	2100 (602)
Protein-RNI (g/day)	72 (12.3)	70 (11.8)	45	66.6 (20.8)	133.8	78.6 (29.1)	55.5	87.4 (29.5)
Fat (g) % total energy intake	66 (16.2) 34	74.2 (15.8) 37	33	62.4 (25.1) 34	110.4 35	73.4 (12.7) 32.1	34	76.6 (28.5) 33
Carbohydrate (g) (%) total energy intake	208 (46) 45.8	211.4 (22.6) 44.7	280-290 47	199 (66) 46	312.4 41.7	258 (65) 47.8	45	249 (78) 45
Fibre Individual minimum	18 (6.7)	18 (4.5)	30	20.7 (7.1)	36.7	15.4 (7)	30	17 (6.2)
Calcium-RNI (mg/day)	811.6 (256)	753.4 (169)	700	746 (288)	930	843 (456)	700	897 (381)
Folate -RNI (µg/d)	222 (102)	264 (76)	200	253 (209)	226.1	218.5 (128)	200	300 (327)
Iron -RNI (mg/day)	12.4 (5.3)	14.4 (4.3)	8.7-14.8	9.3 (3.2)	18.1	14.9 (8.9)	8.7	11.6 (4.3)
Magnesium-RNI (mg/day)	430 (108)	341 (91.5)	270	238 (79)	294	294 (132)	300	302 (109)

EAR: Estimated Average Requirement

DRV: Dietary Reference Value,

NDNS: National Diet and Nutrition Survey (combined 2008/9 ,2011/12, 2013/14); Energy and nutrient intake for males at baseline and follow-up compared with the UK population intakes and dietary reference values.

*Energy, macronutrient intakes taken from 19-64 year cohort

Table 5-7 Energy and nutrient intakes of females and males from habitual diet group and low FODMAP group at intervention compared with NDNS data and DRV EAR: Estimated Average Requirement

Parameter	**Female**				**Male**			
	Habitual diet (n=11)	Low FODMAP (n=11)	DRV	NDNS	Habitual diet (n=1)	Low FODMAP (n=4)	DRV	NDNS
Energy (kcal) EAR (kcal/d)	1359 (279)	1654 (408)	2103-2175	1641 (504)	2652	1503(126)	2581-2772	2100 (602)
Protein-RNI (g/day)	6 2(13)	73.1 (22.3)	45	66.6 (20.8)	137	79 (17)	55.5	87.4 (29.5)
Fat (g) % total energy intake	52 (15) 34.4	70.1 (26) 38.1	33	62.4 (25.1) 34	92.8 50.9	51 (12.6) 30.5	34	76.6 (28.5) 33
Carbohydrate (g) (%) total energy intake	154 (41.5) 43	184 (36) 42.3	280-290 47	199 (66) 46	206 30.6	166.3 (6.9) 42	45	249 (78) 45
Fibre Individual minimum	14.3 (3.3)	18 (4.3)	30	20.7 (7.1)	16.6	15 (2.7)	30	17 (6.2)
Calcium-RNI (mg/day)	611 (300)	795 (299)	700	746 (288)	817	500 (138)	700	897 (381)
Folate -RNI (µg/d)	236 (54.6)	265 (86)	200	253 (209)	226	228 (58)	200	300 (327)
Iron -RNI (mg/day)	9.5 (3.8)	13.2 (5.8)	8.7-14.8	9.3 (3.2)	9.8	10.6 (0.4)	8.7	11.6 (4.3)
Magnesium-RNI (mg/day)	243 (53)	315 (82)	270	238 (79)	380	248 (16.4)	300	302 (109)

EAR: Estimated Average Requirement, DRV: Dietary Reference Value,

NDNS: National Diet and Nutrition Survey (combined 2008/9 ,2011/12, 2013/14); Energy and nutrient intake for males at baseline and follow-up compared with the UK population intakes and dietary reference values.

*Energy, macronutrient intakes taken from 19-64 year cohort

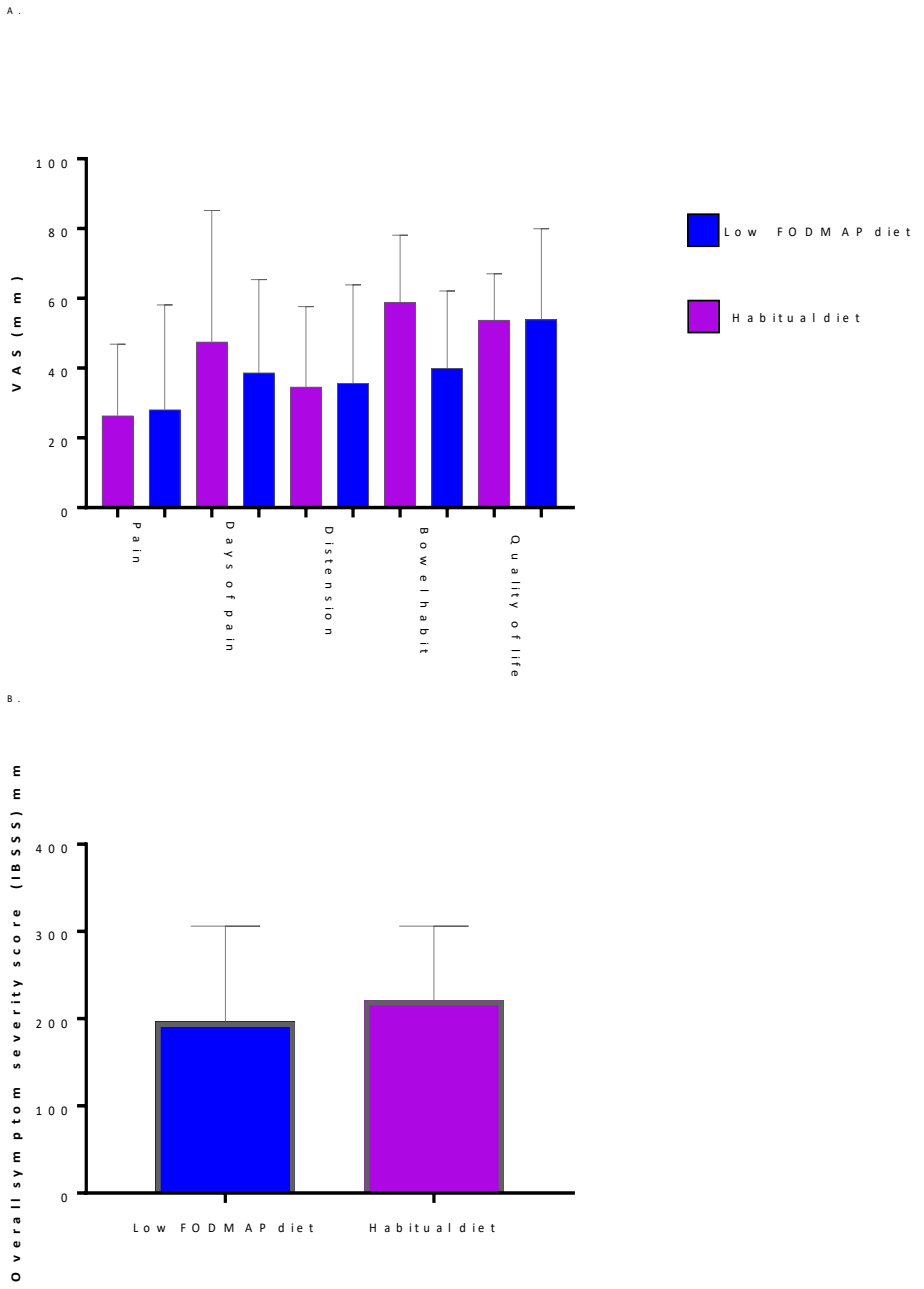
5.8.11 IBS-SSS

The IBS-SSS questionnaire was given to both groups before the start of their diet, and at follow-up after both groups completed their diet. As shown in Figure 5.4, at baseline both groups were categorised as having moderate symptoms (IBS-SSS score between 175-300). As show in Figure 5-4, patients in the low FODMAP group had a significant reduction in symptoms, from 289 (87.2) to 196 (110) with p=0.0017. Although patients in the habitual diet group also experienced a reduction in symptoms, from 221 (85.1) to 177.3 (66.3), with p=0.0561, the difference was not significant.

Figure 5-4: Comparison of IBS-SSS scores between low FODMAP group and habitual diet group.

A: IBS-SSS score for Low FODMAP versus Habitual diet group

B: Overall IBS-SSS score for Low FODMAP versus Habitual diet group



5.8.12 Discussion

The study was aimed to test the hypothesis that there will be a reduction in colonic volume in the low FODMAP group and the reverse for those in the habitual diet group. Colonic volume increased in both groups. However, the increase in volume (calculated as percentage change) in the low FODMAP group was small, compared to the significant increase in the habitual diet group. The results were similar to a study where healthy volunteers who were fed a high residue diet had an significant increase in total colonic volume, compared to those on a low residue diet (Mego et al, 2017). Although the expected outcome was not observed, this study confirms our group's previous findings, in that the habitual diet with high FODMAP content provided fermentable substrates for the colonic microbiota to proliferate, thus increasing total colonic volume. The reverse did not occur in the low FODMAP group, which may be due to type II error or restriction of FODMAPs prior to intervention.

Overall results of the primary outcome coincide with the previous MRI study, confirming variability in segmented colon volumes (Lam et al., 2016., Pritchard et al., 2014). Interestingly, there was an increase from baseline in transverse (27%) and descending volumes (46%) in the habitual diet group, compared to a 7% decrease in transverse volume in the low FODMAP group, but this could be due to individual variation. Feeding studies have shown that the transverse colon plays a role in accommodating material from ascending and descending colons, with rapid volume changes in these colons possibly associated with transit (Lam et al., 2016, Moreno-Osset et al., 1989) and a change in diet.

In order to detect a minimal important difference in total colon volume, it needs to be higher than the baseline volume. Pritchard reported that the fasting volume in healthy adults were around 200mls for each segmented colon. Volume. A 10-20% increase in the ascending colon volume from baseline after a feeding was found to be significant .(Pritchard et al., 2014). This was similar in another study which included rectosigmoid colon volume, a 20% change from baseline was deemed significant in healthy volunteers. (Nilsson et al., 2015).

There is also a possibility of order effect, whereby patients who were initially claustrophobic were less anxious during their second scan. This was observed during the study when some slightly anxious patients were reassured by the scanner operator during their initial scan and had to repeat their breath hold. During their second visits, these patients appeared more relaxed. It is therefore possible that these anxious patients may have emptied their bowels prior to the scan (Libby and Farthing, 1996).

A recent MRI study demonstrated that almost 30% of colon volumes were emptied via defecation, in 10 healthy participants who were fed high residue diet versus low residue diet for 3 days in this crossover study (Mego et al., 2017). This suggests that patients in both groups may have emptied their bowels 24 hours prior to their scan. Perhaps a stool and a stool and symptom diary kept 24 hours prior to baseline and follow-up scans would have been useful, as transit time was not an outcome measured in this study.

Surprisingly, no significant changes in breath hydrogen from baseline were noted in the low FODMAP group, despite a reduction in fermentable substrates. The basal breath hydrogen volume for both groups was less than 10ppm, which may have been due to patients adhering to the MRI guidelines. This was part of the patient information booklet on MRI, advising patients to reduce intake of alcohol and food items the day prior to study day as this may affect the way their bowel behaves. Although the food items were not specified, patients particularly in the habitual diet group may have inadvertently reduced their FODMAP intake during both scans, resulting in low breath hydrogen concentration. Another possibility is that a high concentration of methanogenic bacteria may have reduced breath hydrogen levels, as methanogens consume bacteria. However, methane levels were not tested in this study. Perhaps multiple measures of breath hydrogen may have provided a better understanding of fermentation in both groups. Studies using consecutive measures of breath hydrogen had a higher sensitivity compared to those using >20ppm increase as cutoff to diagnose malabsorption as the latter lacked such sensitivity.

Studies using an increase of >20ppm from baseline lacked in sensitivity, whereby increases of 10ppm on two consecutive measures has a higher sensitivity (Rezaie et al., 2017).

The reduced sample size in both groups may have attributed to loss of power, leading to type II error. Due to the ongoing MRI analysis, 27 patients with complete per protocol analysis were included in this thesis. Of those, only 18

had complete data on colonic volume. 13 of these were from the Low FODMAP and the remaining five were from the habitual diet group, giving a case to control ratio of 2.6:1. This resulted in small sample size and unequal comparison between groups, potentially reducing the power of the study. Clearly this was unplanned, being the result of a high dropout rate (Dumville et al., 2006).

The overall results are inconclusive, possibly due to both groups altering their FODMAP intake prior to their dietary intervention with a view to symptoms management. In addition to a rigorous screening process, patients who had been following a low FODMAP diet or had altered their overall FODMAP intake were excluded from the study. However, most patients in this study had a strong perception that certain type of foods contributed to their symptoms. This coincides with most findings regarding food avoidance behavior in IBS patients (Hayes et al., 2014), (Böhn et al., 2013),(Monsbakken, et al., 2006). 93% (14/15) of patients in the low FODMAP group avoided at least 1 high FODMAP-containing food or beverages, compared to only 42% (5/12) from the Habitual diet group as shown. This was similar to an Irish study, where 135 IBS patients were compared to 111 healthy volunteers with almost 90% of patients avoiding particular foods/beverages, perceiving them as symptom triggers, compared to 50% of healthy volunteers (Hayes et al., 2014).

The most common foods and beverages avoided by patients in the CRAIGS study were wheat and wheat products, onion, garlic, mushrooms, apples, pears, beans and pulses, chickpeas, milk and milk products, apple juice, orange juice and fizzy drinks (Table 5-8). This was consistent with the type of foods avoided in other studies (Böhn et al., 2013, Monsbakken, et al., 2006).

Although patients in the low FODMAP group avoided certain foods, there was no evidence that they were consistent in this behavior. Furthermore, they were neither on a restricted diet nor received dietitian advice on low FODMAP diets, as shown in Table 5-9. More than 80% of patients altered their diet based upon

their own experiences, as shown in Table 5-10. One third of patients also sought information through the internet, with more than 60% of patients having higher education levels (see Table 5-11). These results were similar to the Bohn study, where due to personal experience, 92% of patients restricted their intake. However, patients in this study were not questioned as to their information sources, whether internet or social media etc., as undertaken in the CRAIGS study, (Böhn et al., 2013).

Despite their food avoidance behavior, patients in both groups had a high baseline FODMAP intake (Habitual diet: 16.6g vs Low FODMAP group: 12.2g). Although there was not much difference between groups, the low FODMAP group had lower intake when compared to the 18g baseline intake of IBS patients in a UK study (Staudacher et al., 2017).

Subjective assessment of patients in both ITT groups showed that 80% of patients on the low FODMAP diet reported improvement of symptoms. Meanwhile, 73% of them had adequate bowel relief. Only 17% of those on habitual diets reported improvement in symptoms and as having adequate bowel relief. Therefore, FODMAPs restriction improved symptoms and provided adequate relief among patients in the low FODMAP group.

Although patients in both groups restricted their intakes, they were not at risk of macronutrient or micronutrient deficiency. When energy-adjusted macronutrient and micronutrient intakes were compared to NDNS data, there was no difference in the groups between males and females, although in males

from the low FODMAP group (n=4), energy, protein, fat and calcium intakes were observed to be higher than NDNS data.

Although this study could not directly show evidence that a reduced FODMAP intake alters colonic environment by reducing colonic volume, it was able to demonstrate that the follow-up colon volume was significantly higher than baseline in the habitual diet group. Despite the small increase in colon volume in the follow-up group, the low FODMAP diet appeared to reduce the increase in colonic volume when compared with a habitual diet.

This is the first study to assess the effect of dietary low FODMAP advice on colonic volume using MRI. However, there are some limitations that must be acknowledged. There was a significant dropout rate in this study due to a variety of factors. Most patients who were working were not able to attend multiple visits and complete two food diaries. Others felt the diet was too restrictive and were not able to make dietary changes. A rigorous two-stage screening prior to randomisation may have prevented or at least mitigated this possibility. The MRI should have been made compulsory for all patients, as this was used to assess the primary outcome; additionally, a third MRI scan for patients who served as controls would have been able to assess if multiple scans affect colonic volume.

Patients who reported food avoidance behavior, which impacted on their FODMAPs intake, were not excluded from this study. The dietary trigger questionnaire could have been used as an additional tool to screen patients for eligibility assessment.

The study did not assess changes in stool consistency or faecal microbiota, therefore in both groups the changes in colonic volume could not be justified.

Finally, total FODMAPs intake at baseline and intervention may have been underestimated, due to the methods used to quantify FODMAPs content of food and beverages, as described in the Methods section of Chapter 3.

5.8.13 Conclusion

This randomised controlled trial with stepped wedged design found that a low FODMAP diet reduces colonic volume. It also suggests an order effect not previously recognised for colonic volumes, the cause of which needs further investigation. However, food avoidance behavior, which is common in IBS patients, may mask this effect. Comparison of physiological changes and symptoms response to FODMAPs restriction provided justification that patients had already altered their diet prior to intervention.

Figure 5-5: Comparison of EQ-5D-5L scores between low FODMAP group and Habitual diet group.

A: EQ-5D-5L scores for Low FODMAP vs Habitual diet group

B: Overall percentage health score

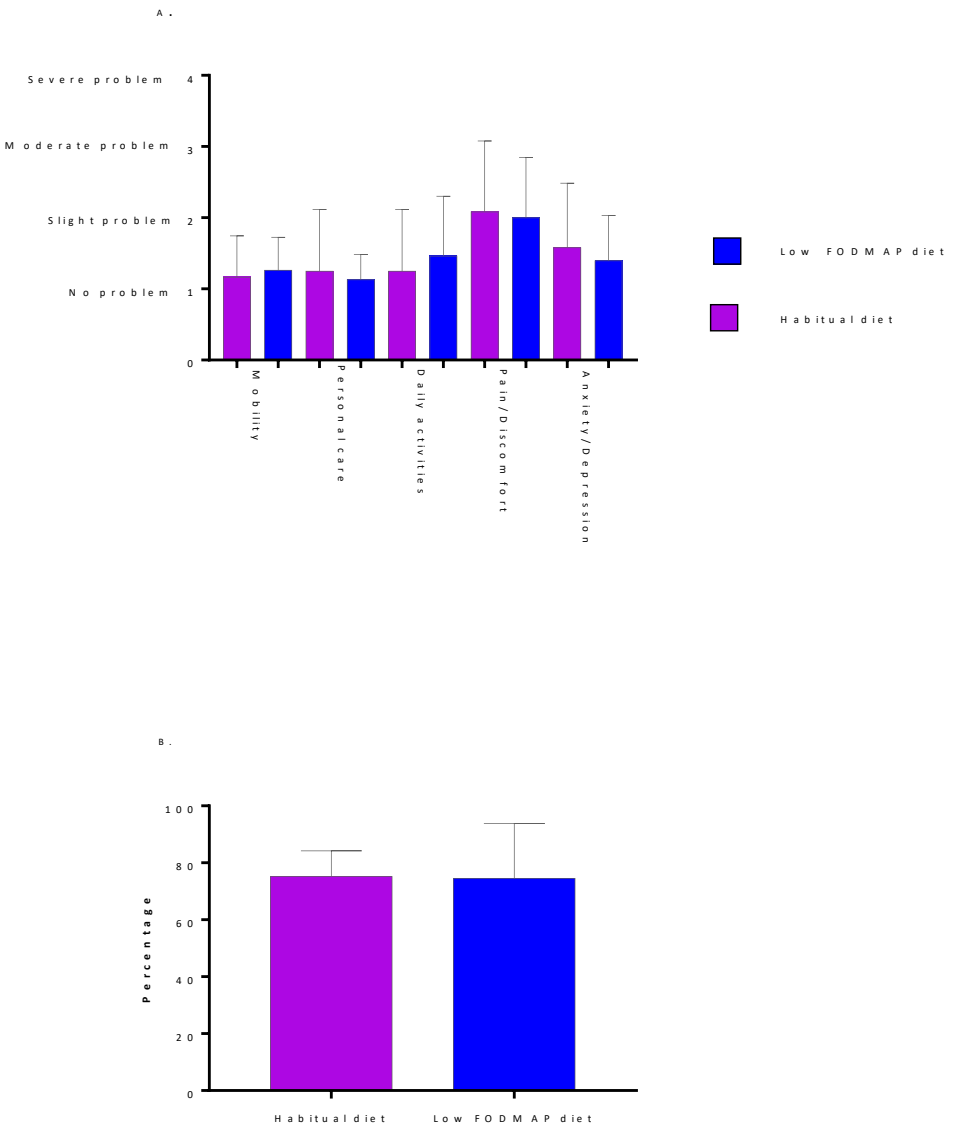


Table 5-8: Self-reported dietary food triggers by patients in low FODMAP group and Habitual diet group

	Low FODMAP	Habitual diet group
Food/Beverages	n (%)	n (%)
Cereal	5 (33)	5 (42)
Vegetables	8 (53)	4 (33)
Fruits	7 (47)	3 (25)
Animal products	4 (27)	3 (25)
Pulses/legumes/nuts/and others	5 (33)	3 (25)
Milk and milk products	7 (47)	4 (33)
Beverages	9 (60)	6 (50)
Others	10 (67)	5 (42)

Table 5-9: Dietary changes reported by patients

	Low FODMAP n (%)	Habitual diet group n (%)
Bowel symptom improved when foods avoided	6 (40)	4 (33)
Bland diet	2 (13)	0
Alternative milk product	7 (47)	3 (25)
Alternative wheat product	3 (20)	0
Elimination diet	2 (13)	0
Skipped meals	2 (13)	1 (8)
Taking supplement to aid digestion	1 (7)	3 (25)
Probiotics	1 (7)	1 (8)
Avoided fatty foods	7 (47)	4 (33)
Increased veg intake	7 (47)	5 (42)
Increased water intake	7 (47)	5 (42)
Increased fibre intake	1 (7)	2 (17)
Avoided eating before bedtime	4 (27)	3 (25)
Eat little and often	4 (27)	2 (17)
Avoided eating out	4 (27)	0

Table 5-10: Factors which influence patients to change their diet prior to recruitment

Source of information	Low FODMAP n (%)	Habitual diet group n (%)
Own experience	13 (87)	8 (67)
Hospital doctor	1 (7)	2 (17)
GP	2 (13)	2 (17)
Nutritionist	1 (7)	1 (8)
Dietitian	1 (7)	1 (8)
Internet	7 (47)	4 (33)
Family/Friends	5 (33)	1 (8)
Books	2 (13)	1 (8)
Social media e.g. Twitter, Facebook	1 (7)	0

Table 5.11: Education levels of patients

Education level	Low FODMAP n (%)	Habitual diet group n (%)
Secondary education	3 (20)	1 (8)
Sixth form	1 (7)	2 (17)
Further education (college)	2 (13)	1 (8)
Higher education (University)	6 (40)	4 (33)
Postgraduate (University)	2 (13)	1 (8)
Doctoral degree	1 (7)	3 (25)

CHAPTER 6 FINAL DISCUSSION

6.1 Summary of Findings

"in the land of the blind a one-eyed man is king"

When Gibson and colleagues first described the low FODMAP diet there was at first incredulity and then a rapid uptake and now perhaps a more balanced realisation that it was a great idea, but actual application is rather complex. Before the diet was introduced, IBS diets had little rationale and were very varied. The FODMAP concept provided a simple rationale which was easy for both patient and dietician to understand which made it seem very attractive to all. However, the details of application prove rather complex (Hill et al., 2017).

The aim of the thesis was, to further understand mechanisms of this underlying changes in luminal volume, symptoms, appetite, on low FODMAP diet exploiting our unique MRI facilities to observe how FODMAPs behaved within the various regions of the GI tract.

Due to the success of the low FODMAP diet in IBS patients, this was the main intervention that was used in the FOG study and CRAIGS study. In the FOG study, FODMAP intake were altered by randomising healthy volunteers to supplements high in FODMAP (oligofructose) versus low in FODMAP (maltodextrin). The low FODMAP diet was administered in both groups mainly to control their dietary FODMAP intake as the aim was to assess the prebiotic effect of oligofructose on luminal volume. Therefore, the intervention group increased their FODMAP intake from oligofructose supplementation around 14g/day for 7 days whilst the control group adhered to their low FODMAP diet together with the same amount of maltodextrin supplement for 7 days.

In the CRAIGS study, the low FODMAP diet was administered to patients with the intervention group following a low FODMAP diet versus the control group following a habitual diet (high FODMAP). In addition, breath hydrogen was assessed in both to measure the effect of fermentation when FODMAP intake was altered. Both studies were carried out to determine if a diet high in FODMAP increased colon volume.

As the low FODMAP diet is quite restrictive it is imperative to also understand how different physical form of FODMAP in the form of solid, puree or liquid behaves in the gut. The Apple study was undertaken to answer this question.

The results of each chapter have already been discussed therefore the focus of this chapter will be on the strengths and limitations of the work carried out and how it compares with other research in similar field. Most importantly, how future work could be improved will also be addressed.

There were many challenges including how best to assess diet and how to assess the impact on the gut and particularly the colon as described below.

6.1.1 Impact of processing and cooking on FODMAP content

Based on the conclusions of Chapter 2, due to the limited data of FODMAP content in cooked foods, there is tendency to underestimate or overestimate FODMAP intake.

FODMAP content particularly in processed foods may differ between countries due to the ingredients and food processing techniques (Varney et al., 2017). Currently in the UK FODMAP content is calculated using food composition tables whereas in Australia it is mostly measured by chemical analysis.

However, both methods only estimate FODMAP content in raw food whereas in reality, FODMAPs is consumed as part of a meal in the form of cooked vegetables, grains and sauces in high temperature. Interestingly some studies has shown that inulin could be degraded by 7-10% when it is cooked in 100^0 Celsius (Böhm et al., 2004), (Pazola and Academy, 1978). Most recently, Tuck et al., demonstrated that pickling, canning, simmering/cooking reduced FODMAP content particularly GOS and fructan. Picking substantially reduced FODMAP content by 97% in garlic, 95% in beetroot, 89% in onion therefore these items classified as low FODMAP. Meanwhile the process of simmering/cooking reduced oligosaccharides content in lentils by 43% and 32% in red kidney beans. However, the beans needed longer cooking time around 30 minutes compared to only five minutes of simmering for lentils. (Tuck et al., 2018). Perhaps future work could be focused on measuring FODMAP content in onion and garlic when cooked in different temperature as it is the main fermentable carbohydrates consumed in large quantity in the UK (Dunn et al., 2011) as well as vegetarians and South Asians. (Hewawasam et al., 2017).

6.1.2 Increased intake of resistant starch and non starch polysaccharides

In the FOG study, there was an unexpected increase in colonic volume in the low FODMAP group. Participants were following a strict low FOMDAP diet and were randomised to receive digestible maltodextrin. Therefore, this did not have an effect on the increase in colonic volume during follow-up.

As part of the low FODMAP diet, participants consumed large amounts of gluten free products as it was an alternative to wheat products and this was

reported in their food diary. Inadvertently this may have increased their intake of resistant starch (RS) and non starch polysaccharides (NSP). RS is present in gluten free bread in the form of maize starch, potato starch and tapioca starch (Horstmann et al., 2017) whereas NSP added as stabilisers are present as ispaghula, xanthan gum, and guar gum.

If a piece of biscuit made from potato starch roughly contains about 10g of RS (Elia and Cummings, 2007), the RS from gluten free bread would have provided larger amounts of RS. In addition, intake of fruits and vegetables, oats and corn which was allowed in the low FODMAP diet would have also contributed to a high NSP intake.

6.1.3 Effect of resistant starch and non starch polysaccharides on luminal volume

A randomised crossover experimental study showed that 14-15g/day intake of psyllium, guar gum or xanthan gum had an effect on faecal output. (Tomlin and Read, 1988). A review by Elia et al., has shown that each gram of NSP fruit and vegetables, oats and corn produce around 3g-6g of faecal output per day. Meanwhile guar gam and isaphagula produces about 3-4g of faecal output/day (Elia and Cummings, 2007)

Both NSP and RS are fermented in the colon thus increasing bacterial proliferation (Cummings and Stephen, 2007),(Cummings et al., 1987)It has been shown that isphagula increases stool mass and lowers transit time. (Tomlin and Read, 1988). Therefore, in the FOG study a high intake of RS and NSP may have increased faecal output and reduced transit time in the

oligofructose group causing a reduction in colon volume. Alternatively, this may have also increased the colon volume in the maltodextrin group.

Due to the reduced fibre intake among patients in the CRAIGS study both RS and NSP may not have had a big effect. The same goes for the apple study as both whole apples and puree only contributed around 8g of total fibre whereas apple juice provided less than 2g of fibre.

6.1.4 Unexpected changes in colon volumes

There was an unexpected relationship between the low FODMAP diet and colonic volume. The principal findings of this study showed an increase in colonic volume, a decrease in breath hydrogen and decrease in breath hydrogen were unexpected requires some thought.

Due to the variation in volumes between participants, measuring colonic volume using MRI has always been challenging. Also, it may be that colonic volume may not be such a helpful measure because it is altered substantially by defaecation (Mego et al., 2017) which is an episodic and not entirely predictable event particularly on a study day. Furthermore, since subjects tend to defaecate when the colon distends this may limit its variability in response to interventions making it less sensitive.

It should be brought to attention that during the study day, participants were required to provide stool samples during Visit 2 and Visit 4 which was one of the primary endpoint for another colleague's study (TS) as well as urine sample on Visit 3 and Visit 5 to measure metabolomes. However, some participants who did not produce their samples on Visit 4 provided their sample on Visit 5

when they had their follow-up MRI. Therefore, some of them may have emptied their bowels before coming to the MRI centre which could explain the variability within participants as this was not controlled during the study.

It has also been reported that patients undergoing MRI are anxious due to the fear of unknown (MRI machine). In one study, around 37% of patients with no functional gut disorder reported moderate to severe anxiety due to claustrophobia and also what the MRI test would reveal (Katz, et al., 1994). A systematic review reported almost 1 out of 100 patients refuse MRI scans or had their scans terminated due to claustrophobia (Munn et al., 2014). In the CRAIGS study during the baseline scan, a few of the patients were claustrophobic and had their scans interrupted. However, they were able to continue with the scan once the radiographer reassured them (AC).

Therefore, it was apparent that the patients in the CRAIGS study were anxious which may have also influenced their baseline colonic volume. However, it is possible that patients were less anxious on the second visit which might lead to greater volumes. A few patients who anxious during their first visit confirmed that they felt much better during their second scan.

This uncontrolled variable may explain why colonic volume varies widely between individuals and between visits and will make it difficult to show consistent effect of interventions unless they are very large as for example seem with large doses of ispaghula (study under revision to be published). Measures of colonic composition like T1 may be more reliable as they are less influenced by defaecation patterns.

The other consideration is the fact that metabolites from dietary residue may alter colonic motility and changes in transit will alter colonic volumes with faster transit reducing and slower transit increasing colonic volume as shown by Lam et al. This study showed no consistent change in transit with diet nor intervention suggesting that this is not the main explanation for our results (Ching Lam et al., 2016).

6.1.5 Low basal breath hydrogen concentration in patient

In the CRAIGS study, the low FODMAP diet reduced colonic volume compared to the habitual diet in patients. Besides low basal breath hydrogen, there were no increment in breath hydrogen concentration in the habitual diet group. This may have occurred due to the effective clearance by respiratory excretion or rectal expulsion through flatulence or defecation. However, as stool and symptom diary were not collected this could not be determined.

The low levels of breath hydrogen at baseline could be due to some patients restricting foods that may cause symptoms prior to the MRI scan. As per the instructions in the MRI participant information sheet (PIS), patients were advised to avoid eating particular food which may alter the way the bowel behaves .This is a standard instruction on the MRI participant information sheet prior to having an MRI scan as gas formation may reduce the image quality of the area being scanned the colon (Pritchard et al., 2014). A recent MRI study of the prostate reported that an gas distension reduces image quality significantly (Caglic et al., 2017) justifying the need to exclude gas forming foods. This needs to be taken into account when planning future studies and perhaps a standard MRI meal could be provided to control this effect.

An increased level of breath hydrogen demonstrates a low rate of gas clearance. In the CRAIGS study, only 3/15 patients had breath hydrogen concentration > 10 suggesting increased gas clearance in other patients. Findings from King et al., demonstrated that a rise in breath hydrogen is inversely related to gas production possibly due to rectal expulsion in order to avoid excessive colonic distension (King, et al., 1998).

In summary breath hydrogen test underestimates hydrogen concentration especially when it is excreted via flatus when there is rapid production. Furthermore, the test may not reflect total gas production as hydrogen is also converted into sulphide and methane (Eisenmann et al., 2008). By taking this into consideration, breath hydrogen measurement after prolonged fasting may not be a suitable marker of fermentation.

6.1.6 Instituting the low FODMAP diet in clinical practice

The apple study showed, how physical form of FODMAP altered behaviour in gut. The study demonstrated that liquid FODMAPs could be expected to influence gut differently from solid so low FODMAP diet might logically make a distinction between different forms liquid preparations of osmotically active FODMAPs such as milk, fruit drinks being more likely to induce symptoms than solid such as cheese or FODMAPs mixed in with solid meal which would empty more slowly.

Therefore, it is worth reviewing if there is a benefit in total restriction of all high FODMAPs food as the diet has only been effective in reducing symptoms in only 75% of patients in an Australian study (Halmos et al., 2014) and 57% in

a UK study (Staudacher *et al.*, 2017). It does not improve overall global symptoms except pain and bloating in in patients with constipation predominant IBS (Eswaran et al., 2016).

Current reviews have suggested a liberal bottom to top approach whereby only few foods which are high in FODMAPs are restricted or only a few targeted FODMAPs such as fructose, polyols and lactose are reduced for short period of time. If needed, patients could follow a more restricted diet. (Halmos, 2017), (Hill et al.,2017). However, this needs to be investigated further with participation of patients from different ethnic groups due to their varied intake of FODMAPs, (Hewawasam et al., 2018).

Dietary adherence to the low FODMAP diet was lower when dietary advice was given compared to when patients were provided with intervention diet. (Marsh et al., 2016). It has been shown that adherence is associated with greater symptoms response. (Shepherd and Gibson, 2006). Consideration needs to be given to working patients as planning is needed before starting the diet. Dietary restriction will mean replacing current diet with other low FODMAP sources which may be costly not be feasible in those from low income group with large families. A recent questionnaire based study in the UK followed-up long term IBS patients on the low FODMAP diet. Even though 82% of patients were still continuing with the diet, 86% reported that the diet was expensive compared to 42% in the habitual diet group. These patients also reported increased difficulty in eating out (O Keeffe et al., 2018).

Food and beverages containing FODMAPs are grouped into high, moderate and low FODMAPs based on the cutoff value derived from clinical experience and has been set by the Monash group. This has enabled food items to be grouped according to their FODMAPs levels and made available to patients worldwide through a smart phone application both in Australia and the UK. (Varney et al., 2017). However, these cutoff were based on Australian patients and it may not appropriate for those who could tolerate larger quantities of FODMAPs particularly ethnic minorities. Also fructan intake in the UK is mainly from wheat, onion and garlic (Dunn et al., 2011). Onion and garlic are often consumed in cooked form as part of a meal, but chemical analysis is only available for raw onion and garlic (Muir et al., 2007). Therefore, future studies should focus on the effect of heat as well as different physical form of FODMAPs on symptoms as findings from the Apple study showed that a changing the physical property of FODMAPs has an effect on luminal volume. Also, dietary restriction should be proportional to symptom severity which needs to be explored further.

The reintroduction phase is carried out when patients have completed their low FODMAP diet which may range from 4-12 weeks (Gibson and Shepherd, 2010) (Whigham et al., 2015) in a clinical setting. A recent study using a food frequency questionnaire (CNAQ) reported that patients on long term low FODMAP diet had an intake of 20g of total FODMAPs per day. Findings from chapter 2 has shown that the CNAQ overestimates both nutrient and FODMAPs intake. It is also unknown how the long term low FODMAP diet which has been

adapted impacts on gut bacteria as previously a diet low in FODMAPs reduces concentration of b*ifidobacteria* (Staudacher et al., 2012).

6.1.7 The low FODMAP diet intervention and limitations

In the CRAIGS study, not many patients had the time or budget to have a balanced diet since they were either working part time or studying. This often led to some opting for readily available processed gluten free food items low in FODMAPs but high in cost. An Austrian study assessed the nutrient composition and cost of the gluten free products in supermarkets. It was found that their protein content was more than twofold lower in 60% of such food (all categories). About 19% of products were high in fibre (more than 6g/100g) but it was alarming to note that the cost was 205% to 270% more than standard (gluten-containing) products (Missbach et al., 2015). Another recent study in the UK showed that gluten free bread was typically 400% more expensive than standard products (Hanci and Jeanes, 2018).

Another limitation in the CRAIGS study was that patients had limited variety of fruits and vegetables in their diet as they often repeatedly ate the same food. In comparison, those who had time to make their own meals and enjoyed cooking and baking consumed a more balanced diet. That said, some patients with limited cooking skills (who previously relied on convenience foods) were still able to maintain a low FODMAP diet by planning their meals and enjoyed the experience of experimenting with new low FODMAP foods and recipes.

During the reintroduction phase, most patients wanted to challenge the foods they missed the most, such as wheat and wheat products, dairy products and

onion and garlic. Interestingly, some patients who did not routinely consume these foods in significant amounts were often content to continue such avoidance. One of the secondary objectives of this study was to assess 'food avoidance behaviour and symptoms post intervention', but due to low response rate from patients, this had to be excluded. However, this is of concern as the long term effect of the diet has been shown to induce growth of unfavourable gut microbiota and needs to be explored further (Hill et al., 2017).

Patients in this study were consuming a low FODMAP diet for only two weeks duration. However, significant reduction in symptoms were indicated from IBSSS scores, which decreased from 289 (87.2) to 196 (110) with p=0.00. However, based on UK dietary guidelines, patients are advised to pursue the diet for 4 to 6 weeks (McKenzie et al., 2016). This not only increases financial burdens but could also reduce patient adherence to the diet.

Evidence shows that between 5%-44% of patients with IBS, IBD and coeliac disease suffer from disordered eating behaviour. This usually results in patients having to constantly monitor and even restrict their dietary intake (Satherley, et al., 2014). One patient in the CRAIGS study passed the screening criteria and completed the study but had to be excluded from the analysis owing to disordered eating behaviour. This was evident from the patient's food diary, with baseline carbohydrate very low due to the patient substituting most of her carbohydrate-based meals with protein flour. Effects of the diet in such

patients is uncertain, putting into question whether a total restriction of FODMAPs is even necessary.

The limitations discussed above should be considered both by researchers and clinicians to ensure that the diet is safe, easy to follow, and accessible to patients from all walks of life.

6.1.8 Implications for practice

1. Based on evidence from current studies, a low FODMAP diet should be administered when first line dietary lifestyle and intervention is ineffective (McKenzie et al., 2016).

2. The low FODMAP dietary intervention should only be carried out by a dietitian trained in administering the diet (Staudacher et al*.,* 2017).

3. Dietary intervention should be individualised, with those able to tolerate lactose and fructose being advised to restrict other FODMAPs instead.

4. Patients with IBS-C may also benefit from the low FODMAP diet; however, strict dietary constraints may not be useful.

5. It is important to provide resources that are simple.

6.2 Summary and Conclusion of this doctoral thesis

The aim of this thesis was to investigate the effect of fermentable carbohydrates on luminal volume and function in both healthy volunteers and patients by using MRI scanning technology.

With significant investment of acumen, this aim was achieved; whilst some of the findings were unexpected, they nonetheless provide some novel insights in the field.

Assessment of nutrient and FODMAP intake is important in any dietary intervention trials. It is an important method to assess compliance as well as understand the effect of diet on physiological changes. However, amongst the biggest challenges are quantifying of both nutrients and FODMAPs. In the UK, the 7 day food diary is the most common method used to record FODMAP intake; in the US, 24 hour multiple pass dietary recalls are commonly used. Another popular method among researchers is the FFQ, there being two FFQ available specifically for FODMAPs. However, one FFQ validated in the UK could only measure inulin whereas the other method was validated in the Australia and may not be appropriate for different populations. Considering the strength and limitations of the dietary assessment methods available, perhaps a 3 day food diary or a 3 days multiple pass dietary recall (covering two weekdays and a weekend) would be more appropriate, as this would not only reduce participant burden but also time and cost.

The placebo in the FOG study was maltodextrin, which may or may not have influenced the rise of the colon volume. It was chosen because it was identical to the intervention (oligofructose), which was also a white powder, with similar texture, aroma and appearance. However, the results from the FOG study raised a few questions regarding both placebo and intervention. It was not clear whether the placebo was able to affect colon volumes, or if food choices during the intervention phase had an effect. A crossover study with a 1 week washout period may have been a better way to study the effects of oligofructose on colon volume.

In the Apple study, altering the physical form of food affected luminal volumes, particularly gastric content and small bowel water; FODMAPs in solid form emptied much slower compared to both puree (semi-solid form) and juice (liquid form). One of the limitations of the study was, the difference in fibre content between the test meals due to processing technique. It would be interesting to see the results if fibre had been standardised in all test meals, by adding a powder-form fibre supplement to water provided to the participants.

The FODMAP content of the test meals in the Apple study was too low to have an effect on SBWC. Some of the mechanistic studies have been able to demonstrate a clear effect on because participants were able to consume large amount of fructose, inulin and mannitol diluted with water. It is not possible to give the same amount of FODMAPs in the form of food as patients would struggle to consume such test meals. Provision of a low or high FODMAP diet (such as pizza and a side salad containing lentils of legumes) may be a better

way to observe any effects. This would be an example of a meal complete with all FODMAPs.

Findings from the CRAIGS study suggest that there is no need for total exclusion of high FODMAP content foods. Small amounts may be well tolerated. Also, compared to the habitual diet, the low FODMAP diet has very little effect on colon volume increase, which may be due to reduced bacterial proliferation. Most of the patients in this study experienced mild to moderate symptoms and were not on an entirely habitual diet. Patients were trying to control their symptoms by changing the diet. These were mostly patients who have been diagnosed for 5 years or more. Perhaps a comparison of their colon volumes would be useful. Another limitation is that patients were not matched with their subgroups. A mixture of patient from primary, secondary and tertiary care with all IBS subtypes would be useful to assess the difference in baseline colon volume and to measure changes from baseline post intervention. Meanwhile, those with disordered eating behavior should be excluded. More information on food avoidance behavior should also be collected, in order to assess any influence on colon volume. It should be noted that the results of this study are incomplete, as participant dietary and MRI data have not yet all been analysed.

Future studies are still needed to understand mechanisms within the gut when dietary intake is altered, especially when patients are following a low FODMAP diet.

A well-designed, double blind, placebo controlled study should be undertaken. With adequate funding, it would be possible to replicate the CRAIGS study and

include a third MRI scan so the effect of reintroduction on colon volume could be observed. An adequate sample size of patients with severe to moderate symptoms with all IBS subtypes should be included. Dietary assessment using FFQ specific to FODMAPs validated in the UK, or a 3 day multiple pass 24 hour recall, would be the best choice to estimate intake at baseline, during intervention, and post reintroduction.

Prior to that, perhaps a validation study is also needed to establish a validated FODMAP FFQ. By replicating the Monash study, it would be quite possible to obtain dietary data of healthy participants over a year, using a 3 day food diary recorded four times to cover each season. In order to detect under-reporters, comparing energy intake with energy expenditure is essential.

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APPENDICES

Screening form: ROME III Criteria Questionnaire

Consent forms

Consent Form: FOG study

IBS Module		
In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?	0 Never → 1 Less than one day a month 2 One day a month 3 Two to three days a month 4 One day a week 5 More than one day a week 6 Every day	Skip remaining questions
For women, For women: Did this discomfort or pain occur only during your menstrual bleeding and not at other times?	0 No 1 Yes 2 Does not apply because I have had the change in life (menopause) or I am a male	
Have you had this discomfort or pain 6 months or longer?	0 No 1 Yes	
How often did this discomfort or pain get better or stop after you had a bowel movement?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	
When this discomfort or pain started, did you have more frequent bowel movements?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	
When this discomfort or pain started, did you have less frequent bowel movements?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	
When this discomfort or pain started, were your stools (bowel movements) looser?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	
When this discomfort or pain started, how often did you have harder stools?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	
In the last 3 months, how often did you have hard or lumpy stools?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	
In the last 3 months, how often did you have loose, mushy or watery stools?	0 Never or rarely 1 Sometimes 2 Often 3 Most of the time 4 Always	

Title of Project: A randomised controlled trial of the effect of the low FODMAP diet and dietary oligofructose on gastrointestinal form, function and microbiota in healthy volunteers.

Investigators: Ms Claire Mulvenna, Ms Shanthi Krishnasamy, Dr Miranda Lomer, Dr Giles Major, Dr Luca Marciani, Ms Susan Pritchard, Dr Caroline Hoad, Prof David Barrett, Prof Penny Gowland, Prof Robin Spiller – Chief Investigator.

Healthy Volunteer's Consent Form

Please read this form and sign it once the above named, or their designated representative, has explained fully the aims and procedures of the study to you.

Please initial the box for each statement

- I voluntarily agree to take part in this study. ☐
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me which is attached. ☐
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result. ☐
- I agree to comply with the reasonable instructions of the supervising investigator and will notify him immediately of any unexpected unusual symptoms or deterioration of health. ☐
- I authorise the investigators to disclose the results of my participation in the study but not my name. ☐
- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for at least 10 years after the end of the study. ☐
- I understand that the image of my body may be used in future studies (approved by an Ethics Committee) beyond the scope of the study explained here. Any images used will be made anonymous, and I will not be identifiable. ☐
- I understand that I can ask for further instructions or explanations at any time. ☐
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing. ☐
- I confirm that I have disclosed relevant medical information before the study. ☐
- I understand and agree that samples of stool and urine will be taken for analysis of their content. ☐
- **Optional: Consent for storage and possible use in future research**. I agree that the samples I give and the data gathered about me can be stored by Prof Spiller's research team at the University of Nottingham for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Prof Spiller, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and I will not be identified in any way. ☐

- I shall receive an inconvenience allowance of £80 and a further £70 on confirmation of compliance with study protocol, totaling £150 if I complete the study as planned. If I withdraw from the study for medical reasons not associated with the study I will receive an inconvenience allowance proportional to the length of the period of participation, but if I withdraw for any other reason, the inconvenience allowance to be received, if any, shall be at the discretion of the investigator. ☐

- I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (e.g. venipuncture >50ml, endoscopy) or exposure to ionising radiation. ☐

Abnormal Findings in Scans

- I understand that the SPMMRC is not a clinical diagnostic facility and so does not routinely inspect images for abnormalities. I understand that my MR scans will NOT routinely be reviewed by a radiologist (or any other medically qualified person) to look for any signs of disease, and it is unlikely that any abnormalities that may be present will be detected. ☐

- On the other hand, I understand that if one of the investigators should happen to notice something on my scan which they think is abnormal then they will show my scans to a medically qualified doctor who will contact me if further action is required. ☐

- I understand that if an abnormality were detected on my scan it may affect my ability to get life insurance. ☐

- I agree to my GP being informed of any medically relevant information gained from the study. ☐

GP Name: ..... Telephone number:.....
Surgery Address: .....

Full Name: …… Telephone number: …….....

Address: ……
Signature: …… Date: ….....

To be filled in by an Investigator

I confirm that I have fully explained the purpose of the study and what is involved to:
……
I have given the above named a copy of this form together with the information sheet.

Investigators Signature: …… Date…….
Name: ……
Ethics Code: ……..... Volunteer Number: ……..…….

Please return a copy of this form to the MR Centre receptionist for storage.

Title: Effect of physical form of apples on gastrointestinal function and satiety:

an MRI study

Investigators: Mrs. Shanthi Krishnasamy, Dr Luca Marciani, Dr Sue Pritchard, Prof Penny Gowland, Prof Robin Spiller (Lead Investigator)

Healthy Volunteer's Written Consent Form

Please initial the box for each statement

- I voluntarily agree to take part in this study. ☐
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me which is attached. ☐
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result. ☐
- I agree to comply with the reasonable instructions of the investigator and will notify her immediately of any unexpected unusual symptoms or deterioration of health. ☐
- I authorise the investigators to disclose the results of my participation in the study but not my name. ☐
- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for at least 10 years after the end of the study. ☐
- I understand that I can ask for further instructions or explanations at any time. ☐
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing. ☐
- I confirm that I have disclosed relevant medical information before the study. ☐
- **Optional: Consent for storage and possible use in future research** I agree that the data gathered about me can be stored by Prof Spiller's research team at the University of Nottingham for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Prof Spiller, who ran the first study. Any samples or data used will be anonymised, and I will not be identified in any way. ☐
- I shall receive an inconvenience allowance of £60 for each visit, totaling £180 if I complete the study as planned. If I withdraw from the study for medical reasons not associated with the study I will receive an inconvenience allowance proportional to the length of the period of participation, but if I withdraw for any other reason, the inconvenience allowance to be received, if any, shall be at the discretion of the investigator. ☐
- I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (e.g. venipuncture >50ml, endoscopy)/exposure to radiation. ☐

Abnormal Findings in Scans

- I understand that the SPMIC is not a clinical diagnostic facility and so does not routinely inspect images for abnormalities. I understand that my MR scans will ☐

☐

NOT routinely be reviewed by a radiologist (or any other medically qualified person) to look for any signs of disease, and it is unlikely that any abnormalities that may be present will be detected.

- On the other hand I understand that if one of the investigators should happen to notice something on my scan which they think is abnormal then they will show my scans to a medically qualified doctor who will contact me if further action is required.

- I understand that if an abnormality were detected on my scan it may affect my ability to get life insurance.

- I agree to my GP being informed of any medically relevant information gained from the study.

GP Name: _____ Telephone number: _____

Surgery Address: _____

Full Name: …… Telephone number: ……..

Address: ……

Signature: …… Date: ……..

To be filled in by an Investigator
I confirm that I have fully explained the purpose of the study and what is involved to:

I have given the above named a copy of this form together with the information sheet.

Investigators Signature: …… Date…….

Name: ……

Ethics Code: …….. Volunteer Number: ……..
Please return this form to the MR Centre receptionist for storage.

Full Study Title: Carbohydrate reduction and impact on gastrointestinal system (CRAIGS)

A pilot randomised controlled trial of the effect of a diet low in poorly digested Carbohydrates on gastrointestinal form and symptoms in irritable bowel syndrome patients.

Name of Researchers: Ms Shanthi Krishnasamy (study investigator), Dr Miranda Lomer, Dr Luca Marciani, Dr Giles Major, and Prof Robin Spiller – Chief Investigator.

Please initial the box for each statement

- I confirm that I have read and understand the information sheet version number 1.1 dated 06 November 2015 for the above study and have had the opportunity to ask questions. ☐
- I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
-
- I understand that data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

- I understand and agree that (*if necessary) a blood sample will be taken for analysis of full blood count and tissue transglutaminase (tTG),IgA antibodies test to exclude for coeliac disease. ☐
    - **Test will only be carried out if participant has not previously* been tested for coeliac disease

- Optional: Consent for possible use of data in future research
    - I agree that the data gathered about me can be stored by the research team at the University of Nottingham for possible use in future studies. I understand that some of these studies may be carried out by researchers other than the current team of Prof Spiller, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and I will not be identified in any way. ☐

- I agree to my GP being informed of my participation in this study ☐

- I understand and agree that I and my GP will be informed if my blood results indicate that I may have Coeliac Disease and I will be referred to my GP for further investigations. ☐

- I understand that the SPMMRC is not a clinical diagnostic facility and so does not routinely inspect images for abnormalities. I understand that my MR scans will NOT routinely be reviewed by a radiologist (or any other medically qualified person) to look for any signs of disease, and it is unlikely that any abnormalities that may be present will be detected. ☐

- I understand that if the investigators do notice any potentially significant abnormality on my MR scans they may refer to the NHS for further investigation ☐

- I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

Original + 1 copy: 1 for participant, 1 for the project notes

MRI screening questionnaire

MR Volunteer Safety Screening Questionnaire

NAME	Date of Scan	Date of Birth
ADDRESS	Volunteer Number	
	Ethics Code	
Phone number	Weight	Height if applicable

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is very important that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear. All information is held in the strictest confidence.

Questions	Y	N
Do you have any implants in your body? e.g. replacement joints, drug pumps		
Do you have aneurysm clips (clips put around blood vessels during surgery)		
Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)*		
Have you ever had any surgery? Please give brief details over		
(We do not need to know about uncomplicated caesarean delivery, vasectomy or termination of pregnancy)		
Do you have any foreign bodies in your body (e.g. shrapnel)		
Have you ever worked in a machine tool shop without eye protection		
Do you wear a hearing aid or cochlear implant		
Could you be pregnant? (Pregnancy tests are available in the female toilets)		
Have you ever suffered from tinnitus		
Do you wear dentures, a dental plate or a brace		
Are you susceptible to claustrophobia		

Do you suffer from blackouts, epilepsy or fits		

Questions	Y	N
Do you have any tattoos? (If yes, you may be asked to read and sign another form)		
Do you have any body piercing jewellery that cannot be removed		
Do you have any skin patches (trans-dermal patches)		
Do you have a coil in place (IUD) for contraception? Do you know what type?		
Do you have any condition that may affect your ability to control your temperature? (e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease?)		
Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing aids etc. before entering the magnet hall? *(lockers available by the changing rooms)*		
Is there anything else you think we should know?		

I have read and understood all the questions Signature:	Date
Verified By: Scanner Operator/MR Assistant Signature:	Date

Tattoo Consent Form

On the safety screening form you have indicated that you have a tattoo. Please read the following information and we will go through it with you, ask if anything is unclear.

What are the risks?

During an MRI scan, certain types of tattoos may heat up if they are inside the region being scanned; in exceptional conditions burning may occur around the tattoo (in extreme cases leading to blistering).
For people with facial or genital tattoos or large tattoos (greater than 7.5cms across), such burning could be inconvenient or uncomfortable and may require medical care.
People may feel tingling at the site of the tattoo during scanning if it is going to heat up; this provides a warning of a potential problem.

What should I do if I feel any heating or tingling?

If you feel any heating or tingling in your tattoo alert the scanner operator using the call bell. We will stop the scan and remove you from the scanner. If there are any signs of heating we will apply a cold compress. In extreme situations we will seek medical help. Terminating the scan does not affect any inconvenience allowance you may be due from participating in the study.

What should I do now?

If you no longer want to participate in the study, just tell the researcher. If you would still like to participate please continue reading.

Statement of consent.

I confirm that:	Please initial box
The researcher has explained the risk of having a scan with a tattoo	☐
That I want to proceed with the scan	☐
That I will alert the operator if I feel any heating or tingling in my tattoo(s)	☐
I understand that if this happens then the MR scan will be terminated	☐
I understand that terminating the scan will not affect any inconvenience allowance due to me for study participation	☐

Participant's Signature: …….. Date.....

Investigator's Signature: …….. Date.....

Participants information sheet

Title: A randomised controlled trial of the effect of the low FODMAP diet and dietary oligofructose on gastrointestinal form, function and microbiota in healthy volunteers: FOG

Names of Investigators: Ms Claire Mulvenna, Ms Shanthi Krishnsamy, Dr Miranda Lomer, Dr Giles Major, Dr Luca Marciani, Ms Susan Pritchard, Dr Caroline Hoad, Prof David Barrett, Prof Penny Gowland, Prof Robin Spiller – Chief Investigator.

Healthy Volunteer's Information Sheet

FOG study Version 1.0 17th July 2014

"You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this."

Background

Oligofructose (OF) is a type of dietary fibre, or carbohydrate, that is derived from the vegetable chicory. Increased amounts of OF in the diet has been proposed to have various benefits to health but recently studies have suggested that in people who suffer with irritable bowel syndrome it may cause symptoms. People who do not suffer with IBS rarely get symptoms.

We want to study the effects that OF has on bowel function, the bacteria that normally live inside the bowels of healthy people, and any chemicals produced by those bacteria. In this study we will use Magnetic Resonance Imaging, a type of scan that is safe to have many times, to look at changes in the bowel. We will also collect samples of stool, urine and breath to study other changes. The whole study is expected to last about 4 months, although we will do further analysis after we finish recruiting people.

Why have I been asked to take part?

You have responded to an advertisement for healthy volunteers, or asked about such studies.

In this study we want to recruit 36 adults (18 or older) who do not have any bowel problems.

What does the study involve for me?

The study will look at the effect of different carbohydrate supplements on bowel function. To do this all participants will be asked to follow a specially designed diet for a week while taking a supplement. The study is a randomised controlled trial. This means that we will compare a group who take the test supplement (OF) with a control group who take a different one, in this

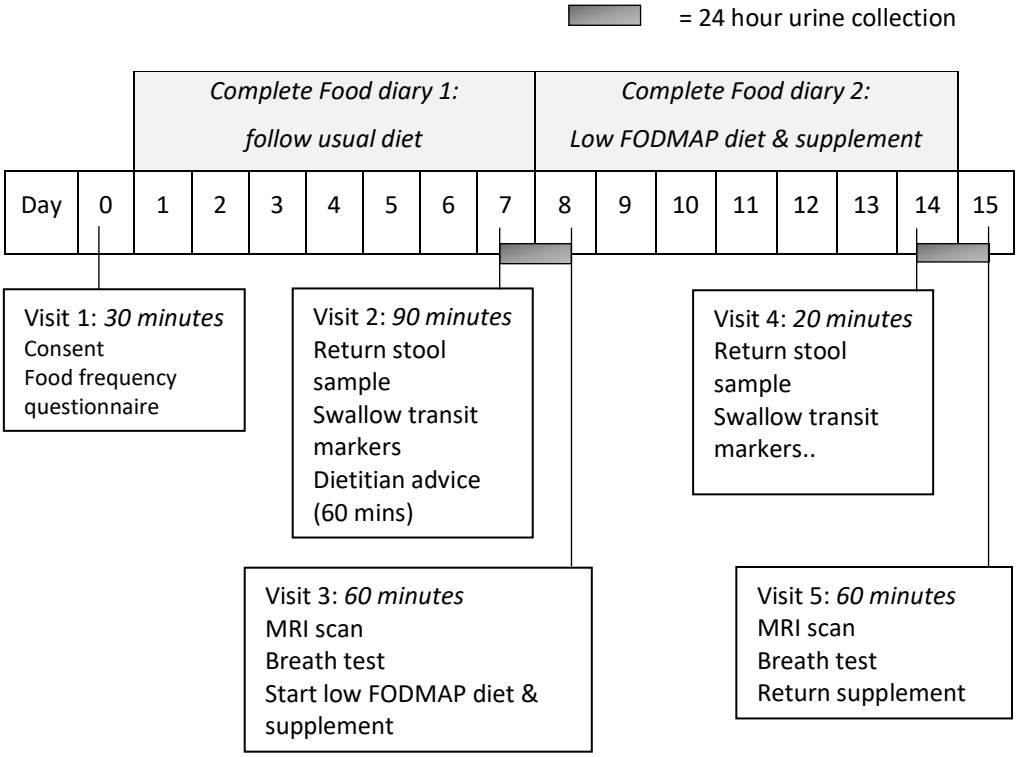
case a type of starch. You will be assigned to one group or the other at random so that the comparison is a fair test. You have a 1 in 2 chance of being in each group.

The study is also a blind trial. This means that neither you nor the research team will know which supplement you are taking. That information is held by someone independent until after the trial. The research team can find out if they need to for safety but all the supplements in this study are very safe so this should not be required.

The study lasts about 15 days from the day that you give your consent to your last visit.

There are 5 visits in total, as shown on the diagram on the next page:

Study Diagram



Visits 1, 2 and 4 will take place in the Nottingham Digestive Diseases Centre (NDDC) in Queen's Medical Centre. Visits 3 and 5 will take place at the 1.5T scanner of the Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) on the University Park Campus. For visits 2-5 you should attend at 8.30am, having fasted (nothing to eat or drink since waking).

Below is a list of the main elements of the study.

- Food frequency questionnaire and food diaries: these are ways to assess your diet. There is 1 questionnaire and 2 food diaries which last a week – one when eating your normal diet; one when following the study diet. You will be told how to do this and we will discuss any problems with you during the study.

- Stool samples: during each of the weeks when you keep your food diaries we will ask you to collect a stool sample for us. We will give you a kit with which to do this. More detail is given on the stool sample instruction sheet.
- Urine collection: between visits 2/3 and 4/5, we will ask you to empty your bladder, then collect your urine for 24 hours in a special container.
- Swallowing transit markers: at visits 2 and 4 you will swallow 5 transit markers, each 2 cm long (the size of a vitamin pill). They have been designed to show up in the MRI scan and are safe to swallow.



- MRI scans: at visit 3 and visit 5 you will have an MRI scan lasting 15-20 minutes. More detail is given in the MRI information sheet that accompanies this sheet.
- Breath tests: at visits 3 and 5 you will also blow into a machine that measures the level of hydrogen and methane gas in your breath.
- Follow a special diet for a week and take a supplement: the diet is designed to minimise the amount of fibre similar to OF in your diet. Half the participants will then have OF added back to their diet while the other half have a different, glucose-based, carbohydrate. These supplements are widely used in processed food and are safe.

Please note that a small quantity of food dye is added to the supplement. This is of animal origin so we are not recruiting vegetarians.

The measurements that we take will only be relevant if participants follow the dietary advice that we give. The next section will outline what we would like you to do

What do I have to do?

Visit 1: once you have given consent to take part in the study we will measure your height, weight and ask for certain health details such as medical history, medications and smoking status. You will also fill in a questionnaire to confirm that you are safe to go in the scanner.

If you are eligible to take part we will then ask you to fill in a questionnaire about your diet. We plan for this to be done on computer but if there is a technical problem we may need to use a paper copy.

We will then give you give you a food diary and a stool collection kit to take home. Please note that participants are asked not to drink more than 8 units (2 pints of beer; half a bottle of wine) per day during the study, and should abstain from alcohol for 48 hours before MRI scans.

The next day you should start filling in the food diary. It is possible to delay this if you cannot make the next visit, or we need to schedule visits around availability of the MRI scanner.

Once you have started the food diary you should write down everything that you eat or drink for 7 days. On the 6th day you should try to collect a stool sample, as described on the information sheet. This can be stored in your freezer in the container provided until Visit 2.

Visit 2: this will be in the NDDC around 8.30am. Once you have woken up please do not eat or drink before you arrive (A small sip of water for essential medicines is permissible). When you arrive we will ask you to empty your bladder and give a urine sample, then ask you to swallow the transit markers. Once you have swallowed the pills we will provide you with breakfast. We will also collect your stool samples at this visit. If you have not managed to collect any please keep trying - you can bring them to visit 3.

During visit 2 you will attend a 1-hour session with a dietitian about a special diet that we would like you to follow called the 'low FODMAP' diet. This diet minimizes the amount of certain carbohydrates in your diet. You will be lent information booklets about how best to follow the diet. *Do not start the diet yet!* We will also supply you with a packed lunch and a meal to have in the evening. Please eat these as your main meals as it make everyone's experience the same. You may have some additional snacks if you need them but please do not eat or drink after 10pm that day. You should still fill in your food diary as for the other 6 days.

Between visits 2 and 3 we want you to collect all the urine that you pass. It is really important that this is accurate. We will give you the kit with which to do this.

Visit 3: this will be at the 1.5T scanner of the SPMMRC. You will be given a time slot of 8.30-9.30 or 9.30-10.30. Please arrive 30 minutes before your time slot. Once you have woken up please do not eat or drink before you arrive (A small sip of water for essential medicines is permissible). You will return your urine collection and your food diary (and your stool sample if it is still outstanding).

More detail about the MRI scanning is given in a special information sheet. You will also give a breath sample as described earlier.



Once you have completed the MRI scans and breath test you will be given a breakfast that complies with the low FODMAP diet. You will also be given a white tub containing your supplement and a scoop to measure servings. The picture shows the type of tub that we will use, from a previous study. The team will watch you prepare your first supplement dose by adding it to a hot/ warm drink of around 250ml. The team will observe you drinking this.

You are then free to leave. You'll take with you the supplement tub, a second food diary that should start with the breakfast provided, and another stool collection kit.

For the next week you should follow the low FODMAP diet and take 7 grams (1 scoop) of the supplement twice each day. You should fill in the second food diary as before. If you have any questions about the diet please get in touch with the study team. The team will also contact you to make sure that you are managing with the diet. On the 6th day of the food diary you should collect a stool sample as before. Again, please do not drink alcohol in the 48 hours before your next MRI scan.

Visit 4: is the equivalent of visit 2, so please come to the NDDC at 8.30am. Once you have woken up please do not eat or drink before you arrive (A small sip of water for essential medicines is permissible). As before, when you arrive we will ask you to empty your bladder and give a urine sample, then ask you to swallow the transit markers. Once you have swallowed the pills we will provide you with a low FODMAP breakfast and ask you to drink the study supplement in front of us. We will also collect your stool samples at this visit. If you have not managed to collect any please keep trying – you can bring them to visit 5.

Please bring along your low FODMAP booklets so that you can return them. We will supply you with a low FODMAP packed lunch and a microwave meal to have in the evening. Please eat these as your main meals as it make everyone's experience the same. You may have some additional snacks if you need them but please do not eat or drink after 10pm that day. Please

also ensure that you have your evening dose of supplement. You should still fill in your food diary as for the other 6 days.

Again, we want you to collect all the urine that you pass between visits 4 and 5. It is really important that this is accurate. We will give you the kit with which to do this.

Visit 5: is the equivalent of visit 3 so will be at the 1.5T scanner of the SPMMRC. You will be given a time slot of 8.30-9.30 or 9.30-10.30. Please arrive 30 minutes before your time slot. Once you have woken up please do not eat or drink before you arrive (A small sip of water for essential medicines is permissible). You will return your urine collection, your food diary and your supplement container which will be weighed.

You will then have the MRI scan and give a breath sample as for Visit 3. At this point you will have finished the study and will be offered a light snack.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What is the drug or procedure that is being tested?

As well as following the diet you will take a carbohydrate supplement. This will be either oligofructose, which is produced from chicory and is commonly added to processed food, or maltodextrin, which is produced from starch. Both have the status of Generally Regarded As Safe and have been used in studies with healthy volunteers at higher doses than here without any side effects. In this study we would like you to take 7 grams (1 scoop) twice daily for a week. You should add the powder to a hot drink and stir well.

Both powders will appear pink. This is because we have added a food colouring called carmine red (E120). Carmine red is not absorbed in the bowel so can be measured in the stool. Do not be alarmed if your stool has had a red tinge. Adding the carmine red will help us to know that you took the supplement.

E120 is of animal origin so may not be suitable for vegetarians. It is considered to be halal by the Gulf States but if you follow halal or kosher practice you may which to check for yourself before taking part in the study.

What are the side effects of any treatment or procedures received when taking part?

The supplements and diet are not associated with any ill effects in healthy volunteers. The safety issues surrounding MRI are described in the accompanying sheet.

What are the possible disadvantages and risks of taking part?

The study involves 5 visits to see the research team which is time consuming. It may be difficult to follow the low FODMAP diet for a week and the food required may be more expensive than your usual shopping. To compensate we will pay an inconvenience allowance of £80 plus a further £70 once we have confirmed that you took the supplement as planned and have returned the low FODMAP information booklets (each set costs us £10).

Please note that we cannot recruit people with pre-existing bowel complaints, those taking medicines that affect bowel function or people who cannot use the MRI scanner (e.g. weight >120kg, implanted electronic devices).

What if something goes wrong? /Who can I complain to?

In case you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach any of the research team or the lead investigator, Prof Robin Spiller, Nottingham Digestive Diseases Centre, University of Nottingham, Nottingham NG7 2UH. Tel 0115 8231090.

If this achieves no satisfactory outcome, you should then contact the Ethics Committee Secretary, Mrs Louise Sabir, Administrative Support, UoN Faculty of Medicine & Health Sciences Research Ethics Committee, c/o School of Medicine Education Centre, B Floor, Medical School, QMC Campus, Nottingham, NG7 2UH. Tel 0115 8231063 or email louise.sabir@nottingham.ac.uk

In the unlikely event that you suffer injury to yourself or damage to your property as a result of taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study. In addition, all medical practitioners taking part in the research have personal medical negligence cover.

What if I decide to leave the study?

You are free to leave the study at any time if you choose to do so without giving a reason. If you withdraw then the information collected so far cannot be erased and this information may still be used in the project analysis.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it.

Please note that if you choose to withdraw from the study data already collected about you cannot be erased. We will also seek your permission to use that data in the analysis of the study. We will not contact your GP unless we find something unexpected and important on the MRI scan, as discussed in the MRI information sheet.

What will happen to the results of the research study?

The results of the study will be presented at scientific conferences and published in a peer-reviewed journal. We plan to make any publication open access, which means that it can be accessed by anyone through the internet. We plan to put a link to the relevant paper on our website www.nddcbru.org.uk and also through our social media accounts. All data will be presented anonymously so that none of the volunteers can be identified.

Who is organising and funding the research?

The project is being funded by the lead investigator Prof Spiller and our collaborators Prof Gowland and Prof Barrett. We may receive additional funding from the Alpro Foundation, an independent charity that funds research on plant-based diets.

Who has reviewed the study?

The study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

Contact for Further Information

If you have any questions please ask the person who gave you this sheet or telephone

Dr Giles Major Ms Shanthi Krishnasamy

Tel: 0115 8231073 Tel: 0115 9249924 x63756

giles.major@nottingham.ac.uk shanthi.krishnasamy@nottingham.ac.uk

Or you contact **Lesley Martin at the SPMMRC (0115 9514747)** who can contact the right person.

Thank you for considering participation in our study. Don't forget you can change your mind and withdraw from the study at any time.

Visit timetable (to be filled in)

Visit 1:

Visit 2:

Come to the Nottingham Digestive Diseases Centre, QMC, for 08.30

Visit 3:

Come to the 1.5T scanner of the Sir Peter Mansfield Magnetic Resonance Centre at

Visit 4:

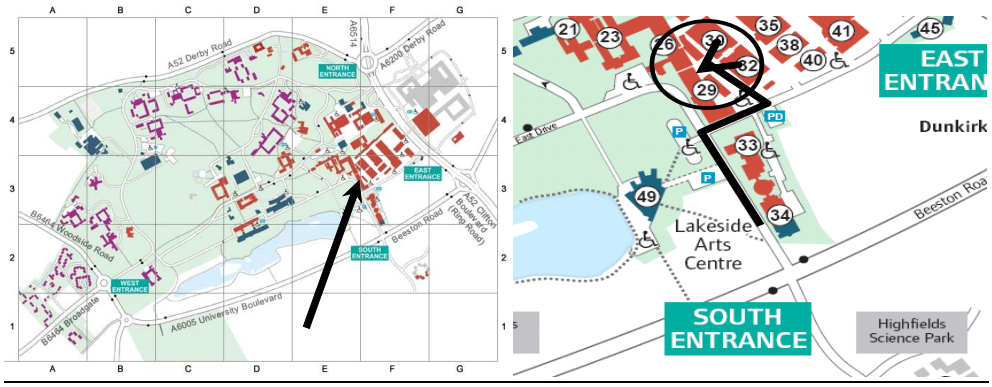
Come to the Nottingham Digestive Diseases Centre, QMC, for 08.30

Visit 5:

Come to the 1.5T scanner of the Sir Peter Mansfield Magnetic Resonance Centre at

DIRECTIONS TO THE SCANNER: The 1.5T Sir Peter Mansfield Magnetic Resonance Centre research MRI scanning Unit is on the main University of Nottingham campus in the Psychology building, shown by the arrow on the first map below here. The entrance to the Unit is on the ground floor at the BACK of the building as shown in the map at the bottom of the page. Proceeding from the South entrance of the University Campus and going past the Arts Centre, take the first road on the right just before the security gate and immediately after take the first left. Walk for about 200 meters and you will see the glass double-doors of the entrance to the Unit signposted inside a small yard on your left.

Should you have problems in finding the Unit or in attending on the morning of your study day, please ring the Unit on 0115 84 68253 or extension 68253 from any University phone.


A52 Derby Road
A6200 Derby Road
A6514
NORTH ENTRANCE
EAST ENTRANCE
SOUTH ENTRANCE
WEST ENTRANCE
A52 Clifton Boulevard (Ring Road)
Beeston Road
B6540 Woodside Road
B6540 Broadgate
A6005 University Boulevard
East Drive
Lakeside Arts Centre
SOUTH ENTRANCE
Highfields Science Park
Dunkirk
Beeston Road

Title: Effect of physical form of apples on gastrointestinal function and satiety: a MRI study

Investigators: Mrs. Shanthi Krishnasamy, Dr Luca Marciani, Dr Kathryn Murray, Prof Penny Gowland, Prof Robin Spiller (Lead Investigator)

Healthy Volunteer's Information Sheet

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet.

Background and summary of the study

Eating exactly the same foods but in different physical forms (solid, puree or juice) may have an effect on how people feel after eating. A study from the 1970s showed that if people eat a whole apple they will feel full for longer than when they eat it juiced. This is probably because the different forms of food will change the way nutrients are delivered to the bowel and how, in turn, the body responds to these nutrients. Some of the nutrients are sugars that can be fermented in the colon and this is known to induce gut symptoms such as bloating, diarrhoea and pain in some people. Avoiding these sugars in the diet usually works but it is not well understood why.

We want here to investigate this effect further using our special medical imaging (magnetic resonance imaging known as MRI) techniques that allow us to image foods in the body.

We plan to feed 3 different test meals in 3 separate morning studies, one week apart, to a group of healthy volunteers. The test meals consist of approximately 3 to 4 Pink Lady apples; whole apple, apple puree and apple juice. We will use MRI at intervals to monitor the behaviour of the different meals in the gut and relate what we see to how people feel as measured by symptom scores.

What does the study involve for you?
If you are interested to take part we will ask you some questions to check you are eligible to take part. Part of this involves measuring your height and weight. If you are eligible we will invite you to join the study. You will be asked to sign a consent form. We will then arrange for you to come to 3 morning study days, approximately 1 week apart.

On the day before your study day: We need you to avoid certain activities for the rest of the day. Drinks like coffee or alcohol and performing too much sport activity may affect the function of the gut. There are also certain minerals and iron supplements that can interfere with the MRI measurements we are going to make.

Therefore please avoid:

- caffeine (tea, coffee, cola) for 18 hours prior to your study day.
- alcohol for 24 hours prior to your study day.
- taking any dietary supplement (e.g. multivitamins and minerals pills, iron supplements) for 24 hours prior to your study day.
- performing any strenuous exercise for 24 hours prior to your study day.

Please eat your normal portion evening meal on the night before your study day and then start fasting (i.e. have no further food or drink other than water) from 10pm that night. A small glass of water is allowed on waking up on the study day but not after 7am.
Please do not eat or drink anything else until your each the study centre for 8.30am (i.e. you will have fasted for about 10 hours and had nothing to drink after 7am).

We will give you a leaflet to help you to remember these instructions and we will ask you to fill in a checklist questionnaire about them on the morning of the study day.

On each study day
We would like you to come to the 1.5T scanner of the Sir Peter Mansfield Imaging Centre on the University Park campus for 8.30am o'clock in the morning. There is a map at the end of this leaflet. You should have had nothing to eat since 10 o'clock the night before. We will check that you have followed the advice above, then ask you to fill in a questionnaire on any appetite symptoms you have that morning. You will then have an MRI scan to look at your tummy.

The MRI scanner is quite noisy and so you will wear ear defenders (i.e. headphones and/or soft earplugs) during the scan. Because the scanner is built around a large magnet, you will have to remove all metal from your body, including jewellery. You may prefer to wear loose non-metallic clothing and you will be asked to change into clothing provided.

After the MRI scan we will give you a test meal each week which could either whole apples, apple puree or apple juice and ask you to eat it within 15 minutes. Immediately after that you will have another MRI scan. Every 45 minutes we will take another MRI scan for 5 hours.

Each MRI scan lasts approximately 10 minutes, you will spend the rest of the time sitting comfortably upright in a volunteers' room next to the scanner.

Satiety scales
Every time you come out of the MRI scanner we will ask you to rate your feelings of fullness, hunger and appetite on 100mm scales printed on a sheet of paper.

Gastrointestinal function
We will also ask you to rate your symptoms such as gas/flatulence, bloating, abdominal pain and diarrhoea each time you are out of the MRI scanner using similar scales as the questionnaire above.

Why have you been chosen?
You have been chosen because you are a healthy volunteer. We are aiming to complete the study up to 18 adult healthy volunteers in total.

Who is organising the research?
This is an academic research study organised by the University of Nottingham. This study has been independently reviewed and approved by the University Medical School Research Ethics Committee.

Are there any risks?
Magnetic resonance imaging (MRI) uses radio waves similar to those used in radio and TV transmission. These have a much lower energy than X-rays and as such are considered biologically safe. Structural MRI using a 1.5 T magnet (about 60000 times the earth's magnetic field) is already a routine procedure in medical practice, and not been found to be harmful in any way. This study will be carried out using a 1.5 T magnet, within national safety guidelines. A few people find being in the scanner claustrophobic, but the scanner does have a relatively wide bore.

IMPORTANT: What happens if we notice something abnormal on your scan?
While it is extremely unlikely that your scan will show any abnormality, it is unlikely that we would notice anything unusual or abnormal on the images since the Sir Peter Mansfield Imaging Centre (the SPMIC) is NOT a clinical diagnostic facility and the scans we collect are NOT the same as scans collected by doctors for medical purposes. The pictures will NOT usually be looked at by a radiologist (a doctor qualified to find abnormalities in scans), so this test does NOT replace any tests that your doctor thinks might be needed.

However, there is a possibility that one of the researchers working with your scans might notice something that they consider abnormal. If we suspect that there is something abnormal on your scan we will ask a specialist to review it. If he/ she thinks that further action is necessary we will inform your GP. Your GP may refer you to other medical specialists for further clinical investigation.

It is a requirement of the SPMIC that you give permission for us to inform your GP of such a finding before we can enrol you into the study.

In the unlikely event that we do notice something abnormal on your scan, giving you that information might have the benefit of allowing you to start treatment earlier than you would have otherwise. However, it is also important for you to realise that by providing you with this information there may be implications for your future ability to find employment and obtain insurance.

What is in it for you?
If you are eligible and enter the study we will give you a picture of your body. You will receive £60 inconvenience allowance for completion of each study day, making a total of £180 if you complete the whole study as planned. You may not receive the entire amount if you stop the study earlier.

Conduct of the study:
This study is carried out according to the principles of Good Clinical Practice and the Declaration of Helsinki.

What if there is a problem?
In case you have a complaint (for example on your treatment by a member of staff) you can initially approach the lead investigator (Prof. Robin Spiller, Nottingham Digestive Diseases Centre, University of Nottingham, Nottingham NG7 2UH).E-mail: Robin. Spiller@nottingham.ac.uk.

If this achieves no satisfactory outcome (or in case of something serious happened during or following your participation in the study) you can then contact the FMHS Research Ethics Committee Administrator, c/o The University of Nottingham, School of Medicine Education Centre, B Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH. E-mail: louise.sabir@nottingham.ac.uk.

What if you decide to leave the study?
You are free to leave the study at any time if you choose to do so without giving a reason.

The Data Protection Act
Data about you will be held on computer, but will be treated in the strictest confidence at all times.

Medical Records
We will not contact your GP to inform him/her that you are going to take part in this study. Any information, which is collected about you during the course of the research, will be kept strictly confidential. Your name will not be disclosed outside the study centre and any information about you, which leaves the study centre will have your name (and address) removed so that you cannot be recognised from it to ensure we comply with Data Protection legislation.

Publication and Dissemination of the Results
The results of this study may be published in the scientific literature and may also be presented at scientific meetings. All such data will be presented anonymously so that none of the volunteers can be identified

Do you have any further questions?
If you have any questions please ask the person who gave you this sheet or contact Lesley Martin (0115 9514747) who can contact the right person.

Don't forget you can change your mind and withdraw from any time.

The 1.5T Sir Peter Mansfield Imaging Centre research MRI scanning Unit is situated in the main University campus (see directions and arrow on the maps in the following page).

DIRECTIONS: The 1.5T Sir Peter Mansfield Magnetic Resonance Centre research MRI scanning Unit is on the main University of Nottingham campus in the Psychology building, shown by the arrow on the first map below here. The entrance to the Unit is on the ground floor at the BACK of the building as shown in the map at the bottom of the page. Proceeding from the South entrance of the University Campus and going past the Arts Centre, take the first road on the right just before the security gate and immediately after take the first left. Walk for about 200 meters and you will see the glass double-doors of the entrance to the Unit signposted inside a small yard on your left.

Should you have problems in finding the Unit or in attending on the morning of your study day, please ring the Unit on 0115 84 68253 or extension 68253 from any University phone.

Title of Study: Carbohydrate Reduction and Impact on Gastrointestinal System

(CRAIGS)

Participant Information Sheet

A pilot single blind randomised controlled trial of the effect of a diet low in poorly digested carbohydrates on gastrointestinal form and symptoms in irritable bowel syndrome patients

Name of Researcher(s): Prof Robin Spiller (Chief Investigator), Ms Shanthi Krishnasamy, Dr Miranda Lomer, Dr Luca Marciani, Dr Giles Major

We would like to invite you to take part in our research study.
Before you decide we would like you to understand why the research is being done and what it would involve for you. If you wish to take part, one of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

Background

FODMAPs (Fermentable Oligosaccharide-Disaccharide-Monosaccharide and Polyols) are a group of poorly-digested carbohydrates found in certain cereals, fruits, vegetables, and dairy products. They are poorly absorbed in the small intestine and enter the large intestine, where they are fermented by bacteria. This results in symptoms such as wind, bloating, diarrhoea and altered bowel habit in Irritable Bowel Syndrome patients.

What is the purpose of the study?

The low FODMAP diet has been recommended by the National Institute for Health and Care Excellence (NICE) as dietary management for IBS patients whose symptoms still persists following general dietary advice and lifestyle. Other research suggests that a reduction of these carbohydrates through a low FODMAPs diet might alter the growth of bacteria that usually live in the large intestine. Therefore, we want to understand how these two approaches work and what effect they have on the bowels.



Why have I been invited?

You are being invited to take part because you have either responded to an advertisement, enquired about such studies or have been referred by a General Practitioner following diagnosis with IBS. We are inviting 40 participants like you who have not received advice on a low FODMAP diet, to take part.
We want to gather more information on a small group of people to help plan bigger studies of diet in a more varied group in the future.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign consent form (if appropriate – completion and return of a Questionnaire can be taken as consent). If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect your medical or legal rights.

How does the study work?

Visit 1: around 60 minutes
Informed Consent. We will check some further details to make sure you are eligible for the study and ask you to sign a consent form agreeing to take part in the study.

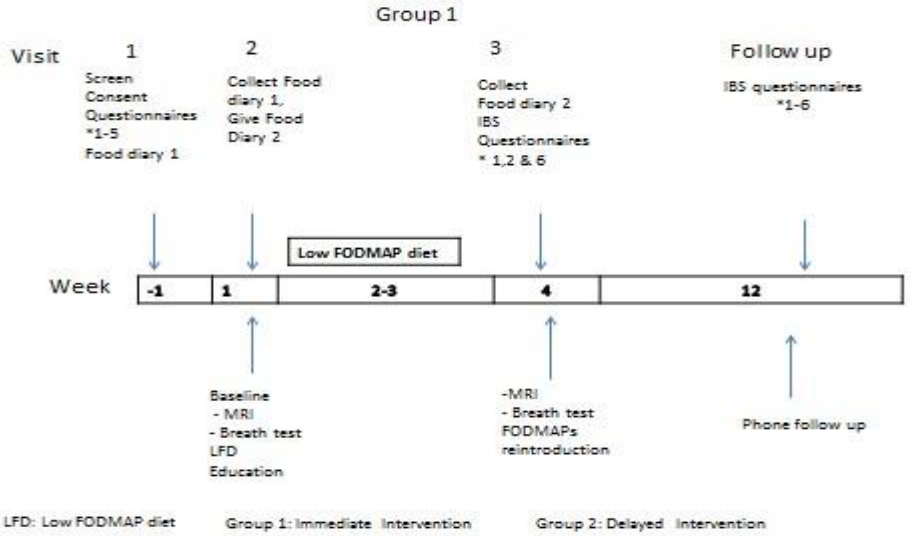
Once you have signed your consent form we will measure your height and weight, and ask for certain health details such as medical history, medication and smoking status.

You will fill in two screening questionnaires. One is to identify if you have IBS symptoms and the other is to see if it safe for you to have an MRI scan as part of the study. If you don't have IBS symptoms you won't be able to take part in the study as we are looking for volunteers who have been diagnosed with IBS. However if you do have IBS but can't have an MRI due to safety reasons, we would still like you to take part in the study without the MRI scans.

You will then fill in 5 short questionnaires: about your bowel symptoms, about how you are feeling generally, your psychological status and foods that you tend to avoid. One of the questionnaires aims to assess anxiety and depression. Participants who have a high score are more likely to have anxiety and depression and we will advise those participants to make an appointment to see their GP.

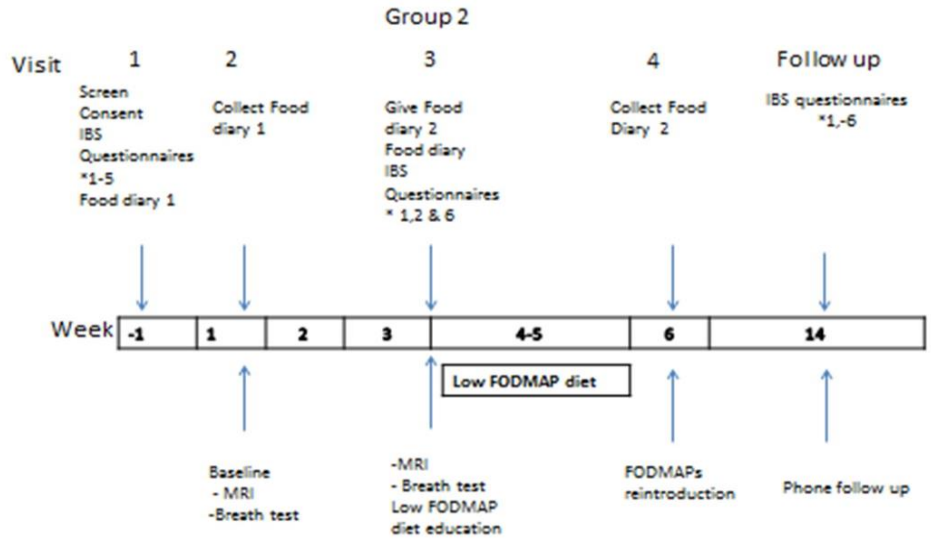
Finally you will take a food diary home. Once you have started your food diary, you should write down everything that you eat or drink for 7 days and bring this with you to your second visit.
If you have done a blood test, you will be contacted following satisfactory receipt of your blood test results and requested to begin completion of the questionnaires and food diary.

Trial Regimen



LFD: Low FODMAP diet Group 1: Immediate Intervention Group 2: Delayed Intervention

IBS Questionnaires :1.Health Questionnaire (EQ-5D-5L) 2.IBS Symptom Severity Scale (IBSS) 3. Hospital, Anxiety and Depression Scale Questionnaire (HADS) 4.Dietary Trigger Questionnaires 5.Gastrointestinal Symptoms Rating Scale (GSRS), 6. IBS Global Improvement Scale (GIS)



LFD: Low FODMAP diet Group 1: Immediate Intervention Group 2: Delayed Intervention

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Visit 2: 90 minutes - 2 hours

You will have an MRI scan, followed by a breath test and then have an appointment with a dietitian.

Visit 2 is at the 1.5T scanner of the Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) on the University Park Campus.
There is a separate sheet about MRI scanning that you should receive along with this information sheet. Please do not eat after 8pm on the day before the study. Drinking water is fine, but please do not eat or drink anything on the morning of the visit apart from a small sip of water for essential tablets.

Shortly after your scan you will be asked to do a simple breath test at the scanner by blowing into a breath test machine.

Dietitian appointment: the low FODMAP diet

You will then be escorted back to the BRU to have a session of 45-60 minutes with a dietitian. The dietitian will talk to you about the low FODMAP diet and give you a booklet describing which foods you should avoid and which you can eat.
At the end of your appointment with the dietitian you will be given a food diary to record what you eat and drink in the week before your next visit. Please start the diary in the second week on the diet.

Visit 3: 90 minutes to 2 hours

Visit 3 is identical to Visit 2. You will be asked to follow the same instructions as for Visit 2.
After your MRI scan you will be escorted to the BRU where you will complete questionnaires about your bowel symptoms, how you feel generally and also return your diaries.

You will then have another appointment with the dietitian where they will go through your diet and discuss how to re-introduce foods to your diet whilst assessing if any food triggers your symptoms. This will take 45-60 minutes.

Visit 4: 60 minutes
Some of you will be asked to come for a 4th visit to do the re-introduction diet; this will take only about 45-60 minutes.

Expenses and payments

To help with travel costs and any extra expense of the diet we will pay an allowance of £25 per visit on completion of the whole study. This may affect you if you are in receipt of benefit payments.

What are the possible disadvantages and risk of taking part?

Some people find that following the dietary advice is restrictive and do not want to do it for two weeks. You will also need to complete 2 food diaries.

It can be inconvenient to come to the hospital, although we have tried to time research visits to coincide with your dietitian appointments. There may also be some specific issues around MRI scanning that are described on the MRI information sheet, but the scans are not harmful.

What are the possible benefits of taking part?

Following the dietary advice that you are given may help your bowel symptoms to improve and make you feel better; which is what we are testing. We are also testing if the diet alters the gut bacteria by doing a breath test and MRI.

What happens when the research study stops?

If you have completed the study, you will be given an inconvenience allowance of £25 for each completed visit. Data collected so far cannot be erased and may still be used if a final analysis is carried out.

What if there is a problem? Who can I complain to?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet.
If you remain unhappy and wish to submit a formal complaint, you can do this by contacting the Patient Advisory Liaison Service (PALS) at Nottingham University Hospitals, tel. 0115 924 9924 extension 65412 or pals@nuh.nhs.uk

Will my taking part in the study be kept confidential?

We will follow ethical and legal practice and all information about you will be handled in confidence.
If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for (12 months to 3 years) after the end of the study so that we are able to contact you about the findings of the study *and possible follow-up studies* (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I don't want to carry on with the study?

This is fine. Although we would like you to complete the study, your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and this information may still be used in the project analysis.

What about my GP (General Practitioner/ Family doctor)?

Your GP will be notified of your participation in the study. We will, however, ask your permission to contact the practice in case something relevant is found on your MRI scans or blood test.

Your data

Any data used will be anonymised: you will not be identified.

What will happen to any samples I give?

Your blood will be taken by a trained health care assistant for pathology analysis. It will be labelled in accordance with the local NHS procedures. After the sample has been analysed it will be destroyed in accordance with the Human Tissue Act, 2004.

What will happen to the research study results?
The whole study will take up to 2 years to complete after which the results will be analysed and presented at scientific conferences. Results will then be published. We plan to make any publication open access, which means that it can be accessed by anyone through the internet. We plan to put a link to the relevant paper on our website www.nddcbru.org.uk and also through our social media accounts. All data will be presented anonymously so that none of the volunteers can be identified. The results will also be written up as part of Ms Krishnasamy's PhD thesis.

Who is organising and funding the research?
This research is organised by the University of Nottingham, working with King's College. It is funded by Professor Spiller's Research Grant.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by East Midlands Nottingham 2 Research Ethics Committee.

Study Advocate Volunteers

The diet has been shown to improve symptoms after a few days. However if you are struggling, please contact the study team or your Study Advocate Volunteers and we will see what we can do.
If you feel uncomfortable or uncertain about speaking to the study team you can contact the Study Advocate. This is a participant in a previous study who has volunteered to be an independent point of contact. They are bound by the same rules of confidentiality and data protection as the study team.

Your Study Advocate Volunteers are:

Stevie Vanhegan – Tel: 07800 716 632

Gemma Barker – Tel: 07800 716 823.

Who to contact for more information

Ms Shanthi Krishnasamy, PhD Student and Registered Dietitian

Tel: 0115 9249924 extension 63756 , 0115 97 09966

Mobile: 07773 237601

Email: msxsk@nottingham.ac.uk

Chief Investigator: Prof Robin Spiller Tel: 0115 8231090

Magnetic Resonance Imaging Information Sheet

Name of Researcher(s): Ms Shanthi Krishnasamy, Dr Luca Marciani, Dr Miranda Lomer, Dr Giles Major, Prof Robin Spiller

This information sheet explains what is involved when a research study includes Magnetic Resonance Imaging (MRI)
Before you decide we would like you to understand what the research would involve for you. If you wish to take part, one of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish.

Ask us if there is anything that is not clear.

You have been given this leaflet in relation to:

The CRAIGS study

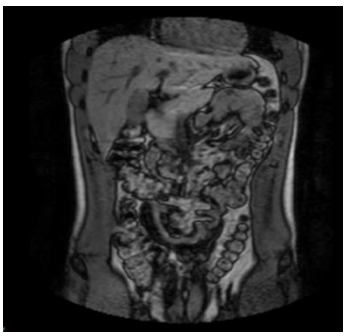
What is MRI?
Magnetic Resonance Imaging uses radio waves and magnetic fields to look at the water and fat molecules inside your body. These have been developed into powerful techniques to diagnose disease and to investigate the workings of the human body. The foundations of the science behind MRI were developed by a Nottingham scientist called Professor Sir Peter Mansfield, for which he was awarded the Nobel Prize in 2003. The Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) at the university continues to be a world leading research centre in the field of MRI.

Why is it useful for research?
Since the 1990s researchers at the Nottingham Digestive Diseases Centre have used MRI scanning to understand more about the workings of the stomach, liver and intestines and how these changes make people feel. The MRI scanner does not use X-rays so it is considered safe to have many scans. This allows us to track what happens over hours or weeks. By working with the physicists at the SPMMRC, medical researchers can develop new ways to assess the body, and gain new insights into the effects of food and medicines.

What will happen to me if I take part?
The MRI scanner is an extremely strong magnet so any metal that gets near it can cause serious problems. We will provide surgical scrubs and ask you to change into them before you go into the scanner (underwear can be worn but bras must be discussed with staff at the scanner and usually need to be removed due to metal clips and wires).



Lying on the scanner table



MRI image of the liver and colon

The MRI scans in our studies look in the abdomen (tummy) so you will lie on a table on your back (See picture) and a special cover will be placed on your tummy, held in place with straps. Your arms will usually be inside the cover. The scanner is quite noisy so during the scan you will wear ear defenders (headphones) and sometimes also earplugs.
The table moves so it will slide you into the scanner under the control of the scan operator. The scan operator can talk to you through the headphones to tell you what is happening and they can hear you talk back. You have a 'nurse call' button to squeeze if there is a problem.



A scan operator at the console. They can see you in the scanner through the window.

Each scan makes a different set of beeping noises. Some scans require you to hold your breath. The scan operator will discuss with you what is needed before going in the scanner and will talk you through every step using the headphones.

Studies requiring more than one scan on any day will involve some waiting between scans. There is a comfortable volunteers' lounge next to the scanner where you can sit between tests. It is worth bringing a book, tablet or something else to pass the time.
Toilets are just next door and easily accessible should you need them during the day.

Preparing for your MRI

What you do on the day before each study visit can make an important difference. Excess alcohol, eating particular foods and extreme sport activity can all alter the way your bowel behaves so please avoid any of this. If necessary we will give you a dietary information sheet that goes into detail. Please ask if you are unsure.

In some studies we will ask you to fast before your scan. Staff will always be close by and you will be able to ask them any questions.

Are there any risks?

Before any scanning takes place you will be asked to fill out a questionnaire to identify any reason that might make if unsafe to scan you, like having metal implants in your body. Piercings may need to be removed while you are in the scanner. You may still be safe if you raise an issue on the questionnaire but it acts as an alert to protect volunteers so please fill it in as accurately as possible. Some studies will not enrol people who cannot go in the scanner; some will enrol you in the rest of the study but exclude you from the scanning part.

A few people feel claustrophobic in the scanner but most do not. Scans rarely last longer than 15 minutes. MRI uses radio waves similar to those used in radio and TV transmission. These have a much lower energy than X-rays and as such are considered biologically safe. This study will be carried out using a 1.5T magnet, within national safety guidelines. Scanning with magnets this size is already a routine procedure in medical practice, and is considered to be safe, apart from certain circumstances (which we ask about in a safety questionnaire) that exclude you from taking part.

What if I am pregnant (or might be)?

If you are pregnant we would advise you do not participate in the MRI portion of this study. This will not prevent you from participating in the remainder of the study. Pregnancy tests are available in the toilets at all magnets if you think you might be pregnant and want to check.

IMPORTANT:

What happens if we notice something abnormal on your scan?

While it is unlikely that your scan will show any abnormality, the Sir Peter Mansfield Magnetic Resonance Centre (the SPMMRC) is NOT a clinical diagnostic facility and the scans we collect are NOT the same as scans collected by doctors for medical purposes. The pictures will NOT usually be looked at by a radiologist (a doctor qualified to find abnormalities in scans), so we may not see something that a radiologist would detect. This test does NOT replace any tests that your doctor thinks might be needed.

However, it is possible that one of the team working with your scans might notice something that they consider abnormal. If this happens we will ask a radiologist to review the scan. If he/ she thinks that further action is necessary we will inform your GP. Your GP may refer you to other medical specialists for further clinical investigation. It is a requirement of the SPMMRC that you give us permission to inform your GP of such a finding before we enrol you into the study.

In the unlikely event that we do notice something abnormal on your scan, giving you that information might have the benefit of allowing you to start treatment earlier than you would have otherwise. However, it is also important for you to realise that providing you with this information may affect your future ability to find employment and obtain insurance.

The chances of detecting an abnormality depend on the region of your body we are scanning, the methods we are using to scan you, your age and your health.

Your Data:
All information collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office and on a password protected database. Any information about you which leaves the research facility will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Who to contact for more information:

Contact details for the research team are given on the main information sheet. You can also contact the SPMMRC directly on 0115 9514747.

Food diary and guide

Food and Drink Diary

A randomised controlled trial of the effect of the low FODMAP diet and dietary oligofructose on gastrointestinal form, function and microbiota in healthy volunteers “FOG”

Study Number										
SPMMRC Volunteer Number										
Date of Birth										
Diary number (1 or 2)										
Date Diary Started										

Thank you for filling in this diary

Filling it in accurately, each day, is crucial to the research that we are doing

If you have ANY questions or concerns about how to fill it in, please contact the research team for advice

Day of the week		Date	

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
6 am to 9am			
9 am to 12 pm			
12 pm to 2 pm			
2pm to 5pm			

5pm to 8 pm			
8 to 10 pm			

Did you take any vitamins, minerals or other food supplements today? Yes ☐ No ☐

If yes, please describe the supplements you took below

Brand	Name (in full) including strength	Number of pills, capsules, teaspoons

Please record details of any recipes or ingredients of made up dishes or take-away dishes (if not already described).

NAME OF DISH:		SERVES:	
Ingredients	Amount	Ingredients	Amount
Brief description of cooking method			

Food and Drink diary Guide

Instructions for completing your Food and Drink Diary

PLEASE READ BEFORE STARTING YOUR FOOD AND DRINK DIARY

This guidance provides information about recording what you eat and drink.

Completing your diary:

1. Day and date

   Please write down the day and date at the top of the page each time you start a new day of recording.

2. Time slots

   Please note the time of each eating occasion into the space provided. For easy use each day is divided into sections, from the first thing in the morning to late evening and through the night.

3. What do you eat?

   Please describe the food you eat in as much detail as possible. Be as specific as you can. Pages 7-14 of this booklet will help with the sort of detail we need, like cooking methods (fried, grilled, baked etc) and any additions (fats, sugar/sweeteners, sauces, pepper etc).

4. Homemade dishes

   If you have eaten any homemade dishes e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking method. Write this down in the recipe section at the end of the record day. Record how much of the whole recipe you have eaten in the portion size column.

5. Takeaways and eating out

   If you have eaten takeaways or made up dishes not prepared at home such as at a restaurant or a friend's house, please record as much detail

about the ingredients as you can e.g. vegetable curry containing chickpeas, aubergine, onion and tomato.

6. Brand name

Please note the brand name (if known). Most packed foods will list a brand name, e.g. Bird's eye, Hovis, or Supermarket own brands.

7. Portion sizes

Examples for how to describe the quantity or portion size you had of a particular food or drink are shown in this booklet.

For foods, quantity can be described using:

- Household measures, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy. Be careful when describing amounts in spoons that you are referring to the correct spoon size. Compare the spoons you use with the life size pictures in your participant diary.
- Weights from labels, e.g. 4oz steak, 420g tin of baked beans, 125g pot of yogurt.
- Number of items, e.g. 4 fish fingers, 2 pieces of chicken nuggets, 1 regular size jam filled doughnut.
- Picture examples for specific foods are shown on pages 15-17 of this booklet.

For drinks, quantity can be described using:

- The size of glass, cup etc (e.g. large glass) or the volume (e.g. 300ml). Examples of typical drinks containers are on page 18 of this booklet.
- Volumes from labels (e.g. 330ml can of fizzy drink).

We would like to know the amount that was actually consumed which means taking leftovers into account. You can do this in two ways:

- Record what was served and make notes of what was not eaten e.g. 3 tbsp of peas, only 2 tbsp eaten; 1 large sausage roll, ate only ½.
- Only record the amount actually eaten i.e. 2 tbsp of peas, ½ a large sausage roll.

8. Supplements

At the end of each recording day there is a section for providing information about any supplements you took. Brand name, full name of supplement, strength and the amount taken should be recorded.

9. When to fill in the diary

Please record your eating and drinking as you go, not from memory at the end of the day. Use written notes on a pad if you forget to take your diary with you. Each diary day covers a 24 hour period, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water.

10. Completed diary examples

On pages 3-6 of this booklet you can see an example day that has been filled in. This shows you how we would like you to record your food and drink, for example a ready meal and a homemade dish.

With thanks to the Medical Research Council for Human Nutrition Research for permission to use this Food and Drink Diary design.

Day the week	**6.2.1** Thurs	***6.2.2*** Date	**6.2.3** 31 March

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
6 am to 9am			
6.30 am 7.30 am	Filter coffee, decaffeinated Milk (fresh, semi-skimmed). Sugar (white) Filter coffee with milk and sugar Cornflakes Milk (fresh, semi-skimmed) Toast, granary medium sliced Light spread Marmalade	Douwe Egberts Silverspoon As above Tesco's own Hovis Flora Hartleys	Mug A little 1 level tsp As above Picture 1b Drowned 1 slice Med spread 1 heaped tsp
9 am to 12 noon			
10.15 am 11 am	Instant coffee, not decaffeinated Milk (fresh, whole) Sugar brown Digestive biscuit – chocolate coated on one side	Kenco McVities	Mug A little 1 level tsp 2

12 noon to 2pm			
12.30 pm	Ham salad sandwich from home Bread, wholemeal, thick sliced Light spread Low fat Mayonnaise Smoked ham thinly sliced Lettuce, iceberg Cucumber with skin Unsweetened orange juice from canteen Apple with skin from home, Braeburn	Tesco's own Flora Hellmans Tesco's own Tropicana	2 slices thin spread on 1 slice 2 teaspoons 2 slices 1 leaf 4 thin slices 250ml carton medium size, core left
2pm to 5pm			
3 pm	Tea, decaffeinated Milk (fresh, whole) Jaffa cake – mini variety	Twinings Tesco's own McVities	Mug Some 6

5pm to 8pm			
6.30 pm 8 pm	Gin Tonic water diet Lager Salted peanuts Spaghetti, wholemeal Bolognese sauce (see recipe) Courgettes (fried in butter) Tinned peaches in juice (juice drained) Single cream UHT Orange squash No Added Sugar	Gordon's Schweppes Draught KP Tesco's own Prince's Sainsbury's own	Single measure 1/2 small glass 1 pint 1 handful Picture 3b 6 tablespoons 4 tablespoons 3 halves 1 tablespoon 200ml glass, 1 part squash, 3 parts tap water
8pm to 10pm			
9 pm	Grapes, green, seedless Chocolates, chocolate creams Potato crisps, Prawn Cocktail	 Bendicks Walkers	15 2 25g bag (from multipack)
10pm to 6am			
10.30 pm	Camomile tea (no milk or sugar) Fairy cake	Twinings homemade	1 mug 1

Did you take any vitamins, minerals or other food supplements today? Yes ☐ No ☐

Brand	Name (in full) including strength	Number of pills, capsules, teaspoons
Holland & Barrett	Evening Primrose Oil – 1000 mg	1 capsule
Holland & Barrett	Vitamin E – 400 IU	1 capsule

Please record details of any recipes or ingredients of made up dishes or take-away dishes (if not already described).

NAME OF DISH: *Fairy cakes*			SERVES: *makes 20 cakes*
Ingredients	Amount	Ingredients	Amount
Tate & Lyle caster sugar	175g	Silver Spoon icing sugar	140g
Anchor butter, unsalted	175g	Yellow food colouring	3 drops
Eggs	3	Water	2 tablespoons
Homepride self-raising flour	175g		
Baking powder	1 teaspoon		
Brief description of cooking method Mix together and bake for 15 min. Mix icing sugar with water and add colouring. Approx. 1 teaspoon of icing on each cake			

Examples and advice on food descriptions

This table explains the detail we'd like you to provide in your Food and Drink Diary for the different foods and drinks listed.

Food/drink	Description and preparation	Portion size or quantity
Bacon	Back, middle, streaky; smoked or un-smoked; fat eaten; dry-fried or fried in oil/fat (type used) or grilled rashers	Number of rashers
Baked beans	Standard, reduced salt or reduced sugar	Tablespoons, weight of beans marked on tin label (e.g. 420g)
Beefburger (hamburger)	Home-made (ingredients), from a packet (brand name) or take-away; fried (type of oil/fat), microwaved or grilled; economy; with or without bread roll	Number, large or small, ounces or in grams if info on package
Beer	What sort e.g. stout, bitter, lager; draught, canned, bottled; low-alcohol or home-made	Number of pints or half pints, size of can or bottle
Biscuits	What sort and brand e.g. cheese, wafer, crispbread, sweet, chocolate, shortbread, home-made	Number, size (standard or mini variety)
Bread (see also sandwiches)	Wholemeal, granary, white or brown; currant, fruit, malt; large or small loaf; sliced or unsliced loaf; give brand	Number of slices; thick, medium or thin slices
Bread rolls	Wholemeal, white or brown; alone or with filling; crusty or soft	Size, number of rolls
Breakfast cereal (see also porridge)	What sort and brand e.g. Kellogg's cornflakes; any added fruit and/or nuts; Muesli – added sugar and/or fruit	Tablespoons or picture 1

Food/drink	Description and preparation	Portion size or quantity
Bun	Iced, currant or plain, homemade or bought (brand name)	Large or small, number
Butter, margarine & fat spreads	Give full product name	Thick/average/thin spread; spoons
Cake	Individual or piece of large; type and brand; fruit (rich), sponge, fresh cream, buttercream, iced; type of filling	Number, slices, packet weight, see picture 10 for sponge cake
Cheese	Name, brand and type e.g. cheddar, cream, cottage, soft; low fat	picture 9, or number of slices, thick or thin cut, number of spoons
Chips	Fresh, frozen, oven, microwave, take-away (where from); thick/straight/crinkle/fine cut; type of oil/fat used for cooking give brand name	picture 4, as A, B, or C or 2 x B, etc
Chocolate(s)	What sort e.g. plain, milk, white, fancy, diabetic; type of filling; give brand name	Number, weight/size of bar
Coffee	With milk (see section on milk); half milk/half water; all milk; ground/filter, instant; decaffeinated; give brand name	Cups or mugs
Cream	Single, whipped, double or clotted; dairy or non-dairy; low-fat; fresh, UHT/Longlife; imitation cream e.g. Elmlea	Tablespoons

Food/drink	Description and preparation	Portion size or quantity
Crisps	What sort e.g. potato, corn, wheat, maize, vegetable etc; give brand; flavour; low-fat or low-salt; premium variety e.g. Kettle chips, Walker's Sensations	Packet weight
Custard	Pouring custard or egg custard; made with powder and milk/sugar, instant, ready to serve (tinned or carton); low fat, sugar free, brand	Tablespoons
Doughnut	Plain, jam, cream or iced; round or ring, where bought/brand name	Number, size e.g. mini, large
Egg	Boiled, fried (type of oil/fat), scrambled (type of fat used, with or without added milk), poached, omelette (with or without filling, type of oil/fat used), etc	Number of eggs, large, medium or small eggs
Fish (including canned)	What sort and brand e.g. cod, tuna; fried (type of oil/fat), grilled, poached (water or milk) or steamed; with batter or breadcrumbs; canned in oil, brine or tomato sauce	Size of can or spoons (for canned fish) or picture 7 for battered fish
Fish cakes & fish fingers	Type of fish; plain or battered or in breadcrumbs; fried, grilled, baked or microwaved; economy	Size, number, packet weight
Fruit -fresh	What sort; eaten with or without skin	Size, number

Food/drink	Description and preparation	Portion size or quantity
Fruit - stewed/canned	What sort; sweetened or unsweetened; in fruit juice or syrup; juice or syrup eaten	Tablespoons Size of can or weight on can
Fruit – juice (pure)	What sort and brand e.g. apple, orange; sweetened or unsweetened; pasteurised or UHT/Longlife; freshly squeezed; added vitamins/minerals, omega 3?	Glass (size or volume) or carton size
Ice cream	Flavour; dairy or non-dairy; brand name; luxury/premium; added nuts, fruit	Number of tablespoons/ scoops
Jam, honey	What sort; low-sugar/diabetic; shop bought/brand or homemade	Teaspoons, heaped or level, or thin or thick spread
Marmalade	Type and brand; low-sugar; thick cut; shop bought/brand or homemade	Teaspoons, heaped or level, or thin or thick spread
Meat (see also bacon, burgers & sausages)	What sort; cut of meat e.g. chop, breast, minced; lean or fatty; fat removed or eaten; skin removed or eaten; how cooked; with or without gravy	Large/small/medium, tablespoons, or picture 6 for stew portion
Milk	Brand and type (whole, semi-skimmed, skimmed); fresh, sterilized, UHT, dried; soya milk (sweetened/unsweetened) , goats' milk, rice milk; flavoured; fortified with added vitamins and/or minerals	Pints, glass (size or volume) or cup. For milk on cereal: *damp/normal/drowned* . For milk in tea/coffee: *a little/some/a lot*

Food/drink	Description and preparation	Portion size or quantity
Nuts	What sort and brand; dry roasted, ordinary salted, honey roasted; unsalted	Packet weight, handful
Pie (sweet or savoury)	What sort and brand; individual or helping; one pastry crust or two; type of pastry	Individual or slice, or picture 8
Pizza	Thin base or deep pan or French bread; topping; brand name and type	Individual, slice, fraction of large pizza e.g. ¼
Porridge	Brand name; made with oats or cornmeal or instant oat cereal; made with milk and/or water; with sugar or honey; with milk or cream	Bowls
Potatoes (see also chips)	Old or new; baked, boiled, roast (type of oil/fat); skin eaten; mashed (with butter/spread and with or without milk); fried/chips (type of oil/fat); instant; any additions e.g. butter	Mash – tablespoons, number of half or whole potatoes, small or large potatoes, or picture 4 for chips portion
Pudding	What sort; e.g. steamed sponge; with fruit; mousse; instant desserts; milk puddings	Tablespoons, picture 10 for slice of sponge
Rice	What sort; e.g. basmati, easy cook, long or short grain; white or brown; boiled or fried (type of oil/fat); brand name	Tablespoons or picture 2
Salad	Ingredients; if with dressing what sort (oil and vinegar, mayonnaise); brand name of dressing	Amount of each component; e.g. number of tomatoes, slices of cucumber, leaves; tablespoons of dressing

Food/drink	Description and preparation	Portion size or quantity
Sandwiches and rolls	Type of bread/roll (see Bread & Rolls); butter or margarine; type of filling; including salad, mayonnaise, pickle etc. If shop-bought, where from?	Number of rolls or slices of bread; amount of butter/margarine (on both slices?); amount of filling
Sauce – cold (including mayonnaise)	Tomato ketchup, brown sauce, soy sauce, salad cream, mayonnaise; low fat; brand name	Teaspoons, tablespoons
Sausages	What sort; e.g. beef, pork; fried (type of oil/fat) or grilled; low fat; economy; brand name	Large or small, number
Sausage rolls	Type of pastry; brand name	Number, size e.g. jumbo, standard, mini
Scone	Fruit, sweet, plain, cheese; type of flour; bought/brand or homemade	Number, small, medium or large
Savoury snacks -in packet	What sort: e.g. Cheddars, cheese straws, Twiglets, Pretzels; give brand name	Size (standard or mini variety), packet weight, number
Soft drinks – squash/ concentrate/cordial	Give brand name & flavour; no added sugar/low calorie/sugar free; “high” juice; fortified with added vitamins and/or minerals	Glass (size or volume)
Soft drinks – carbonated/fizzy	Give brand & flavour; diet/low-calorie; canned or bottled; cola – caffeine free	Glass, can or bottle (size or volume)
Soft drinks – ready to drink	Give brand & flavour; no added sugar/low calorie/sugar free; does it contain real fruit juice, if so, how much?; fortified with added vitamins and/or minerals	Glass, carton or bottle (size or volume)

Food/drink	Description and preparation	Portion size or quantity
Soup	What sort; give brand name; cream or clear; canned, packet, instant or vending machine, home-made	Tablespoons, bowl or mug
Spaghetti, other pasta	What sort; fresh/chilled or dried; white, wholemeal; canned in sauce; type of filling if ravioli, cannelloni etc	Tablespoons (or how much dry pasta used per portion in grams/packet size) or picture 3
Spirits	What sort: e.g. whisky, gin, vodka, rum	Measures as in pub
Sugar	Added to cereals, tea, coffee, fruit, etc; what sort; e.g. white, brown, demerara	Heaped or level teaspoons
Sweets	What sort: e.g. toffees, boiled sweets, diabetic; give brand name	Number, packet weight
Tea	With/without milk (see section on milk); decaffeinated, herb	Mugs or cups
Vegetables (not including potatoes)	What sort; how cooked or raw; additions e.g. butter, other fat or sauce	Tablespoons, number of florets or sprouts, weight from tins or packet as guidance
Water	Tap, filtered, bottled: give brand name	Glass or bottle (size or volume)
Wine, sherry, port	White, red; sweet, dry; low-alcohol; give brand name	Glass (size or volume)

Food/drink	Description and preparation	Portion size or quantity
Yoghurt, fromage frais	What sort: e.g. natural/plain or flavoured; creamy, Greek, low-fat, very low fat/diet, soya; with fruit pieces or just fruit flavoured; twinpot with separate cereal/crumble; fortified with added vitamins and/or minerals; brand name	Pot size or tablespoons
Home-made dishes	Please say what the dish is called (record recipe or details of dish if you can in the section provided) and how many persons it serves	Tablespoons – heaped or level, number, size
Ready-made meals	Please give brand name and full description of product; did it contain any accompaniments e.g. rice, vegetables, sauces; was it chilled or frozen; microwaved, oven cooked, boil-in-the-bag; was it low fat, healthy eating range. Enclose label and ingredients list if possible in your plastic bag	Packet weight, if not whole packet describe portion consumed
Take-away food or food eaten out	Please say what the dish is called and give main ingredients if you can. Give name of a chain restaurant e.g. McDonalds	Tablespoons, portion size e.g. small/medium/large

Pictures for food portion guidance

Use the pictures to help you indicate the size of the portion you have eaten. Write on the food record the picture number and size A, B or C nearest to your own helping.

Remember that the pictures are much smaller than life size. The actual size of the dinner plate is 10 inches (25cm), the side plate, 7 inches (18cm), and the bowl, 6.3 inches (16cm).

The tables in your food diary guidance booklet also give examples of foods that you might eat and how much information is required about them.

Breakfast cereals







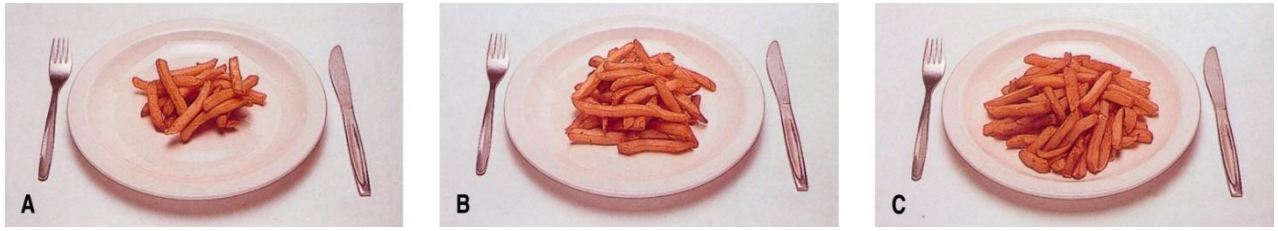
Rice



Spaghetti



Chips



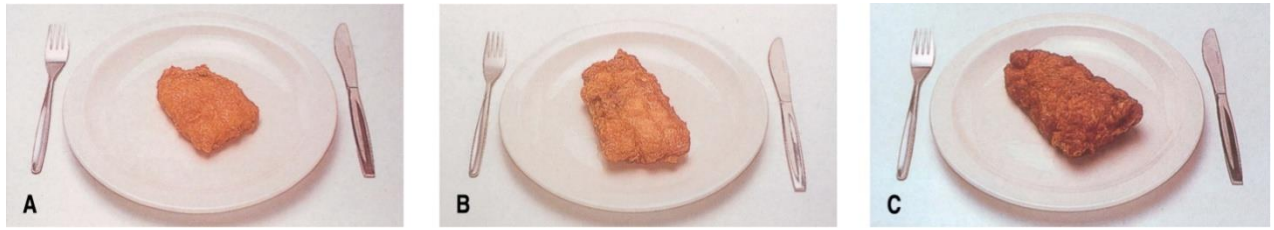
Broccoli or cauliflower



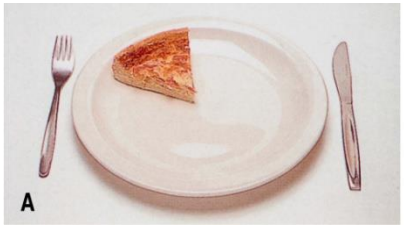
Stew or curry

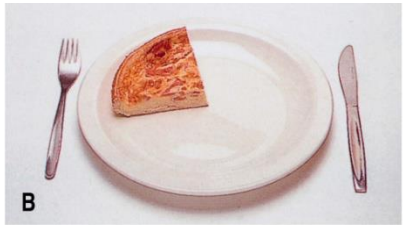


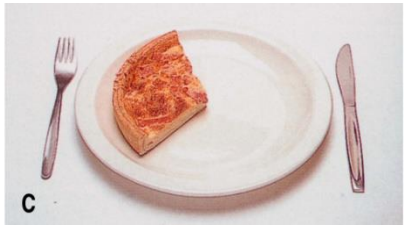
Battered fish



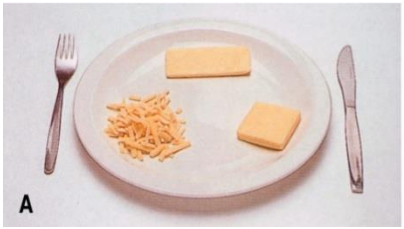
Quiche or pie

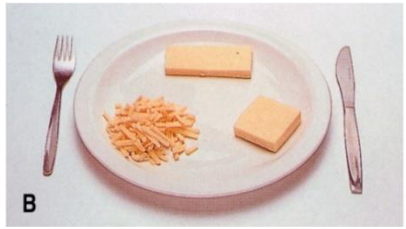


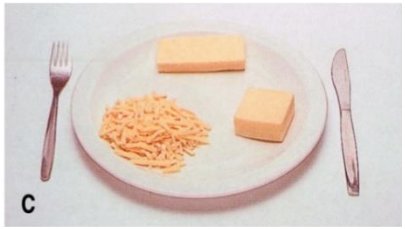




Cheese







Note: in each picture the slice, cube and pile of grated cheese represent the same weight of cheese. If you record the amount of cheese you've eaten as e.g. 'Picture 9a', your record will refer to just one of the three (slice, cube, grated) on the plate in picture 9a.

Sponge cake

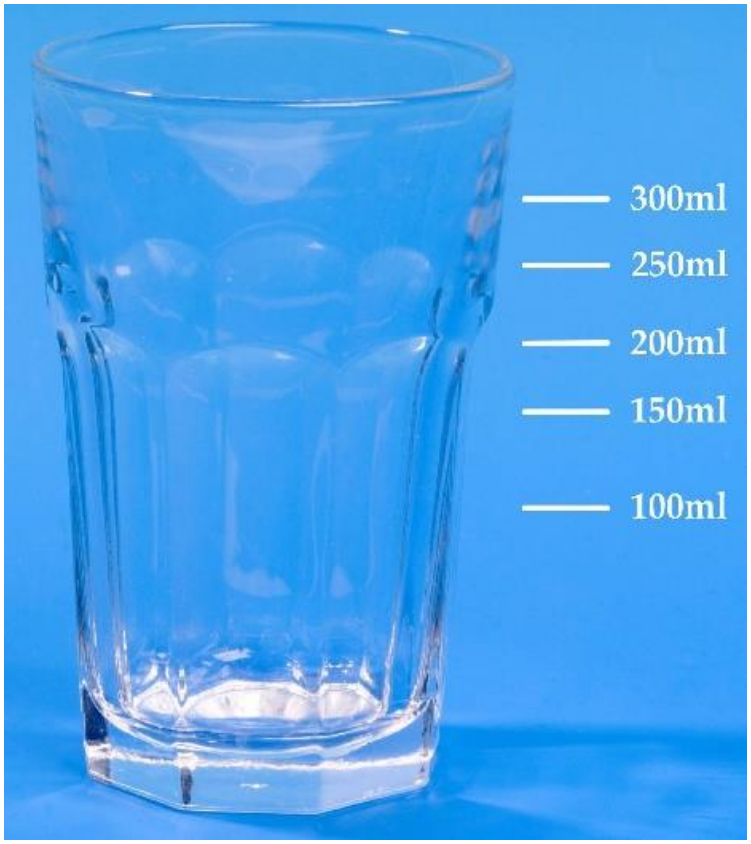


Typical quantities of drinks in various containers measured in millilitres (ml)

	Small glass	Average glass	Large glass	Vending cup	Cup	Mug
Soft drinks	150	200	300			
Wine	125	175	250			
Hot drinks				170	190	260

Glasses come in different shapes and sizes. On this page is a life-size glass showing approximate volumes. You can use this picture as a guide for estimating how much volume of drink the glass holds that you are drinking from.

Life size glass



Life size spoons

Teaspoon

(5 ml)



Dessert spoon

(10 ml)



Tablespoon

(15 ml)

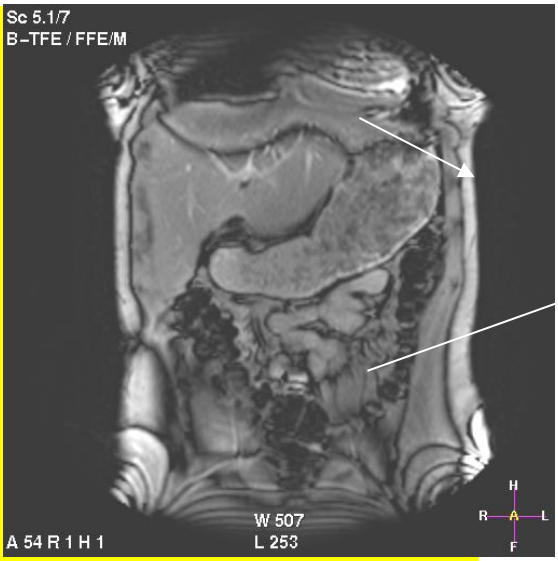


Poster FOG study

Sir Peter Mansfield Magnetic Resonance Centre

and Nottingham Digestive Diseases Centre

University of Nottingham



Healthy volunteers required for an MRI study of a food supplement.

We can email you a picture of your scan, and a disturbance allowance will

Call 0115 9514747 and ask for Lesley Martin or

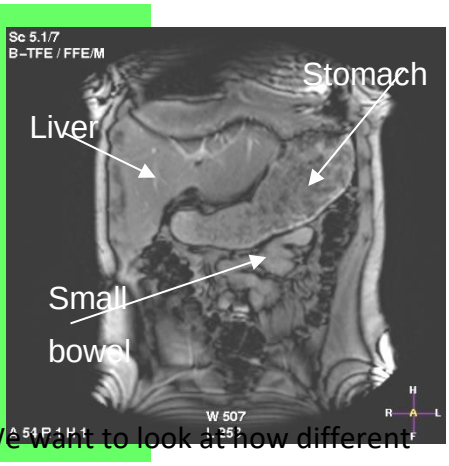
email lesley.martin@nottingham.ac.uk and ask about the FOG study, or text FOG to 07786 203250 (text only) and we will call you back

Apple Study Poster

Nottingham Digestive Diseases Centre

and Sir Peter Mansfield Imaging Centre

University of Nottingham



Healthy volunteers required for a MRI study. We want to look at how different physical form of apples effect absorption in the gut.

We'll give you a picture of your scan, and a disturbance allowance will be provided.

Call 0115 9514747 and ask for Lesley Martin or

email lesley.martin@nottingham.ac.uk and ask about the APPLE study, or

text APPLE to 07786 203250 (text only) and we will call you back

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Lesley.Martin @nottingham.ac.uk Lesley.Martin @nottingham.ac.uk Lesley.Martin @nottingham.ac.uk Lesley.Martin @nottingham.ac.uk

CRAIGS study poster

The University of Nottingham

NDDC
Nottingham Digestive Diseases Centre



NHS
National Institute for Health Research

How is your tummy feeling?

Managing Irritable Bowel Syndrome with the Low FODMAP diet

Irritable Bowel Syndrome (IBS) patients needed!

We seek to assess food intake and provide dietitian-delivered dietary intervention which ***may help to relieve your symptoms***

The trial involves 3-4 visits to the NDDC, MRI scans, breath tests and possibly 1 blood test if required; ***you will also be provided with an inconvenience allowance*** for your participation.

CONTACT: SHANTHI KRISHNASAMY (msxsk@nottingham.ac.uk)

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Carbohydrate Reduction and Impact on Gastrointestinal System
Study flyer Final version 1.0
10 September 2015

Questionnaires

Satiety questionnaire

Time point: …… Visit Number:……

Date:……

Participant Number				Initials				DoB	D	D	M	M	Y	Y

Please make a vertical mark through the horizontal line to show how you feel at the moment.
Left and right extremes represent minimum and maximum values respectively.

How hungry do you feel?

I am not hungry at all — I have never been more hungry

How satisfied do you feel?

I am completely empty — I cannot eat another bite

How full do you feel?

Not at all — Totally full

How strong is your desire to eat?

Very weak — Very strong

How much do you think you can eat?

Nothing at all — A lot

Baseline Symptom Questionnaire

Effect of different physical form of apples on gastrointestinal function and satiety: a MRI study

Visit Number ……

Participant Number				Initials				DoB	D	D	M	M	Y	Y

Baseline Symptom Questionnaire We want to know about any symptoms you are having before you consume the drink

Symptom category: consider if you are experiencing each symptom at the present time	**Visual Analogue Scale:** mark the point on the line that best describes how bad each symptom is 0 = 'not at all' ; 100 = 'as bad as it could be'
Gas/ Flatulence:	0 100
Bloating:	0 100
Abdominal Pain:	0 100
Diarrhoea:	0 100

IBS-SSS Questionnaire

Carbohydrate reduction and impact on gastrointestinal system (CRAIGS)
(Final Version 1.1 06.11.15)
CRAIGS STUDY
Irritable Bowel Syndrome Severity Score (IBSSS) Part 1

BRU-ID			-							
Study Number										
Date of Birth										
Visit										
Date										

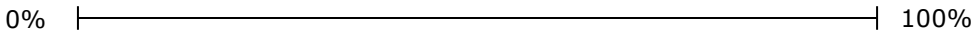
INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your symptoms. Please try and answer the questions based on how you currently feel (i.e. over the last 10 days or so).

The questions require you to put a cross on a line which enables us to judge the severity of a particular problem. For example:

How severe was your pain?
Please place your cross (×) anywhere on the line between 0-100% in order to indicate as accurately as possible the severity of your symptom.

This example shows a severity of approximately 90%:



Please turn over and complete the questionnaire

Research team to fill in:

Symptom	Pain	Bloating	Bowel Habit	QoL	Days of pain	Total

1. Pain

a. Do you currently suffer from abdominal (tummy) pain?

YES	*NO*

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

0 100

% not very quite severe very severe severe %

c. Please enter the number of days that you get the pain in every 10 days. For example, if you enter 4 it means you get pain 4 out of 10 days. If you get pain every day enter 10

Number of days with pain

2. Bloating/ Distension

a. Do you currently suffer from abdominal distension*?
(bloating, swollen or tight tummy)
*women, please ignore distension related to your periods

YES	*NO*

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

0 100

% no distension not very severe quite severe severe very severe %

3. Bowel Habit

How satisfied are you with your bowel habit?

0% 100%

Very happy quite happy quite unhappy very unhappy

4. Life in General

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

0% 100

0%

Not at all not much quite a lot completely

IBS-GIS Questionnaire

Carbohydrate reduction and impact on gastrointestinal system
Final Version 1.1 06.11.15
CRAIGS STUDY
IBS-GIS+: Irritable Bowel Syndrome Global Improvement Score (+ adequate control)

BRU-ID			**-**							
Study Number										
Date of Birth										
Visit										
Date										

INSTRUCTIONS

Compared to the way you usually felt during the 3 months before you entered the study, are your bowel symptoms over the past 2 weeks:

Substantially worse	☐
Moderately worse	☐
Slightly worse	☐
No Change	☐
Slightly improved	☐
Moderately improved	☐
Substantially improved	☐

Over the past 2 weeks have your bowel symptoms been adequately controlled?

Yes	☐
No	☐

EQ-5D-5L

Carbohydrate reduction and impact on gastrointestinal system

Final Version 1.1 06.11.15
CRAIGS STUDY

BRU-ID			-				
Study Number							
Date of Birth							
Visit							
Date Today							

Health Questionnaire

English version for the UK

UK (English) v.2 © 2009 EuroQol Group. EQ-5D™ is a trade mark of the EuroQol Group

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

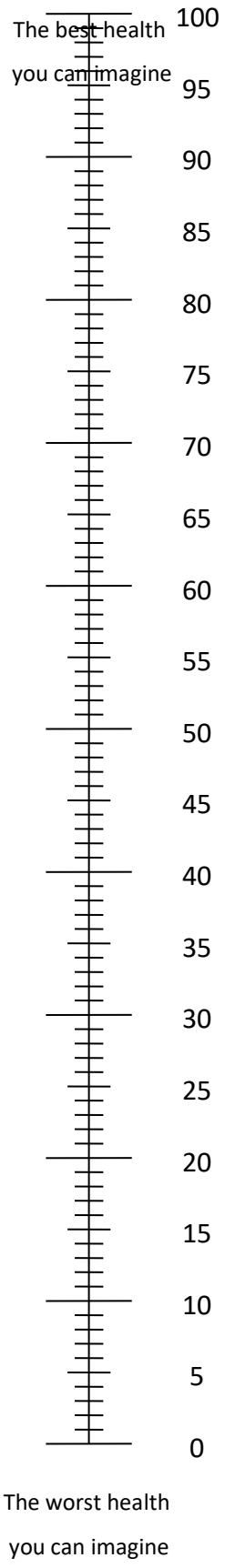
PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is



TODAY.

- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY =

HADS Questionnaire

Carbohydrate reduction and impact on gastrointestinal system Final Version 1.1 06.11.15
CRAIGS STUDY

BRU-ID			-				
Study Number							
Date of Birth							
Visit							
Date							

1. I feel tense or 'wound up':		2. I still enjoy the things I used to enjoy:	
Most of the time		Definitely as much	
A lot of the time		Not quite as a much	
Sometimes		Only a little	
Never		Hardly at all	
3. I get a sort of frightened feeling as if something awful is about to happen:		4. I can laugh and see the funny side of things:	
Definitely and quite badly		As much as I always could	
Yes, but not too badly		Not quite as much now	
A little, but it doesn't worry me		Definitely not as much now	
Never		Never	
5. Worrying thoughts go through my head:		6. I feel cheerful:	
All of the time		Never	
A lot of the time		Not often	
Sometimes, but not too often		Sometimes	
Rarely		Most of the time	
7. I can sit at ease and feel relaxed:		8. I feel as if I am slowed down:	
Definitely		Nearly all the time	
Usually		Very often	
Not often		Sometimes	
Never		Never	
9. I get a sort of frightened feeling like 'butterflies' in the stomach:		10. I have lost interest in my appearance:	
Never		Definitely	
Occasionally		I don't take as much care as I should	
Quite often		I may not take quite as much care	
Very often		I take just as much care as ever	
11. I feel restless, as if I have to be on the move:		12. I look forward with enjoyment to things:	
Very much		As much as I ever did	
Quite a lot		Somewhat less than I used to	
Not very much		Definitely less than I used to	
Never		Hardly at all	
13. I get sudden feelings of panic:		14. I can enjoy a good book or TV program:	
Very often		Often	
Quite often		Sometimes	
Not very often		Not often	
Rarely		Rarely	

Please complete each of the following questions, checking the one response that comes closest to how you were feeling **one month ago**.