Managing irritable bowel syndrome

Dietary approaches and food intolerance

Sanna Nybacka

Department of Internal Medicine and Clinical Nutrition
Institute of Medicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2021

Cover illustration: Good gut feelings by Sanna Nybacka

Managing irritable bowel syndrome – dietary approaches and food intolerance

sanna.nybacka@gu.se

ISBN 978-91-8009-260-9 (PRINT)
ISBN 978-91-8009-261-6 (PDF)

Printed in Borås, Sweden 2021
Printed by Stema Specialtryck AB

To my dear son Stellan,
for constantly reminding me of the joy in learning new things.

“Student: Dr. Einstein, aren’t these the same
questions as last year's final exam?

Dr. Einstein: Yes; But this year the answers are different.”

-Albert Einstein

Managing irritable bowel syndrome

Dietary approaches and food intolerance

Sanna Nybacka

Department of Internal Medicine and Clinical Nutrition, Institute of Medicine
Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden

ABSTRACT

Irritable bowel syndrome (IBS) is a complex disorder where diet plays a pivotal role in symptom generation for many patients. The aim of this thesis was to explore how diet and self-perceived food intolerance relate to gastrointestinal (GI) symptoms among patients with IBS, and within different manifestations of IBS.

In Paper I, reported dietary intake of fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) were characterized among patients with IBS. Intakes varied more between subjects than within, leading to an acceptable precision in diet estimates when data rankings are used. In Paper II cross-sectional data were used to explore how FODMAP intake relates to GI symptoms in IBS patients with different subtypes. Although reported FODMAP intake appeared to be similar between IBS subtypes, only in unsubtyped IBS a strong relationship between excess fructose intake and GI symptom severity was found. NMR metabolomics from serum and urine derived from a randomized controlled dietary trial among patients with IBS in Paper III, did not reveal any consistent pattern in principal component analysis (PCA) regarding reported baseline dietary intake. When evaluating the changes in metabolite concentrations, several metabolites seemed to distinguish responders and non-responders to the dietary modifications. In Paper IV, it was shown that atopic disease is a common IBS comorbidity, but presence of self-reported food intolerance/allergy or atopic disease did not relate to IBS symptom severity. In this analysis, female gender, other somatic symptoms and the number of food items reported to cause GI symptoms were associated with increased IBS symptom severity.

In conclusion, both habitual intake of FODMAPs and how one reacts to FODMAP intake seem to vary in this heterogenous patient group. Studies evaluating separate FODMAP components and individual tolerance to these are further warranted.

Keywords: Irritable bowel syndrome, diet, FODMAP, food intolerance

ISBN 978-91-8009-260-9 (PRINT)
ISBN 978-91-8009-261-6 (PDF)

SAMMANFATTNING PÅ SVENSKA

Irritable bowel syndrome (IBS), eller irritabel tarm, är en mycket vanlig funktionell mag-tarmsjukdom - inte bara i Sverige utan även i resten av världen. Sjukdomen kan yttra sig på olika vis men det alla med IBS har gemensamt är en upplevelse av återkommande smärta i magen, samt att avföringskonsistens och frekvens är onormala. Varför man drabbas av IBS är fortfarande till stor del oklart, men många anger att symptomen triggas igång eller förvärras utav mat. Besvären kan uppstå i samband med måltid, men symptom kan också vara förknippat med intag av enskilda livsmedel. Den här avhandlingen syftar till att bidra med kunskap kring hur kost och födoämnesintolerans kan påverka symptom hos patienter som lider av IBS.

I **delarbete I** beskrivs det rapporterade intaget av fermenterbara oligo-, di-, monosackarider och polyoler (FODMAP) bland 197 individer med IBS, med fokus på att karakterisera variation i intag inom och mellan individer. Med hjälp utav variationskoefficienterna gick det sedermera att beräkna att antalet dagar som krävs för att fånga intag av FODMAP på individnivå är svår att uppnå med god precision. Dock fanns det en stor variation mellan individer i rapporterat intag, vilket gör det statistiskt lämpligt att rangordna individer i kvartiler av kostintag.

Hur habituellt intag av FODMAPs var kopplat till symptomsvårighet hos individer med IBS, och inom olika subtyper av IBS, beskrevs i **delarbete II**. Vi fann att intag av FODMAPs var relativt likartat inom de olika subtyperna, förutom att kvinnor med mestadels lös avföring rapporterade ett något lägre intag av laktos. Det fanns ingen tydlig koppling mellan intag av FODMAPs och grad av IBS symptom, förutom hos kvinnor som har en okategoriserad IBS; där var intag av fruktos starkt kopplat till symptomsvårighet.

I **delarbete III** använde vi serum- och urinprover från individer som medverkat i en randomiserad kontrollerad kostinterventionsstudie. Med hjälp av metabolomik försökte vi särskilja metabolitmönster bland individer som svarat väl på behandling, kontra individer som inte svarat på behandling. Flertalet metaboliter tycktes särskilja grupperna åt, framför allt bland individer som behandlats med en låg-FODMAP kost. Flera av metaboliterna skulle kunna kopplas till livsmedelsgrupper eller specifika livsmedel som ingick i interventionskosten.

I **delarbete IV** såg vi att atopiska besvär var vanligt bland patienter med IBS, men det tycktes inte förekomma i högre grad jämfört med en kontrollgrupp av individer utan IBS. Atopi och självrapporterad födoämnesintolerans/allergi var inte kopplat till symptomsvårighet av IBS, men det var däremot kvinnligt kön, förekomst av andra kroppsliga symptom och antalet livsmedel man upplevde orsaka symptom.

Sammanfattningsvis så varierar intaget av FODMAP, och även hur man reagerar på FODMAP-intag, i denna heterogena patientgrupp. Framtida studier bör fokusera på enskilda komponenter av FODMAP och utvärdera individuell tolerans gentemot dessa.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Nybacka S, Störsrud S, Liljebo T, Le Nevé B, Törnblom H, Simrén M, Winkvist A.
Within- and between-subject variation in dietary intake of fermentable oligo-, di-, monosaccharides, and polyols among patients with irritable bowel syndrome.
Curr Dev Nutr. 2018;3(2):nzy101.

II. Nybacka S, Störsrud S, Lindqvist H, Törnblom H, Simrén M, Winkvist A.
Habitual FODMAP intake in relation to symptom severity and pattern in patients with irritable bowel syndrome.
Nutrients. 2020;13(1):27.

III. Nybacka S, Simrén M, Störsrud S, Törnblom H, Winkvist A, Lindqvist H.
Changes in serum and urinary metabolomic profile after a dietary intervention in patients with irritable bowel syndrome.
Submitted.

IV. Nybacka S, Öhman L, Störsrud S, Mybeck M, Böhn L, Wilpart K, Winkvist A, Bengtsson U, Törnblom H, Simrén M. **Neither self-reported atopy nor IgE-mediated allergy are linked to gastrointestinal symptoms in patients with irritable bowel syndrome.**
Neurogastroenterol Motil. 2018;30(10):e13379.

Appendix. Nybacka S, Törnblom H, Simrén M, Störsrud S

The role of CARbohydrates in Irritable Bowel Syndrome (CARIBS): Protocol for a randomized controlled trial comparing three different treatment options
Submitted.

CONTENT

ABBREVIATIONS

BDA	British dietetic association
BSFS	Bristol stool form scale
CBT	Cognitive behavioural therapy
CV-ANOVA	Cross-validated analysis of variance testing
CV_b	Coefficient of variation between subjects
CV_w	Coefficient of variation within subjects
FODMAP	Fermentable oligo-, di-, monosaccharides, and polyols
FDR	False discovery rate
GI	Gastrointestinal
IBS	Irritable bowel syndrome
IBS-C	Constipation predominant irritable bowel syndrome
IBS-D	Diarrhea predominant irritable bowel syndrome
IBS-M	Irritable bowel syndrome with mixed/alternating bowel habits
IBS-U	Unsubtyped irritable bowel syndrome
IBS-SSS	Irritable bowel syndrome severity scoring system
NICE	National Institute of Health and Care Excellence
NMR	Nuclear magnetic resonance
OPLS	Orthogonal projections to latent structures
OPLS-DA	Orthogonal projections to latent structures discriminant analysis
OPLS-EP	Orthogonal projections to latent structures with effect projections
PCA	Principal component analysis
PHQ-15	Patient health questionnaire-15
PI-IBS	Post-infectious irritable bowel syndrome
SCFA	Short chain fatty acids
VIP	Variable importance in projection

DEFINITIONS IN SHORT

Atopy	A hereditary predisposition to develop atopic dermatitis, allergic rhinitis, and asthma. Atopy is associated with heightened immune responses to common allergens
Biomarker	Any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease
Coefficient of variation between subjects	An expression of the true variation in food intake between individuals
Coefficient of variation within subjects	A reflection of the true variation in food choices of an individual
FODMAPs	Fermentable carbohydrates, i.e., oligosaccharides, disaccharides (lactose), monosaccharides (fructose), and polyols (sorbitol and mannitol)
Habitual diet	Average long-term intake of nutrients and/or foods
Metabolomics	The comprehensive study of all small molecules, i.e., metabolites, within a biological sample
Orthogonal projections to latent structures discriminant analysis	A supervised regression method that may be used for identifying discriminatory variables between classes of subjects
Principal component analysis	A dimensionality-reduction method often used for exploratory data analysis
Prebiotics	A substrate that is selectively utilized by host microorganisms conferring a health benefit
Probiotics	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host
Somatization	The expression of psychological distress into physical symptoms

INTRODUCTION

Irritable bowel syndrome (IBS) affects the life of many individuals in Sweden, but also worldwide. The disorder is acknowledged to impair working capability, leading to substantial costs for both the individual as well as the society. Currently there are no available diagnostic biomarkers of IBS, thus leading to patients being misdiagnosed and undergoing unnecessary invasive examinations. The search for novel biomarkers also involves the pursuit for understanding the pathophysiology of IBS, which is yet only partly understood.

Food intake in general, and some foods in particular, have a tendency to generate symptoms in patients with IBS. In fact, a vast majority of patients with IBS report that specific foods, or eating *per se,* induces the onset of symptoms. So, if diet is a part of the problem, it might as well be a part of the solution?

This thesis focuses on different aspects of symptom generation in patients with IBS, including dietary triggers and food intolerance. Hopefully, this can lead to a piece in the puzzle on how we can manage symptoms for patients with IBS in the future.

AIM

To gain a higher understanding of the intricate relationship between food intake and gastrointestinal (GI) symptoms, the aim of this thesis was to explore how diet and self-perceived food intolerance relate to GI symptoms among patients with IBS, and within different manifestations of IBS. The specific aims were to:

I. Characterize habitual intake of fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs) and intake variability among individuals with IBS

II. Investigate if habitual FODMAP intake is associated with IBS symptom pattern and severity

III. Explore if dietary intake could be revealed by metabolite profiles in serum and urine samples from patients with IBS, and evaluate if metabolite profiles changed during a dietary intervention

IV. Investigate if self-reported atopic disease and food intolerance are related to IBS symptom severity

IBS – A MULTIFACETED DISORDER

IBS is one of the most common functional GI disorders, with a global prevalence of approximately 4-15%, depending on definition and with regional variations[1]. It is also a complex disorder that can manifest in different ways. IBS is more common among women than men, with a ratio of approximately 2 to 1[1,2], and most common among individuals below 50 years of age[2].

The diagnosis of IBS is based on the presence of well-defined clinical features and absence of abnormal findings on clinical routine investigations. The Rome criteria are currently used as the standard diagnostic tool in clinical settings, and the criteria were updated to Rome IV in 2016, Table 1[3]. According to the new criteria, IBS is characterized by recurrent abdominal pain together with abnormal bowel habits. There is also an element of chronic character as the symptoms persist over time, and the symptom onset should be at least six months before the diagnosis is set.

Table 1. Rome IV diagnostic criteria for irritable bowel syndrome

Recurrent abdominal pain on average at least one day per week in the last three months* associated with two or more of the following:
1. Related to defecation
2. Associated with a change in frequency of stool
3. Associated with a change in form (appearance) of stool

***Symptom onset must have been at least 6 months prior to diagnosis[3].**

SUBTYPES OF IBS

For research purposes, IBS is often classified into different subtypes based on the predominant bowel habit; constipation predominant IBS (IBS-C), diarrhea predominant IBS (IBS-D), having a mix of both constipation and diarrhea (IBS-M) or unsubtyped IBS (IBS-U). To assist with subtyping, a stool diary based on the Bristol Stool Form scale (BSFS) may be used, Figure 1[3].

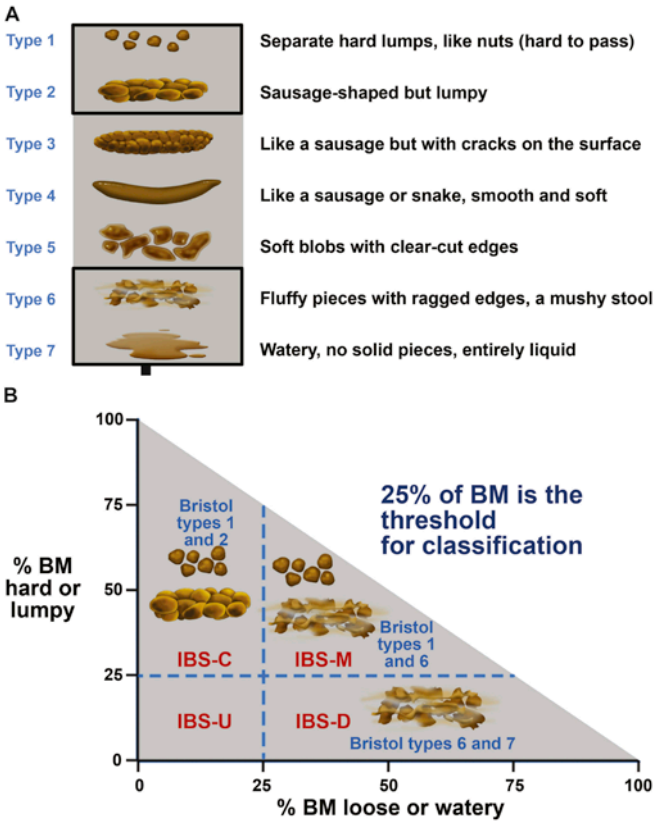

Figure 1. A) Stool type 1+2 and 6+7 are deviating from normal stool consistency. B) IBS subtypes should be established according to stool consistency, using the BSFS; IBS with predominant constipation (IBS-C, ≥25% hard stools (BSF 1 or 2) and <25% loose stools (BSF 6 or 7)), IBS with predominant diarrhea (IBS-D, ≥25% loose stools and <25% hard stools), IBS with alteration between constipation and diarrhea (IBS-M, ≥25% of reported stools hard and ≥25% loose); and unsubtyped IBS (IBS-U, insufficient abnormality of stool consistency to meet criteria for IBS-C, -D, or -M). From Lacy et al. Bowel Disorders. Gastroenterology 2016, 150(6):1393-1407.e1395. Reprinted with permission.

As IBS is a multifaceted disorder that can manifest in various ways, there is an ongoing discussion whether IBS should be considered as one single disorder, or if there are specific groups that experience anomalies that should be considered as distinct separate subtypes, or even as different disorders.

Post-infectious IBS

Apart from the four abovementioned subtypes, post-infectious IBS (PI-IBS) is considered as a distinct subgroup of IBS patients, where diarrhea predominance and low psychological comorbidity are common[4]. PI-IBS is triggered after an episode of infective gastroenteritis, typically after a bacterial infection, where patients develop IBS symptoms after the acute illness and where the symptoms persist long-term in 3-36% of patients after recovery of the gastroenteritis[5]. A meta-analysis concluded that the odds for developing IBS was sixfold increased after having an infective gastroenteritis[6].

Atopic IBS

In 2008, Tobin et al. suggested "Atopic IBS" as a new subgroup of IBS, i.e., IBS patients that also exhibit atopic manifestations[7]. Atopy is defined as having a genetic predisposition to produce IgE-antibodies in response to allergen exposure, causing asthma, atopic dermatitis and rhino-conjunctivitis[8]. Studies have reported that increased atopic sensations, such as worsened rhino-conjunctivitis during pollen season, have caused increased GI symptoms in some individuals[9]. Also, GI symptoms are more prevalent in patients with asthma and allergic rhinitis compared to other chronic diseases[10]. In Paper IV, we studied the prevalence of self-reported atopic disease among individuals with IBS compared to a non-IBS control group. Although atopic disease was commonly reported among patients with IBS, it was also common in the general population (55% vs. 40%, p=0.07). Clinical traits that were predictive of atopic IBS were female gender, having elevated IgE levels, and reporting severe somatic symptom.

Subtyping that include comorbidities

While subtyping patients based on their predominant bowel habits may be of relevance for GI motility and for determining diet treatment, it might also be necessary to include other comorbidities to provide a more personalized treatment approach. Using multivariate analysis to characterize a cohort of IBS patients diagnosed according to Rome IV, Polster et al. were able to identify five distinct subgroups of IBS patients; a) constipation-predominant, b) diarrhea-pain-predominant, c) mixed-high psychological symptoms, d) mixed-moderate psychological symptoms, and d) overall mild symptoms[11]. In subgroups with high comorbidity and psychological distress, patients were more likely to seek health care, i.e., in group c, but also in a and d. This kind of subtyping might provide more information about the underlying pathophysiology of the individual, which could be of relevance in studies aiming to evaluate treatment effects.

PATHOPHYSIOLOGY IN IBS

The pathophysiological features in IBS are only partly understood, although some traits have been distinguished and are found in subsets of patients, Figure 2. A central feature of the disorder is that symptoms arise in the absence of abnormal organic or biochemical features, therefore also known as a functional GI disorder. In recent years, there has been a shift towards naming IBS as a **disorder of gut-brain axis**[12], which indicates that there is a bidirectional connection between the GI system and the central nervous system. For some, the peripheral abnormalities are probably dominating while for others, disturbed central processing of signals from the periphery are more important.

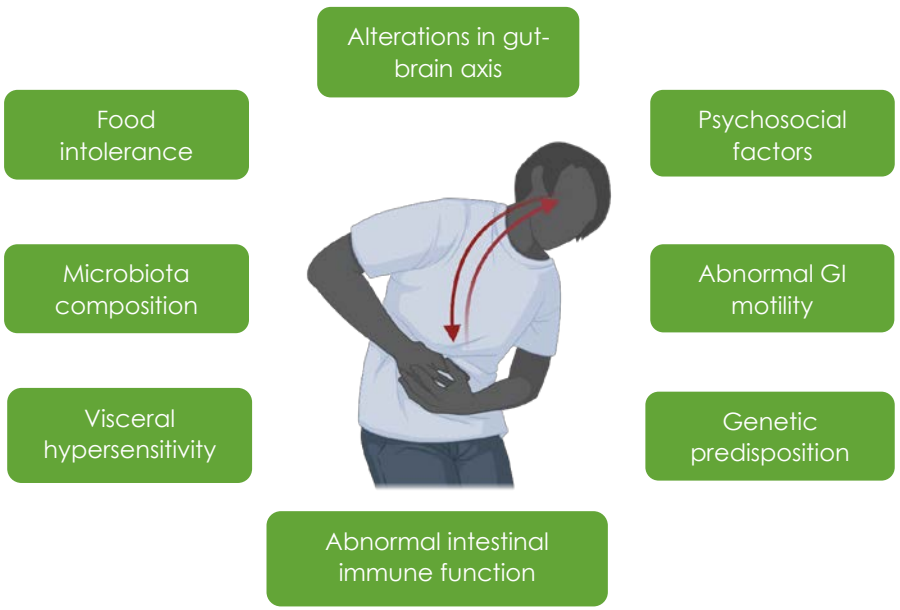

Figure 2. Pathophysiological traits in IBS. Image created with BioRender.com

IBS is considered to be chronic, but symptoms tend to come and go, and may even change over time within individuals[13]. Currently there are no available biomarkers to facilitate with diagnosis or to monitor the progression of the disease.

A range of other GI and non-GI symptoms are often concurrently present in patients with IBS, such as dyspepsia, migraine, back pain, anxiety and fibromyalgia[14-17]. Some of these comorbidities share pathophysiologic traits with IBS, like having a sustained immune activation after infection, disturbances in the gut-brain axis, altered gut microbiota and psychiatric distress.

In this thesis, aspects of diet-induced symptoms have been focused on and will therefore be covered more in depth.

INCREASED PAIN PERCEPTION

According to the Rome IV diagnostic criteria, all patients with IBS experience recurrent abdominal pain by definition[18]. The increased pain perception to stimuli within the GI tract is often referred to as **visceral hypersensitivity,** meaning that patients with IBS are more sensitive to pain stimuli than are healthy subjects[19]. Visceral hypersensitivity can be demonstrated by rectal balloon distention, which provides a measure of the GI sensitivity[20]. In a large multicenter study, it was demonstrated that visceral hypersensitivity was associated with GI symptom severity, independently of psychological distress[21]. Food induced intestinal distention may explain why IBS patients report food intake to trigger abdominal pain. For instance, it has been demonstrated that colonic gas production in response to fermentable carbohydrate intake generate similar amounts of gas in IBS as in healthy controls, but the symptom response to luminal distention is much more pronounced in IBS[22]. This imply that the gas production per se is not abnormal in patients with IBS, but rather the increased sensitivity to colonic distention[22].

ABNORMAL INTESTINAL IMMUNE AND BARRIER FUNCTION

So why do patients with IBS suffer from increased visceral hypersensitivity? There are multiple mechanisms that alone or in combination can help explain this phenomenon. For instance, it has been demonstrated that patients with IBS have increased numbers of **mucosal mast cells** within proximity to enteric

nerve endings[23,24]. Mast cells can be activated via IgE-dependent pathways to release inflammatory mediators such as histamine, prostaglandins and tryptase. Also, food[25] and stress[26] have been shown to stimulate mast cell degranulation. Mast cell numbers correlate to both intestinal permeability, abdominal pain perception and rectal sensitivity in patients with IBS[23,27]. Moreover, mucosal tryptase content has been found to be increased in IBS and mast cells release more tryptase and histamine as compared to controls[23].

As mast cell counts have been linked to increased pain in IBS, we hypothesized that IgE-levels measured in serum could also be associated with IBS symptom severity (Paper IV). However, we could not see any association between IgE-levels and severity of GI symptoms, which is also illustrated in Figure 3.

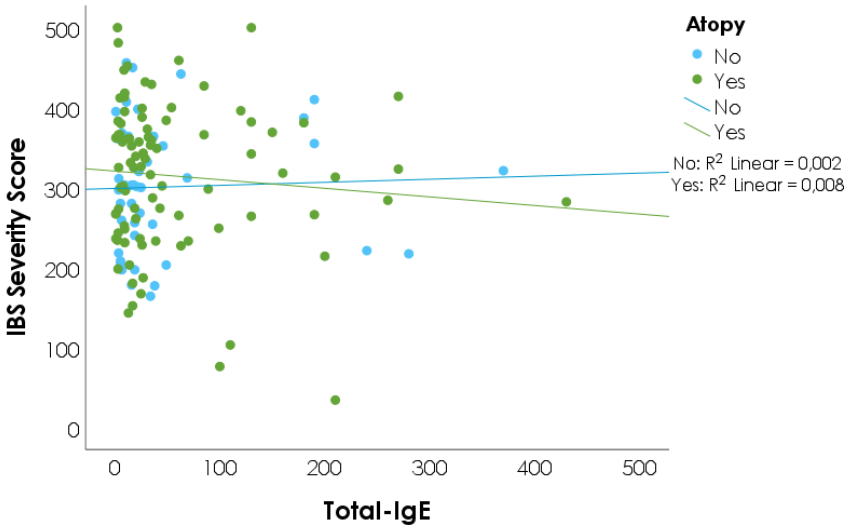

Figure 3. IBS severity, as measured by IBS severity scoring system (IBS-SSS), do not correlate to total IgE-levels measured in serum among patients with IBS. This holds true regardless of having self-reported atopic disease or not.

However, it has recently become more evident that the mast cell activation may be limited locally to the gut[28]. In the paper by Aguilera-Lizarraga et al., it was demonstrated that a bacterial infection could trigger an immune response that resulted in production of dietary-antigen specific IgE antibodies in mice, which were also limited to the intestine. After the sensitization, oral intake of the dietary antigen resulted in increased visceral pain. The same study revealed

that food antigens in form of gluten, wheat, soy and milk that were injected into the rectosigmoid mucosa of patients with IBS, caused a local mast cell activation and oedema. Although this study only enrolled 12 patients with IBS, it was noteworthy that all 12 patients showed signs of mucosal reactions to the foods that were tested, providing evidence that local IgE antibodies may be involved in food induced abdominal pain.

Activation of intestinal immune cells can cause **mucosal barrier disruption**, which allows the passage of luminal antigens into the mucosa[29]. This is often referred to as an **increased intestinal permeability**, or a "leaky gut". In patients with IBS exhibiting an increased permeability, an association to increased visceral hypersensitivity has been demonstrated[30]. Especially among patients with PI-IBS, acute infections have been linked to increased intestinal permeability that is sustained over time[31]. However, altered intestinal permeability have been observed in the entire intestinal tract, with disruptions in the expression of tight junction proteins, in all subtypes of IBS[32,33].

Further, a dysfunctional intestinal barrier has been associated with **low-grade inflammation** in the gut mucosa of IBS patients[34]. The colonic mucus layer forms a defense by protecting the epithelium from direct contact with the gut content[35,36]. To maintain the colonic mucosal integrity, the **gut microbiota** composition plays a pivotal role[35,37,38]. Colonic bacteria produces a great number of metabolites, including short-chain fatty acids (SCFAs) and vitamins, which has a beneficial effect on the host[39,40]. SCFAs are produced by the gut bacteria during the fermentation of undigested carbohydrates, also known as prebiotics.

Butyrate, one of the SCFAs, has especially proven to be of importance in providing and maintaining a healthy gut mucosa. A study by Hamer et. al demonstrated that administration of butyrate reduced oxidative stress in colonic mucosa compared to placebo[41]. Oxidative stress has been linked to increased intestinal permeability and inflammation[42]. Also, administration of prebiotics that produce butyrate[43] and the probiotic *Lactobacillus plantarum*[44] has proven to enhance the epithelial barrier function and to reduce intestinal permeability. Furthermore, a defect colonic epithelial oxidation of butyrate has been linked to inflammatory bowel disease and has been implicated in the pathogenesis of ulcerative colitis[45].

ABNORMAL INTESTINAL MOTILITY

IBS has commonly been associated with altered **GI motility**, where irregular bowel contractions, abnormal frequency of bowel movements and altered transit time are common characteristics. Much attention has been on the motility of the colon, where approximately 20-25% of patients with IBS have shown to have either accelerated or prolonged colonic transit time[46,47]. Accelerated transit time has been linked to IBS-D and prolonged transit time has been associated with IBS-C, but abnormal transit time *per se* displays weak correlations to abdominal pain[46]. However, in conjunction with other pathophysiological traits of IBS, disrupted intestinal motility has been found to have a cumulative effect on IBS symptom severity[47].

IBS has also been related to prolonged gastric emptying time[48] and small intestinal motility alterations[49,50].

Several foods and food components can influence GI motility, thereby causing postprandial symptoms. Among these, **coffee** intake is commonly reported to induce GI symptoms[51,52], and increase rectosigmoid motility in some individuals[53]. Caffeinated coffee can also stimulate colonic motor activity in the same magnitude as a 1000 kcal meal[54]. Similarly, **alcohol** reduce the oro-cecal transit time in individuals with a high alcohol consumption[55], and alcohol intake may exert effects on gastric emptying, mucosal barrier function and nutrient absorption[56].

Intake of spicy foods, or **capsaicin**, is also a well-known stimulant and dietary trigger in IBS[52,57]. In one study, patients with IBS were found to have increased numbers of capsaicin receptors as compared to controls[58], and capsaicin can exert a local motor effect in colon[59].

Further, intake of **fat** and **carbohydrates** can affect small intestinal motility and the gastrocolonic reflex[60], and the induced motor activity persist longer after intake of fat than carbohydrates[60]. Lipids delivered directly into the duodenum of patients with IBS can cause an exaggerated sensory component of the gastrocolonic response, with enhanced colonic sensitivity[61], and also, exert an inhibitory effect on intestinal gas transit[62].

FOOD HYPERSENSITIVITY AND INTOLERANCE

Food induced symptoms are common in IBS and having adverse reactions to foods may involve both immune mediated food allergy as well as non-immune-mediated food intolerance, Figure 4. Prevalence of self-reported intolerance to foods range between 62 and 80 percent in IBS[51,57,63-66]. As previously mentioned, coffee, alcohol, spicy foods, high-fat foods and carbohydrate-rich foods are commonly reported to induce symptoms, and so are also dairy products and wheat-containing foods[51,52,63,67]. The pathophysiological mechanisms involved in the adverse effects of these foods in IBS may involve both immune and non-immune mediated pathways.

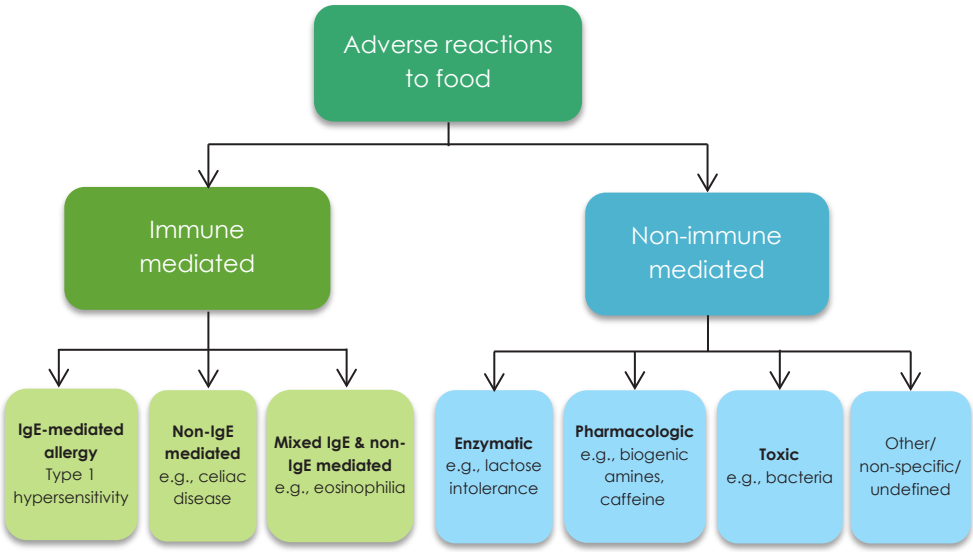

Figure 4. Adverse reactions to foods can be both immune mediated and non-immune mediated.

Immune mediated type 1-hypersensitivity allergic reactions occur when cell-bound IgE are cross-linked by allergens in sensitized individuals[68]. True immune mediated food allergy is believed to have a prevalence of 1-4% in the adult population[69]. At present, the only method to diagnose food allergy is by a double-blind, placebo-controlled food challenge[70], but performing these are time consuming, expensive and the individual is at risk of having an anaphylactic reaction.

A study performed in a general adult population in western Sweden showed that approximately a third of all participants reported self-perceived food hypersensitivity to at least one of the 56 food items that were assessed, with a higher prevalence among women and in younger individuals[71]. In this cohort, 6% of individuals were IgE-sensitized to at least one food allergen while at the same time reporting symptoms after ingestion, indicating true food allergy. Symptoms were most commonly reported to arise from the GI tract (15%), followed by the skin (2.7%). Interestingly, none of the individuals that reported symptoms of fish, wheat and soy showed to be IgE-sensitized to these foods. In general, correlation between self-reported hypersensitivity and IgE-sensitization was week[71].

In the IBS patient cohort in Paper IV, 55% (n=123) of all patients reported having a history of atopic disease (Figure 5), while 19% (n=43) had self-reported food intolerance/allergy. A vast majority of patients reporting food intolerance, 79% (n=34), also had atopy. Further, 15% (n=33) of all patients had elevated IgE levels, but only 2.7% (n=6) had atopy, food intolerance and elevated IgE combined. As previously reported, we did not see an association between IgE levels and IBS severity score. However, when combining all three allergic factors, there is a trend towards higher IBS severity scores (IBS-SSS) compared to patients without any of the allergic factors, p=0.059, Figure 5.

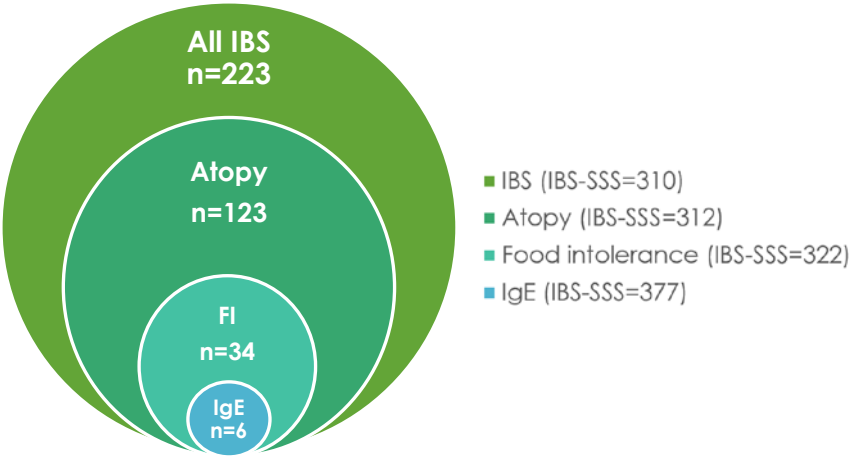

Figure 5. IBS severity symptom score tends to increase with increasing number of allergic factors. FI, food intolerance; IBS-SSS, IBS severity scoring system

There is overall a large discrepancy between asymptomatic individuals who exhibit IgE-sensitization, and individuals who experience symptoms without being IgE-sensitized[65]. This further strengthens the potential relevance of local immune reactions that are not detectable in peripheral blood samples.

Food intolerance on the other hand covers a wide span of physiological reactions, which may be caused by enzyme deficiency (e.g. lactase deficiency), pharmacologic agents (e.g. biogenic amines, caffeine), or toxins (e.g. food poisoning), or other undefined reactions induced by unknown mechanisms.

Malabsorption of carbohydrates, such as lactose, is common both among IBS patients and in the general population[72]. Lactose malabsorption include any cause of failure to digest or absorb lactose, which may be caused by genetics or due to infection, or any other condition affecting the intestinal mucosa[73]. However, the diagnosis of lactose intolerance is only fulfilled when an individual with lactose malabsorption experiences symptoms[73]. Some studies suggest that IBS is associated with an increased risk of having lactose intolerance[74], but results are conflicting and a recent review article concluded that there is insufficient evidence to routinely suggest a lactose-free diet to patients with IBS[75].

The symptoms of IBS-D resemble the symptoms described as being typical for lactose intolerance, i.e., abdominal pain, bloating, and diarrhea. In Paper II, we observed that a higher proportion of women with IBS-D, 17.5%, reported eating very low amounts of lactose (<2 g/day) compared to women with IBS-C (0%), IBS-M (0%) and IBS-U (5.6%), p=0.004. Reported intake of lactose did not correlate to IBS symptom severity in our study, but as this was a cross-sectional study, we could not determine a causal relationship. In a study by Yang et al., lactose hydrogen breath tests were used to establish the relation between lactose malabsorption and intolerance, and the severity of symptoms in patients with IBS-D and in controls[76]. Prevalence of lactose malabsorption was similar in both groups, but presence of lactose intolerance was higher in IBS patients. The excretion of H^2 increased with increasing dose of lactose, as did the severity of abdominal symptoms. This imply that there is a dose-response relationship between lactose intake and severity of symptoms, when participants are blinded to the intervention. However, there was a poor conformity between individuals with self-reported lactose intolerance and signs of lactose intolerance during the lactose hydrogen breath test[76].

MANAGING IBS SYMPTOMS

A diagnosis of IBS is made based on typical symptoms and absence of organic diseases that explain the symptoms. This might be perceived as unsatisfactory for the patient who is suffering from a range of symptoms, causing stress and anxiety. Therefore, a central part of a successful management of IBS is a positive confirmation of an IBS diagnosis, which can lead to more effective treatment and less anxiety for the patient[77]. As IBS is of chronic character, the treatment should aim at identifying successful strategies to minimize symptom burden. While several simultaneous processes can interact, with a cumulative effect on symptom burden, a holistic, multidisciplinary treatment approach is believed to be a success factor.

Most patients will benefit from having a good physician-patient relationship, receiving education about IBS and general lifestyle advice regarding dietary triggers, alcohol intake, stress, etc. If that does not generate adequate symptom relief, a more personalized treatment should be employed (Figure 6). A stepwise approach is recommended, with focus on the predominant symptoms. For patients with severe and persisting symptoms, a team with a multi-disciplinary treatment approach involving diet, pharmacotherapy and psychological treatment may be utilized.

Since diet is the main focus of this thesis, other non-pharmacological and pharmacological treatments will be described briefly.

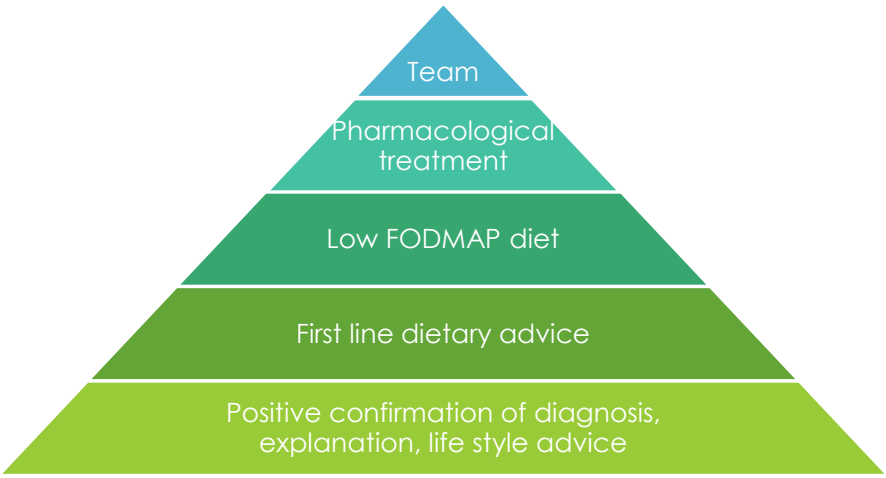

Figure 6. Management of IBS symptoms in a stepwise approach.

BEHAVIORAL/NON-PHARMACOLOGICAL TREATMENT

For some patients with IBS, psychological distress, anxiety and a high level of somatization is closely related to their IBS symptoms. Somatization occurs when psychological distress transforms into physical symptoms[78], such as having headache when being stressed, or throwing up from anxiety. Somatic symptoms, measured with the Patient Health Questionnaire 15 (PHQ-15), explained as much as 25.5% ($p<0.001$) of the variance in IBS symptom severity in a simple linear regression analysis in Paper IV. It indicates that somatization indeed is closely related to the severity of IBS symptoms, at least for some patients, and that behavioral therapy might be central in the treatment for these individuals.

Several behavioral treatment approaches have shown to be successful in alleviating symptoms of IBS. Among these, cognitive behavioral therapy (CBT), and gut-directed hypnotherapy are among the most well-studied.

A meta-analysis published in 2018 showed that CBT in IBS had medium-to large effects on IBS symptom severity. In fact, CBT had larger effect on IBS symptom severity than for reducing psychological distress[79]. In a study by Windgassen et al., the mediating effects of CBT on IBS symptoms were elucidated. The study demonstrated that affecting GI-related safety behaviors (such as use of medication to prevent symptoms, or an excessive straining on the toilet) preceded a reduction in anxiety, and not vice versa, indicating the importance of targeting such behaviors in therapy[80]. Similarly, gut-directed hypnotherapy have displayed promising effects on reducing symptoms of IBS[81], and gut-directed hypnotherapy were as effective as a low FODMAP diet[82]. CBT and hypnotherapy treatments are however not readily accessible for most patients. Therefore, other more easily accessible treatment alternatives, such as yoga, has been evaluated and found to be a good alternative. Some of the potential benefits with yoga include a relaxation of the sympathetic nervous system through structured breathing and relaxation[83]. Although studies performed on yoga for IBS show a large heterogeneity, a recent review article conclude that yoga is a safe and effective therapy to enhance both physical and mental outcomes of patients with IBS among all subtypes[83].

PHARMACOLOGICAL TREATMENT

If initial lifestyle/dietary treatment efforts fail to improve symptoms to a satisfactory level, the next step could be to try a pharmacological treatment option. The choice should be based on the patient's predominant symptom, whether it is the altered bowel habit, abdominal pain or bloating/distention. An overview of pharmacological agents can be seen in Figure 7[84]. It is however noteworthy that these pharmacological agents are used in order to improve symptoms of IBS and are not expected to cure the disorder. Sometimes a combination of pharmacological agents may be necessary for an optimal effect, and in patients with persisting and severe symptoms, psychotropics in combination with other behavioral treatment options may be utilized.

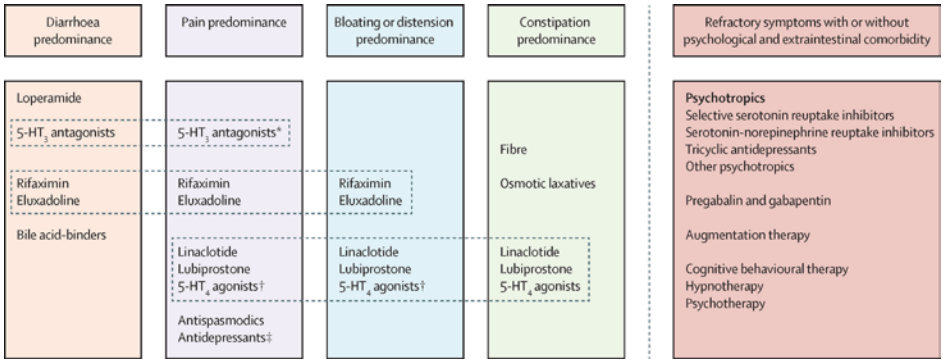

*Figure 7. Overview of treatment options for patients with irritable bowel syndrome, based on the predominant symptom. Some treatments can be used to target several symptoms, which is indicated in the figure. SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors. *Only alosetron and ramosetron, and not ondansetron, improved pain. †Tegaserod is not available, except for in emergency situations; prucalopride is indicated for chronic constipation and improves pain and bloating in patients with constipation. ‡Primarily tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors have analgesic effects. From Simrén M, et al. 2017. 'Management of the multiple symptoms of irritable bowel syndrome', The Lancet Gastroenterology & Hepatology, 2: 112-22. Reprinted with permission.*

DIETARY TREATMENT

Even though IBS is a heterogeneous disorder, a majority of patients report an association between food intake and onset or worsening of GI symptoms. A diet is complex in the sense that it consists of a combination of different nutrients and foods; also, it contains different textures and cooking methods that affect the digestibility and absorption of a food. Furthermore, diet intake is dynamic and varies within individuals. It is therefore a cumbersome task to rule out which foods are most potent in triggering symptoms, and which foods one should avoid. Avoidance of foods that are recognized to cause symptoms is however central for dietary treatment. In Paper IV, we saw that the number of food items that the patients reported to trigger symptoms were statistically significantly related to more severe GI symptoms measured by IBS-SSS. Consequently, individuals who experience a lot of symptoms might exclude more foods from their diet, leading to a risk of nutritional inadequacies and poorer diet quality.

When trying to manage GI symptoms, many patients and especially women, have developed "self-care strategies" to cope with their GI symptoms , which include a "trial and error" method to exclude foods believed to trigger symptoms, and to reintroduce them again if the symptoms did not improve[85].

Traditional dietary advice to patients with IBS are based on the National Institute for Health and Care Excellence (NICE) guidelines[86] along with the systematic reviews performed by the British dietetic association (BDA)[87]. These focus on eating behavior, emphasizing "how" and "when" to eat, and limiting intake of foods believed to cause bloating (cabbage, onions, fizzy drinks etc.). A list of recommendations from both NICE and BDA can be seen in Table 2. These recommendations should be employed as first line dietary treatment as they are relatively simple, not too restrictive and have proven to be effective in around 40 to 50 percent of patients with IBS[88,89]. In 2016, the BDA updated their guidelines adding new insights regarding the role of fermentable carbohydrates (FODMAPs) for triggering GI symptoms[87].

Table 2. British dietetic association (BDA) and National institute of Health and Care excellence (NICE) dietary guidelines for irritable bowel syndrome

Diet factor	BDA recommendations [87]	NICE recommendations [86]
Eating pattern	Insufficient evidence to make a recommendation. Provide advice for a healthy balanced diet with regular meal pattern.	Have regular meals and take time to eat. Avoid missing meals or leaving long gaps between eating.
Fluids	No evidence to make a recommendation. Aim for a total intake of 1.5-3.0 L/day	Drink at least 8 cups of fluid per day, especially water or other non-caffeinated drinks.
Caffeine	Insufficient evidence to make a recommendation. If related to symptoms, consider reducing intake.	Restrict tea and coffee to 3 cups per day.
Alcohol	Assess intake and screen for signs of binge drinking. Ensure alcohol intake is kept at safe national limits.	Reduce intake of alcohol and fizzy drinks.
Fibre	Avoid using dietary wheat bran to treat IBS. In IBS-C, try supplementation of linseed up to 2 tablespoons per day for 3 months. Improvement may be gradual.	May be helpful to limit intake of high-fibre foods, especially bran and wholegrains. Limit intake of resistant starch. People with wind and bloating may find it helpful to eat oats and linseeds (up to 1 tablespoon per day).
Milk/dairy products	If sensitivity to milk is suspected, try a low lactose diet. Use a low lactose diet in individuals with a positive lactose hydrogen breath test.	-
Fat	If related to symptoms, assess fat intake and ensure it is in line with national healthy eating guidelines.	-
Spicy food	If related to symptoms, assess spicy food intake and trial restriction	-
Fruit	-	Limit fresh fruit to 3 portions per day (a portion should be approximately 80 g).
Sweeteners/ sorbitol	-	People with diarrhoea should avoid sorbitol
Gluten	No recommendations can be made	-
Probiotics	Probiotics are unlikely to provide substantial benefit to IBS symptoms. It is however safe to try.	-

IBS, irritable bowel syndrome; IBS-C, IBS with constipation predominance

THE LOW FODMAP DIET

The low FODMAP diet as a concept was introduced in the beginning of 2000s and focuses on limiting fermentable carbohydrates from the diet. These carbohydrates include oligosaccharides (fructo-oligosaccharides/fructans and galacto-oligosaccharides), disaccharides (lactose), monosaccharides (fructose in excess of glucose) and polyols (such as mannitol, sorbitol and xylitol).

A common approach to define foods that are low or high in FODMAPs is to use thresholds that have been defined at the Monash University[90]. The cut-off values are based on standard serving sizes of individual foods, which are then coded into a traffic light system – foods marked with green are allowed during the exclusion phase, whereas foods marked with red, such as garlic, asparagus, watermelon, apples, stone fruits etc., are above the cut-off values and should be omitted.

Table 3. Cut-off values for oligosaccharides (fructans and galacto-oligosaccharides), polyols (sorbitol and mannitol), fructose in excess of glucose and lactose. The values are based on standard portion sizes per sitting [90].

Individual FODMAPs	Examples of food sources	Grams per standard serving size
Oligosaccharides (grains, legumes, nuts, and seeds)	Wheat and rye-based products, almond, cashew nut, soybean	<0.30
Oligosaccharides (vegetables, fruits)	Asparagus, artichoke, beetroot, peas, fennel, garlic, onion	<0.20
Polyols (sorbitol and mannitol)	Cauliflower, celery, sweet corn, button mushroom, snow peas	<0.20
Total polyols	Xylitol and sorbitol as sweeteners	<0.40
Excess fructose [a, b]	Apple, cherries, mango, pear, watermelon, sugar peas	<0.15
Lactose	Milk, yoghurt, ice cream, milk chocolate	<1.0

[a] fructose-glucose
[b] when excess fructose is the only FODMAP present, the cut-off value is <0.40 grams per serve.

Mechanistic action of fermentable carbohydrates

Carbohydrates are mainly absorbed in the small intestine as monomers, after undergoing a process of mechanistic and enzymatic hydrolyzation[91]. Some carbohydrates will however remain unabsorbed (Figure 8). This is mainly due to the fact that humans lack enzymes that can break down complex carbohydrates such as fructans and oligosaccharides[92], or if there are insufficient amounts of the enzyme lactase to break down lactose. Furthermore, absorption of fructose is dependent on passive diffusion via glucose transporter 5 (GLUT5) receptors, which for many individuals lead to poor uptake and malabsorption of fructose[93,94]. However, studies have suggested that fructose can be co-absorbed with glucose via GLUT2-receptors that are translocated to the brush-border membrane, which facilitate uptake of fructose[91,95]. For this reason, only fructose in excess of glucose normally counts as a FODMAP[96]. Sugar alcohols, or polyols, are generally slowly and passively absorbed and will thus partly remain unabsorbed[94,97]. These incompletely absorbed carbohydrates will pass through to the colon where they will act as substrates for bacterial fermentation[98].

During the fermentation process where SCFAs are produced, an increase in gas production can be seen[98]. Also, lactose, fructose and polyols that remain unabsorbed will cause an increased water retention by osmosis[99]. These processes will lead to a distention of the colon, which in turn can cause an increase in nerve signaling and pain perception, especially in patients with visceral hypersensitivity[100]. Therefore, it is believed that excluding these fermentable carbohydrates from the diet will lead to less GI symptoms in patients with IBS.

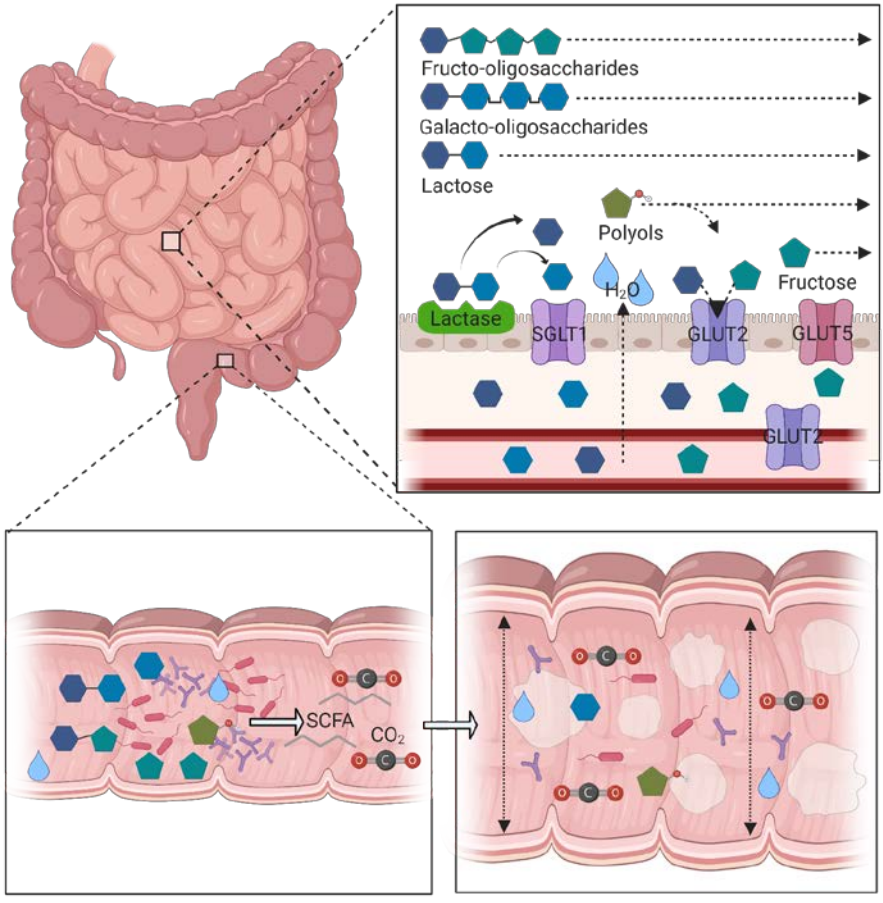

Figure 8. Illustration of the absorption of fermentable carbohydrates and how intake may cause gastrointestinal symptoms. Carbohydrates are hydrolyzed and absorbed as monosaccharides in the small intestine, but some carbohydrates will remain unabsorbed. These incompletely absorbed carbohydrates are fermented in the colon by bacteria, leading to a production of short chain fatty acids and gas. Increased water retention and luminal gas formation will lead to distention of the colon, causing pain in patients with visceral hypersensitivity. Image created with BioRender.com

Implementation of a low FODMAP diet

An extensive review with practical guidelines on implementation of the low FODMAP diet has previously been described by Whelan et al.[101]. In short, the low FODMAP diet can be structured into three phases of treatment. During the first phase of treatment, which normally lasts 4-6 weeks, intake of FODMAPs is kept at minimum. Patients or study participants should be given dietitian led counselling and education before entering the elimination phase. Foods high in FODMAPs should be replaced with low-FODMAP equivalents to ensure that energy and nutrient intake will not decrease during the elimination phase. To facilitate with food choices, food lists, cookbooks, smart phone applications, or food composition tables may be used.

During the second phase, or the re-challenge phase, FODMAPs are tested one by one to evaluate individual tolerance. As individuals may experience varying symptoms to different FODMAP compounds, it is of importance to evaluate each compound separately so that foods that are well tolerated can later be re-introduced again. This second phase can be designed in numerous ways, but commonly the preference of the patient determines which FODMAP will be re-challenged first. A food item that contains high amounts of only one FODMAP is selected to represent the category of FODMAP re-challenged, and the amount can be stepwise increased for three days. At the same time, all other FODMAPs should remain eliminated. After the rechallenge period, a short wash-out period of a few days can be implemented before re-challenging the next category of FODMAPs.

The third phase is the personalization phase, where a long-term strategy should be implemented. As close to a “normal diet” as possible should be pursued, and only FODMAPs found to cause symptoms should be avoided. This will allow for a less restrictive diet, where FODMAPs that are found to be tolerated in certain amounts can be reintroduced, and a larger diversity of foods can be consumed.

POTENTIAL RISKS OF A DIET LOW IN FODMAPs

Nutritional implications

As the low FODMAP diet is an exclusion diet, it poses potential risks concerning both energy and nutrient intake. Foods that are rich in FODMAPs are generally considered as healthy foods; it includes a wide range of vegetables and fruits and foods with high dietary fiber content. These foods are also prebiotics[102] and rich in vitamins, minerals and bioactive compounds such as polyphenols and antioxidants. Removing these foods and not replacing them with other nutritious foods that are low in FODMAPs may lead to worsened nutritional intake of less quality.

A few studies have investigated how the implementation a low FODMAP diet have affected the nutritional composition and diet quality among patients with IBS. Some studies have reported a reduction in energy intake after adapting a low FODMAP diet compared to baseline[88], but not all[103]. Nutrient intake have reportedly been negatively affected following a low FODMAP diet regime, where especially intake of dietary fiber was reduced[88]. Although one study did not see any reduction in energy or nutrient intake following a low FODMAP diet, the overall diet quality had declined[103].

As a short-term treatment, it is unlikely that nutritional inadequacies will pose a negative impact on the individual, but if treatment is prolonged over time, one must take into consideration the potential risks of malnutrition. The only study so far reporting long-term effects on diet and nutrient intake after receiving a low FODMAP diet, did however not find any alarming signs of nutritional inadequacies. This study by O´Keefe et al. reported that participants that had maintained a modified low FODMAP diet did meet the recommendations for a nutritionally adequate diet as defined by the UK dietary reference values[104].

Increased risk of eating disorders

To avoid or prevent GI symptoms, some patients with IBS develop disordered eating habits, such as skipping meals, limiting foods or adhere to restrictive diets[105]. Disordered eating is not the same as an eating disorder *per se*, as it differs in severity and impact on medical and psychosocial wellbeing. Although, complying to a restrictive diet might progress the disordered eating behaviors and pose risk to develop eating disorders[105].

A study by Melchior et al[106] showed that approximately 25% of patients with IBS are at risk of an eating disorder. Those patients had a poorer quality of life and more stressful life events as compared to IBS patients without risk of an eating disorder. It has also been shown that patients with IBS with risk of eating disorders adhere to the low FODMAP diet to a higher degree than patients without risk of eating disorders (57% vs. 35% adherence, respectively)[107].

There are contraindications to when a low FODMAP diet might not be suitable for the patient, and that include having an active eating disorder[108]. Therefore, it might be warranted to screen for eating disorders before recommending a restrictive diet to a patient.

Impact on microbiota

Exclusion of FODMAPs from the diet, which are naturally occurring prebiotic fibers, can potentially have negative impact on the gut microbiota. Prebiotics are by definition promoting an increase in specific bacteria that bring health benefits for the host[109].

If substrates for our colonic bacteria are eliminated, there is a strong risk that the richness of our bacterial composition will be affected. When FODMAPs are restricted, colonic bacteria are deprived of carbohydrate substrates, which could lead to a switch from saccharolytic to proteolytic metabolism[110]. This could impact the types of SCFAs that are being produced and markedly reduce butyrate levels.

In a study by Staudacher et al., a low FODMAP diet led to changes in abundance of saccharolytic genera compared with sham diet, including higher *Bacteroides* and lower *Bifidobacterium*[111]. Similarly, our research group have reported that a reduction in *Bifidobacterium* and *Actinobacteria* was seen in patients after receiving a low FODMAP diet[112]. Lower abundance in *Bifidobacterium* after a low FODMAP diet has also been reported elsewhere[113,114]. Probiotic supplementation administered during dietary intervention was shown to increase the abundance of *Lactobacillus* and *Streptococcus,* which indicates that there might be a beneficial effect on microbiota composition by adding probiotics while adhering to a low FODMAP diet[111].

MEASURING DIETARY INTAKE

Assessing dietary intake in free-living individuals is a complex task, as all accessible dietary assessment methods have their pros and cons. However, none of the existing dietary assessment methods are able to accurately and reproducibly assess dietary intakes without any measurement error, so the results should often be seen as estimates rather than absolute values[115,116].

There are essentially four different methods of dietary assessment: diet records, 24-h recalls, dietary history interviews and food frequency questionnaires. To decide on the most appropriate assessment method, one must consider the purpose of the study and take into consideration how precise data one needs, i.e., if absolute amounts are required, or if ranking of individuals according to dietary intake levels is sufficient.

Weighted dietary records are sometimes considered as the "golden standard" among dietary assessment methods[117]. A diet record is a prospective method where all foods and drinks consumed during a specific period of time is weighed and written down. Depending on the study aim the number of registration days may vary, but usually diet is recorded between 4 and 7 days. To be able to assess nutrient intakes as correctly as possible, detailed information about the foods consumed is required; for instance, product name, cooking method and fat content should be stated. If diet records are performed properly, a good estimate of an individual's dietary intake, especially energy and macronutrient intake, can be obtained. The reliability of diet records is strongly related to the individual's ability to accurately report and specify the amounts and type of foods eaten. An alternative to the weighted record is to estimate the amounts of foods consumed instead, which can be done with either standard household measurements or by using pictures of portion sizes. A disadvantage of diet records of prospective nature is that under-reporting of diet intake might increase, as the participant can for various reasons choose to change the eating pattern during the registration period, a so called “reactivity bias”[118]. Also, the method requires that the respondents are motivated as the method is time-consuming.

Instead of recording diet intakes prospectively, retrospective methods can be used. One option is the **24-h recall method**, in where all foods and drinks consumed during the preceding 24 hours are reported. It can either be conducted by reporting all intakes between midnight to midnight, or all foods consumed from when the respondent wakes up in the morning until the last meal before the next morning. The information can be obtained either by

interviewing the respondent (face-to-face or by telephone), or by using self-administered computer-based methods. The recall method is an open-ended method, but usually structured in a standardized way such as the "multiple pass method"[119]. This method employs a five step approach: step 1 is a "quick list" of foods and drinks consumed during the previous 24 hours; step 2 goes through foods that might have been forgotten during the first step; step 3 records the time and occasion when the foods have been consumed; during step 4, detailed descriptions about amounts and types of foods are obtained; step 5 is a final probe for anything else consumed during the last 24 hours that might have been missed out. Thus, actual and detailed information about dietary intakes of individuals can be obtained. One 24-h recall is however not sufficient to assess habitual dietary intake of an individual but can be used to calculate the average consumption of a group, provided that different days of the week are covered. However, if multiple non-consecutive 24-h recalls are completed over the course of a few months, variability in diet intake can be captured that provide more reliable and accurate estimates of an individual's habitual diet. A downside with 24-h recalls is that they rely on memory and the ability of the respondent to correctly recall what has been eaten.

Another retrospective assessment tool is the **dietary history method**, which is used to describe usual food intakes and variations over a longer period of time. The dietary history method usually comprises both an interview of the respondents usual eating patterns, and a predefined food list together with frequencies of consumption. Occasionally, a food record is added as well. These interviews require a trained interviewer, as knowledge about local food culture and age specific food intake patterns are needed. Also, data entry and coding are intricate. Performing dietary history interviews are time consuming and are most often used in health care settings rather than for research purposes. The accuracy of the data relies on the respondent's memory and may lead to recall bias.

In epidemiological studies, **food frequency questionnaires** (FFQ) are often used to assess dietary intake as it provides a simple and cost-effective tool. FFQs aim to assess frequencies in intake of certain foods during a specified period of time, normally for a few months up to a year retrospectively. The questionnaires contain a predefined list of foods, which for various reasons need to be limited, together with frequencies of intake. Therefore, it is essential that the included foods succeed to capture what is intended to be measured, i.e., intake of the specific macro- and micronutrients that are of interest. Questions regarding portion sizes may be included, providing with semi-

quantitative estimates of food intake. Although, intakes assessed with FFQs should not be interpreted as absolute amounts, but rather as estimates. Energy adjustment of intakes may reduce misreporting related to energy intake, and to use rankings based on intakes instead of absolute amounts are advised[120,121]. FFQs have several advantages; long term intake can be assessed, they can capture intake of foods eaten less regularly, they pose little burden on the respondent and are easy to administer. Nevertheless, FFQs need to be study-specific, and self-administered questionnaires require that respondents are literate and have numeracy skills. Also, as dietary habits and food choices change over time, questionnaires become outdated and need to be revised.

DIET INTAKE VARIABILITY

All individuals have their own dietary intake patterns – these dietary habits are a prerequisite when aiming to study the relationship between diet and health outcome in epidemiological studies. One of the major challenges with dietary assessment is that diet intake fluctuates by nature. Besides the difference in diet intake between individuals, there is also a normal variability in diet intake within individuals. This variability can be noted across the different seasons of a year, during weekdays vs. weekends, and in sickness and in health[122]. It is also highly dependent on personal preferences for foods and cultural belonging. Therefore, to determine how to best capture intake of the nutrient of interest, knowledge about the nutrient is required; both in terms of food sources and the variability in intake.

The variability is divided into **within-subject variation** (CV_w), and **between-subject variation** (CV_b). CV_w captures the true variation in food choices of an individual and takes into account the standard deviation (SD) in estimated intakes. The CV_w can be expressed as a percentage[123,124]:

$$SD_w / mean\ intake \times 100$$

In general, nutrients that are abundant in few foods and that are consumed occasionally have high CV_w (e.g., some micronutrients), whereas macronutrients (except alcohol) have low CV_w. The CV_w normally ranges between approximately 26% for reported energy intake, to 147% for reported vitamin A intake[125].

The between-subject variation is an expression of the true variation in food intake between individuals. CV_b can be expressed as a percentage[123,124]:

$$SD_b / mean\ group\ intake \times 100$$

The CV_b normally increases when studying heterogenous populations with variation in age and gender, because younger persons tend to eat more than older, and men tend to eat more than women. Because of this, it is generally advised to stratify dietary intake data within different age groups, and also, according to gender.

Determining the mean nutrient intake of a group

Estimating the mean intake of a specific group is the easiest objective to achieve. Normally, one single day of observation is required as long as all days of the week are represented and that sufficient number of individuals are included[126]. The number of subjects that needs to be included depends on the desired precision, as well as the day-to-day variation in nutrient intake between subjects. The equation to calculate the number of subjects is[127]:

$$n = s^2_b / e^2$$

where s^2_b denotes the between-subject variance, and e is the desired standard error. In Paper I, we estimated the s^2_b for total FODMAP intake among women to be 11.0, and the standard error of the mean was 1.8. According to Gibson (2005)[124], if a precision of 95% is required, then the margin of error is ± twice the standard error, and e is 3.6/4 = 0.9. Thus, 149 subjects would be required to estimate the group mean FODMAP intake with a high level of precision.

Ranking of individuals according to dietary intake

Consideration of CV_w and CV_b is essential when attempting to rank individuals according to dietary intake data, as a high CV_w together with a low CV_b makes it difficult to estimate an individual's true intake and to distinguish intakes between individuals. To improve the precision in rankings, the number of observations for each subject may be increased. The number of observations needed can be calculated and takes into account the ratio of the within- and between subject variances. The equation to calculate this is[128,129]:

$$n = (r^2/(1-r^2)) \times (s^2_w / s^2_b)$$

where n is the number of days required, r denotes the unobservable correlation between the observed and true mean intakes of observations (for instance, r=0.85, would give 72% correctly classified into quartiles of intake; and r=0.95, would give 84% correctly classified into quartiles of intakes), and s^2_w and s^2_b are the observed within- and between-subject variances, respectively.

Assessing nutrient intake for an individual

When estimating an individual's nutrient intake, one must consider two factors: the precision of the estimation (e.g., +/- 10%, 20%, or 30% of the true mean intake) and the CV_w of the nutrient. The equation is[123,130]:

$$n = (Z_\alpha \, CV_w / D_0)^2$$

where n is the number of days required for each subject, Z_α is the normal deviate for the percentage of times the measured value should be within a specified limit (e.g., 1.96), CV_w the within-subject coefficient of variation and D_0 is the precision in intake.

Variation in FODMAP intake

Most of the basic research regarding variability in diet intake has been performed several decades ago, where variation in energy and dietary intake of macro- and most micronutrients have been described[128,131-133]. Over time, new micronutrients have gained interest within nutrition research, of which we lack information about diet intake variability. For instance, information about variability in FODMAP intake had not been previously described, and thus, the focus of Paper I was to address this.

In Table 4, the within- and between-subject variation in energy and FODMAP intake is presented among women and men with IBS and stratified in IBS subtypes. For most FODMAPs, the CV_w is large. This has implications when attempting to assess habitual intakes of FODMAPs, as it will lead to difficulties in capturing the true mean intake of an individual. But, as the CV_b is even larger, ranking of individuals according to nutrient intakes is feasible.

Table 4. The within- and between-subject variation in energy and FODMAP intake among women and men with irritable bowel syndrome, for all combined and divided into subtypes of IBS.

Women	**All (n=151) ***		**IBS-C (n=36)**		**IBS-D (n=40)**		**IBS-M (n=33)**		**IBS-U (n=36)**	
	CV_w	CV_b	CV_w	CV_b	CV_w	CV_b	CV_w	CV_b	CV_w	CV_b
Energy	23.8	24.6	23.4	25.1	23.0	25.1	24.8	26.3	23.9	21.7
FODMAP	45.7	54.9	39.6	43.2	48.7	48.9	44.7	55.4	49.4	60.1
Fructose	104.9	109.5	93.5	72.7	107.7	100.5	116.5	144.6	104.6	114.8
Lactose	67.4	74.9	56.9	61.8	82.3	86.6	58.5	59.3	71.6	83.0
Fructans	46.9	51.9	47.0	43.3	46.8	51.4	42.6	58.4	45.1	45.2
GOS	72.8	89.6	78.4	87.4	75.2	81.3	65.3	104.7	64.6	56.9
Polyols	107.5	124.8	107.5	113.9	115.9	143.7	105.4	116.8	102.0	115.1
Men	**All (n=45) ***		**IBS-C (n=8)**		**IBS-D (n=14)**		**IBS-M (n=12)**		**IBS-U (n=10)**	
Energy	22.2	24.0	21.0	15.4	22.1	22.3	24.0	29.2	20.5	21.8
FODMAP	41.5	62.8	44.2	46.0	39.7	62.2	47.6	84.5	33.1	41.6
Fructose	100.5	108.3	123.8	98.4	95.4	78.9	119.1	172.0	70.3	58.3
Lactose	74.0	88.0	66.4	70.9	78.0	90.7	79.3	94.6	71.6	86.8
Fructans	45.6	58.9	51.4	53.6	33.9	66.3	46.2	42.3	50.7	57.9
GOS	73.6	84.8	69.7	51.5	71.9	79.7	75.2	105.5	65.1	85.2
Polyols	122.1	168.4	120.9	149.8	122.0	137.9	126.9	176.2	113.6	147.5

Abbreviations: CVw, coefficient of variation within subjects: CVb, coefficient of variation between subjects; FODMAPs, fermentable oligo-, di-, monosaccharides and polyols; GOS, galacto-oligosaccharides; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, mixed type IBS; IBS-U, unsubtyped IBS. *6 women and 1 man lack subtyping

From subjects participating in Paper I, intake of lactose and polyols are plotted from ten random female participants during four consecutive days, Figure 9. The corresponding CV_w were 67.4 and 107.5, respectively. For polyols, it is apparent that few food items contribute with very high levels of polyols, and that intakes on most days are very low (<1g/day). This means that several repeated observations are required to estimate an individual's habitual intake with a precision of +/-20% of true mean intake. The number of days range from 19 days for assessing total FODMAP intake to 46 days for lactose, and up to 117 days for intake of polyols. The comprehensive list can be seen in Paper I, Table 5.

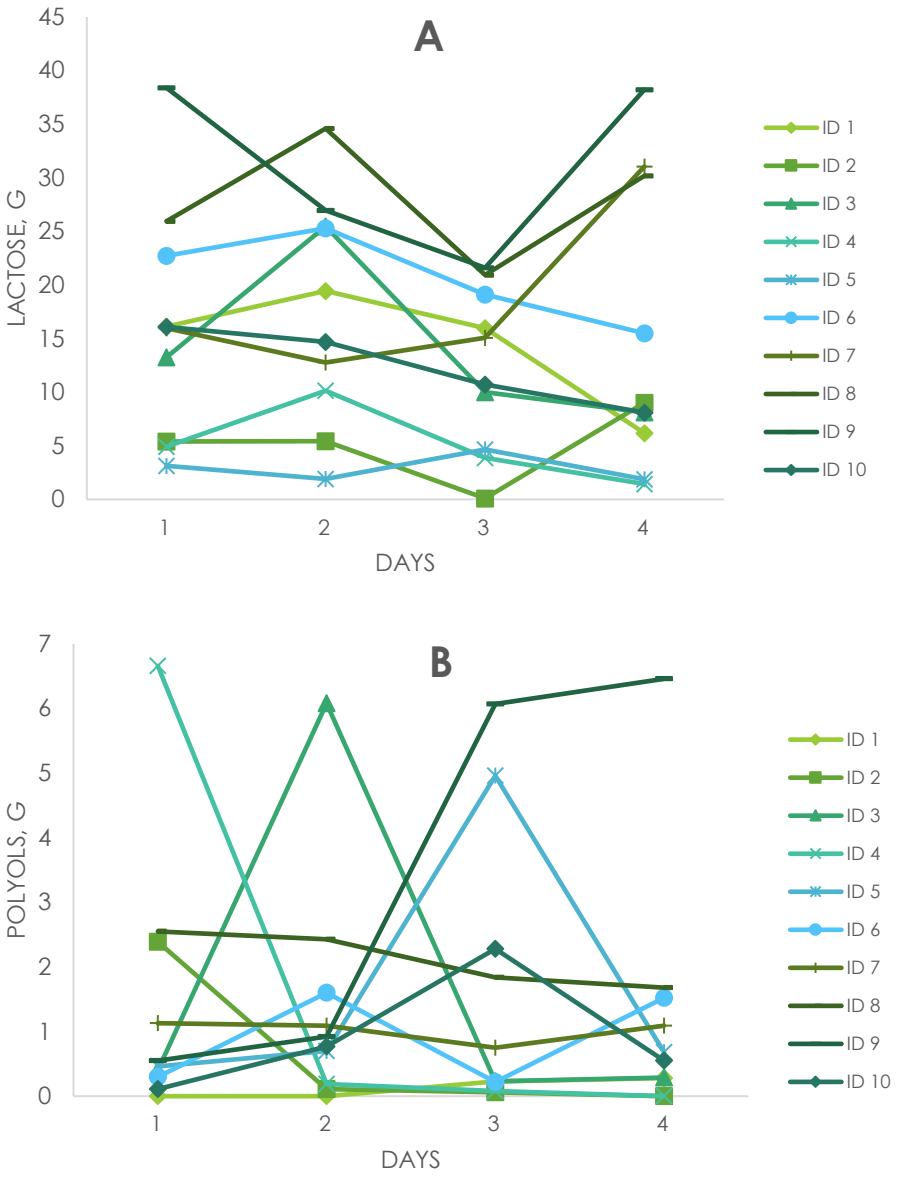

Figure 9. Variation in intake of a) lactose and b) polyols during four days for ten random female subjects with IBS, participating in Paper I. Intakes exhibited a large within-subject variation but even larger between-subject variation.

Recommendations for FODMAP intake assessment

The variability in FODMAP intake does impact how intake is best assessed, and what data precision different assessment methods generate. Nevertheless, food choices and dietary patterns are culturally conditioned, so these guidelines will only apply within study settings similar to Sweden.

In Table 5, an overview of three different data levels and recommendations in how to achieve FODMAP intake assessment are presented.

Table 5. Overview of three different levels of dietary data and how to assess dietary FODMAP intake.

Data level	Method	Comment
Estimating group mean intake in population-based studies or in large cohorts	One day of dietary data, provided that different days of the week are covered	This only applies provided that the sample size is sufficiently large [126]. In Paper III, Figure 1, no differences in group mean intake was noted during the four recording days.
Ranking individuals according to diet intake	Food record (4-7 days) or semi-quantitative food frequency questionnaires, or repeated 24-h recalls	Five days of repeated observations would provide a high level of accuracy (84% correctly classified into quartiles of intake), Paper III, Table 4.
Assessing absolute amounts of FODMAPs for correlation or regression analyses	Not feasible, unless combining information from food frequency questionnaires with e.g., repeated 24-h recalls, or by using dietary history method	This is highly dependent on the precision in data estimates needed. If +/-30% of true mean intake is acceptable, then 9 days of food records would be sufficient for assessment of total FODMAP intake (Paper I, Table 5).

Abbreviations: FODMAPs, fermentable oligo-, di-, monosaccharides and polyols

REPORTED DIETARY INTAKE IN PATIENTS WITH IBS

Several studies have described how habitual nutrient intake and diet quality appear among patients with IBS[52,103,134-141]. Of notice, these studies are performed in different countries with different food cultures, which will largely affect the dietary habits of the responders. Also, different dietary assessment methods have been used which may impact on the results.

Studies comparing dietary intakes in IBS compared to non-IBS controls have shown some conflicting results. In two studies, a Dutch and a French study, patients with IBS have reported a lower diet quality compared to controls, including lower fiber, higher energy, fat and sugar intake[135,136]. Some studies have indicated that dietary intake do not differ in IBS and controls[140,142]. Two studies, one in Sweden and one in the UK, have reported that on the group level, patients with IBS manage to achieve national recommendations for most nutrients (except for fiber intake in the Swedish study)[134,140]. In another study in the UK, assessing habitual diet in IBS using different indices to evaluate diet quality, many patients failed to meet dietary reference values for multiple nutrients[103].

A study among Iranian adults identified four distinct eating patterns in their study, which were named as: “traditional”, “western”, “fast food” and “lacto-ovo vegetarian”. Those exhibiting a fast food type of meal pattern had an increased odds of having IBS, whereas having a lacto-ovo vegetarian dietary pattern seemed to be protective of IBS[139]. In the French “NutriNet” cohort, a “western” kind of food pattern was associated with increased risk of having IBS[143]. Of notice, the “fast food” meal pattern in the Iranian study resembles that of the “western” meal pattern in the French study (with a high amount of “junk food”), so these results point in the same direction. A study from southern Sweden reported that IBS patients had irregular meal patterns and frequent intake of fast food and sugary drinks, and low intakes of fish and vegetables[141]. Intake of sugar-sweetened soda was correlated with increased IBS symptom severity in this study[141].

In summary, there seem to be room for improvement in diet quality within this patient group, where unhealthy eating pattern seems to increase the risk of IBS and having more severe IBS symptoms. Healthy eating patterns should be emphasized along with other IBS-specific dietary advice.

REPORTED FODMAP INTAKE IN PATIENTS WITH IBS

Only few studies have so far described dietary FODMAP intake among patients with IBS[136,144]. Therefore, we defined intake patterns of FODMAPs in Paper I, and described how much FODMAPs are consumed among patients with different subtypes of IBS in Paper II. An overview of FODMAP intake among women and men, divided into IBS subtypes, can be seen in Figure 10. Reported intakes appeared to be similar within the different subtypes. Only among women with IBS-D, a significantly lower lactose intake (compared to IBS-M) was reported (p=0.009).

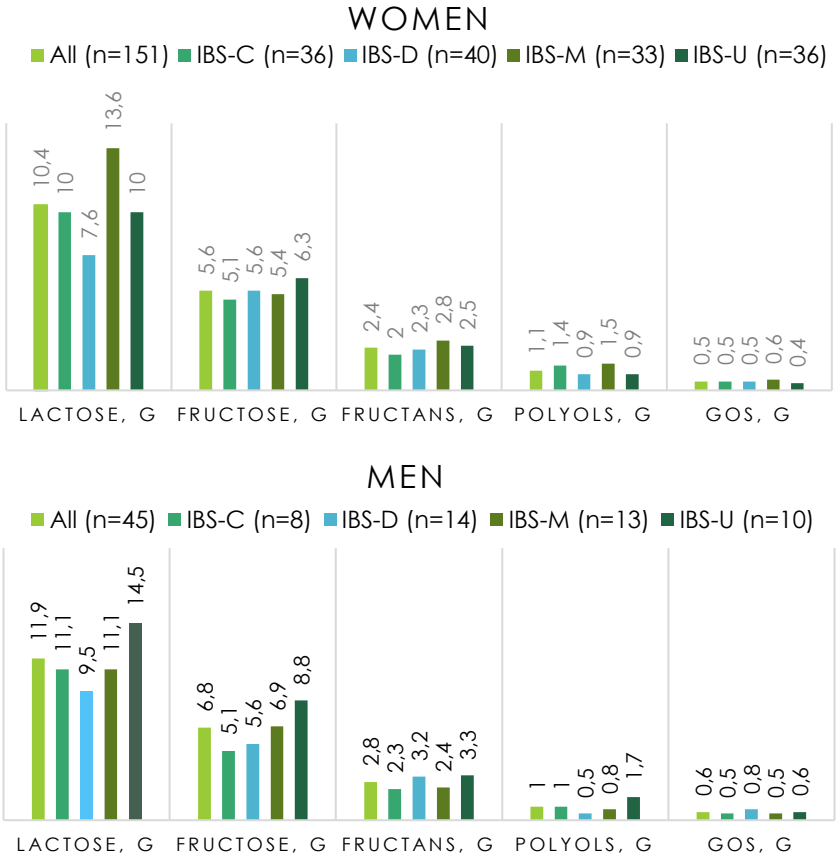

Figure 10. Reported FODMAP intake among women and men, within different subtypes of IBS. Six women and one man had missing information about IBS subtype. GOS, galacto-oligosaccharides. IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, mixed type IBS; IBS-U, unsubtyped IBS

The total amount of reported FODMAPs at group level was 20.0 g/day for women (mean 18.7 g, range 3.7-73.4), and 23.1 g/day for men (mean 22.8 g, range 3.6-63.2). The comprehensive list can be seen in Paper I, Table 2. Intakes were positively skewed and approximately 7% of reported intakes were ≥40 g/day, Figure 11.

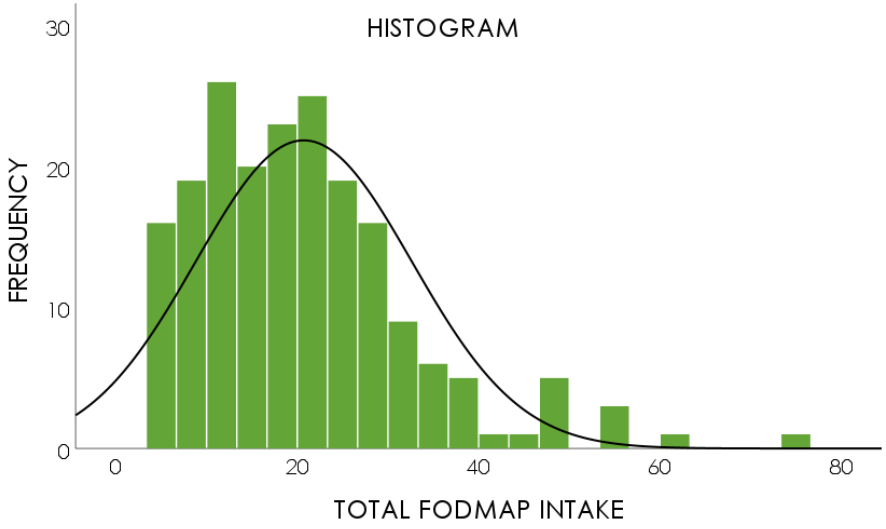

Figure 11. Histogram over the distribution of reported FODMAP intake (g/day) in women and men with IBS.

More than half of the total reported FODMAP intakes, 52%, derived from lactose both in women and men, as seen in Figure 12. Of notice, a large degree of adults living in Scandinavia are lactase persistent[145], which probably affect the amount of lactose tolerated and thus consumed.

A study in the UK reported that habitual FODMAP intake was 17 g/day in patients with IBS[144], which is well in line with our findings. A study in adults in Spain reported a daily FODMAP intake of 21.4 g, which was lower than that of children in their study, 33.4 g/day[146].

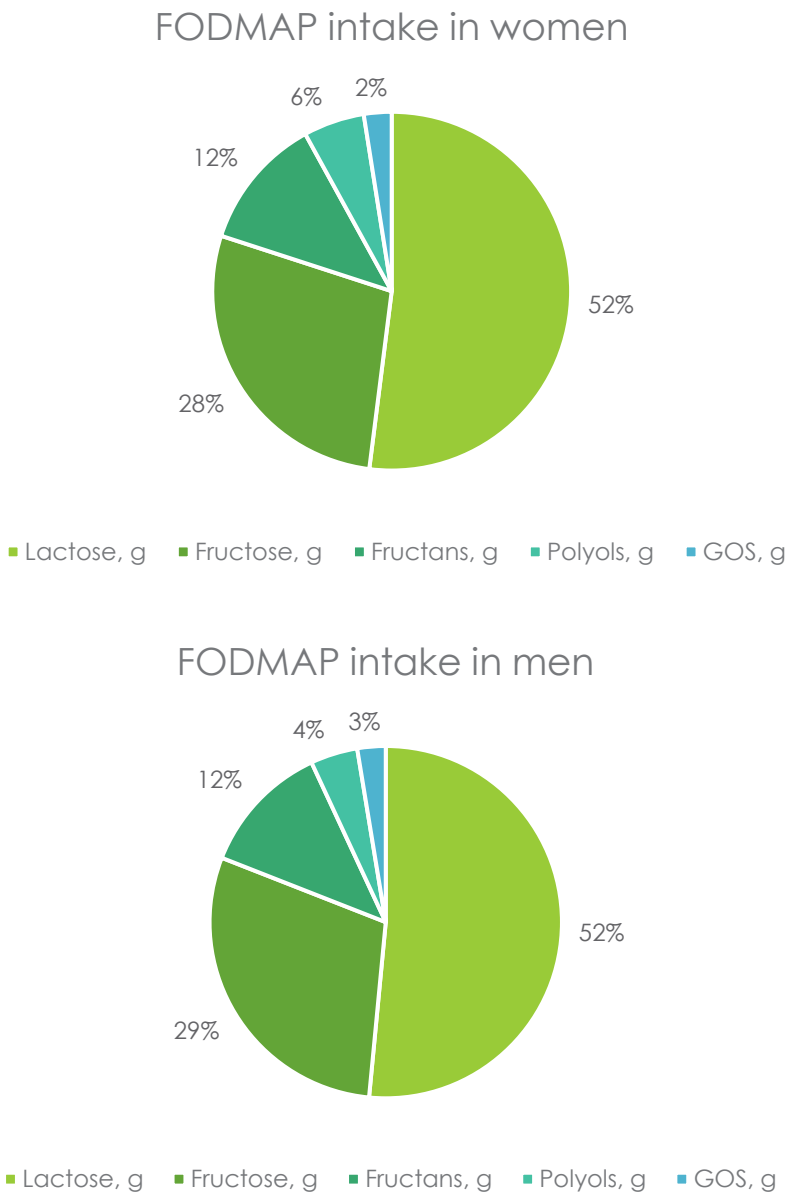

Figure 12. The proportion of FODMAPs that contribute to total FODMAP intake in women and men with IBS. GOS, galacto-oligosaccharides.

BIOMARKERS OF DIETARY INTAKE

As all dietary assessment methods include aspects of subjectivity and hence are prone to biases, it is of great interest to find biomarkers of diet intake that are objective, valid and applicable to different study populations with diverse dietary habits. Many definitions of dietary biomarkers exist, and one of them states: "any biological specimen that is an indicator of nutritional status with respect to intake or metabolism of dietary constituents. It can be biochemical, functional or clinical index of status of an essential nutrient or another dietary constituent"[147].

Dietary biomarkers have many applications within diet research, and except that they function as markers for dietary exposure, they can be used for measuring compliance in dietary intervention studies, or in validation and calibration studies. As to date, several biomarkers for dietary exposure have been identified and are in use. Some of these include; doubly labeled water for assessing total energy expenditure, urinary nitrogen for protein intake, alkylresorcinols as markers for whole grain intake, vitamin C and carotenoids as markers of fruits and vegetable, etc.[148,149]. The downside with these existing biomarkers is that they only measure one aspect of dietary intake, which complicates studies where the exposure is unknown.

In Paper III, we found that urinary polyols had decreased in the group receiving a low FODMAP diet, and levels were significantly lower in post-intervention samples compared to pre-samples. As dietary intake of polyols is difficult to assess and would require >100 days of repeated observations to estimate individual polyol intake (Paper I), it seems promising if polyols measured in urine could function as a biomarker for polyol intake. Polyol excretion in urine have previously been characterized[150,151], but whether excretion correlate to intake of polyols in a way that it may act as a dietary biomarker remain to be established. These findings will need to be validated in studies investigating the specificity, sensitivity and dose-response relationship of dietary intake versus biomarker concentration.

METABOLOMICS

Metabolomics is one of several ”omics” fields within biology that are used to characterize and quantify pools of molecules. Metabolomics can be defined as “the quantitative measurement of the multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification”[152]. A metabolite is usually defined as any molecule less than 1500 Da in size[153], and these are products of chemical reactions, our metabolism, that continually take place within the human body. Because of the dynamic nature of metabolic processes, we are only able to capture snapshots of metabolite patterns in each measurement. On the other hand, by applying metabolomics, all metabolites are measured concurrently which enables the study of several exposures simultaneously.

Several factors affect our metabolome, such as our genetic makeup and gut microbiota composition. But more importantly, we can affect our metabolome by external factors in our exposome, that is our diet, drugs, lifestyle factors, etc. A schematic overview can be seen in Figure 13. All these factors taken together will form our metabolic phenotype, or metabotype.

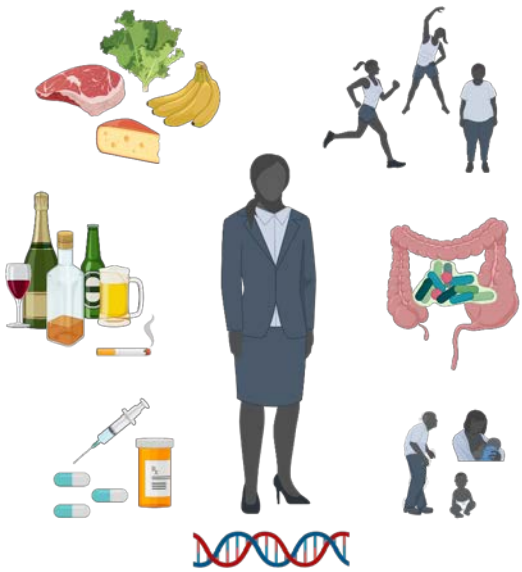

Figure 13. An individual’s metabotype is determined by several factors that interplay. It is to some part determined by our genes, but foremost it is affected by diet, lifestyle, drug use, physical activity, body composition, gut microbiota composition and age. Image created with BioRender.com

There are basically two approaches in metabolomics: non-targeted and targeted metabolomic profiling. The former measures all available metabolites in the sample and is considered to be hypotheses-generating, whereas the latter is used for testing hypotheses and focuses on identification and quantification of a predefined set of metabolites. As metabolomics generate "big data", and several predictor and outcome variables are analyzed simultaneously, multivariate statistics may be used.

Principal Component Analysis (PCA) is often applied to large data sets as a dimensionality-reduction method, i.e., by reducing the number of variables of a data set, while preserving as much information as possible. PCA is an unsupervised method that does not consider any underlying assumptions of the variables included, but merely generate a correlation matrix of all variables in the data set. Variables that correlate will form the new variables, or the principal components, that are used to interpret the data. The output of an PCA is used to visualize systematic trends and clusters. In paper III, PCA was used to explore clustering trends in baseline metabolites from serum and urine on patients with IBS, participating in a dietary intervention trial. No clear trends could be envisioned, and Figure 14 is an example of a PCA where no distinct clustering patterns was seen. In this particular case, metabolite patterns of subjects with similar subtypes of IBS failed to cluster.

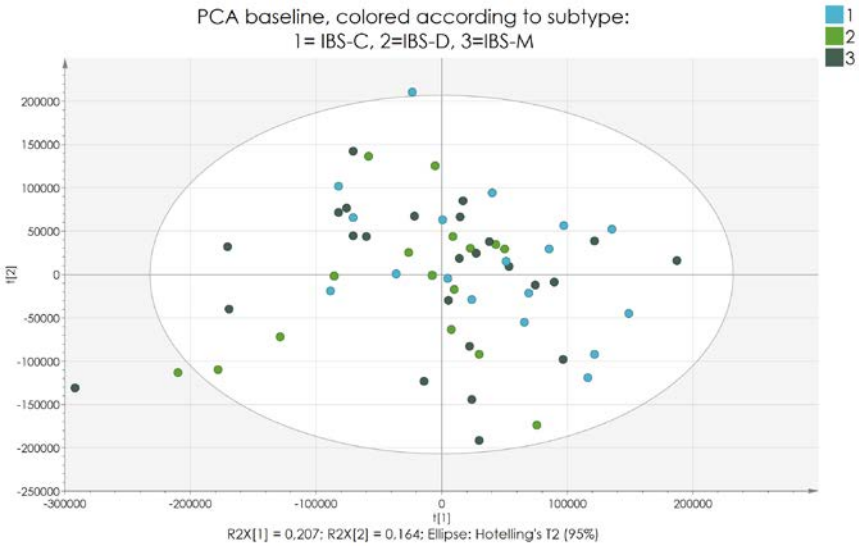

Figure 14. A principal component analysis in urine samples from patients with IBS, colored according to IBS subtype. IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, mixed type IBS.

Multivariate models do not generate a "p-value" like traditional correlation or regression analyses do, but the models can be evaluated by their R^2 and Q^2 values. The R^2 value describes the "goodness of fit", and when using biological samples, an R^2 value ≥ 0.5 is considered sufficient. The predictive ability of the model is represented by the Q^2 value, which ideally should be ≥ 0.4[154]. The R^2 and Q^2 should however not be too distant from each other, because a large discrepancy means that the model has many irrelevant terms[154].

Orthogonal partial least square discriminant analysis (OPLS-DA) is a supervised regression method that may be used for identifying discriminatory variables between classes of subjects, such as "responders" and "non-responders", or "treated" and "controls". These variables are visualized in the loading plots, in which those that discriminate the most are found furthest from the origin. In paper III, OPLS-DA was performed to distinguish between responders and non-responders to a low FODMAP diet, and the score plot with corresponding loading plot can be seen in Figure 15 as an example of an OPLS-DA. This model presented with good fit (R^2X= 0.58) and acceptable predictive capacity (Q^2X=0.447). Metabolites that contributed most to the separation between responders and non-responders were in this case identified as glucose (serum), 2-hydroxybyturate (serum), arginine (serum), and pantothenate (urine).

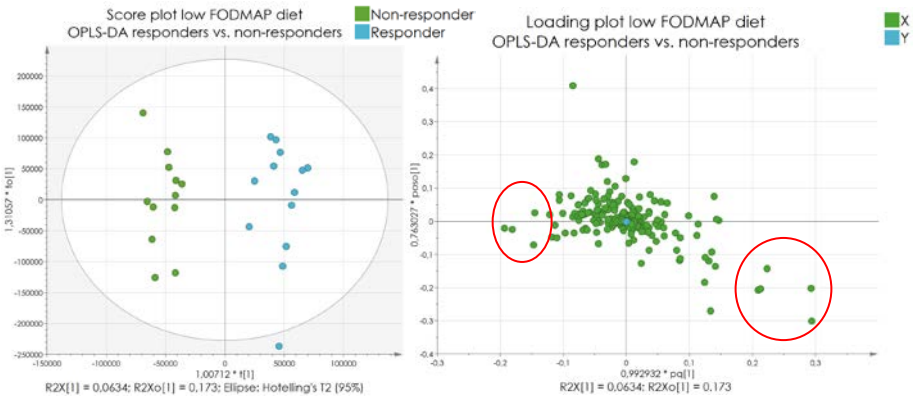

Figure 15. Examples of an orthogonal projection to latent structure discriminant analysis (OPLS-DA) using metabolomics in urine samples. The score plot envisions the subjects according to their response to a low FODMAP diet, and the corresponding loading plot visualizes the metabolites that are mostly responsible for separation between these groups.

In recent years, metabolomics has become more widely used within the field of gastroenterology. As IBS lack diagnostic biomarkers and objective markers for disease progression, metabolomics may provide new opportunities to facilitate diagnostics using limited invasive interference. Also, it may reveal new insights into the pathophysiology of IBS.

Several attempts have been made to metabolically distinguish patients with IBS from healthy control subjects. Numerous metabolites have been shown to differ in concentration between these two groups, but the studies have not yet been able to show a consistent pattern. For instance, a study by Ponnusamy et al[155] applied gas chromatography mass spectrometry (GC/MS) metabolomics in fecal samples, and found that amino acids (alanine and pyroglutamic acid) and phenolic compounds (hydroxyphenyl acetate and hydroxyphenyl propionate) were higher in concentrations in IBS patients compared to controls. A study by Yamamoto et al.[156] identified IBS-specific metabolites in urine using multisegment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS), where hydroxylysine metabolites, mannopyranosy-l-tryptophan, imidazole propionate, glutamine, serine, ornithine, dimethylglycine and dimethylguanosine seemed to distinguish IBS patients from healthy controls. Further, a study by Baranska et al.[157] used a breath analysis test with volatile organic compounds to distinguish patients with IBS from healthy controls using GC/MS metabolomics. They found that a set of 16 volatile organic compounds correctly predicted 89.4% of the IBS patients and 73.3% of the healthy controls, where 1-ethyl-2-methyl-cyclohexan, 6,10-dimethyl-5,9-undecadien-2-one, benzyl-oleate, methyl-cyclohexane, tetradecanol, n-hexane and butane were among the identified compounds.

These studies exemplify some of the major challenges with metabolomics, namely that all kinds of biofluids or volatile organic compounds may be used in order to identify metabolites. Also, several different methods are used for metabolomics identification. These include spectroscopic (NMR), spectrometric (MS) and separation techniques (LC, GC, supercritical fluid chromatography, CE)[158]. Applying different methods, using different types of biofluids and in heterogenous populations like IBS cohorts, make comparisons of findings difficult.

METHODOLOGICAL CONSIDERATIONS

Study participants

The papers included in this thesis were based on data assembled within three clinical studies performed at a specialized unit for functional GI disorders at Sahlgrenska University Hospital, Gothenburg, Sweden (study B in collaboration with Karolinska University Hospital, Stockholm, Sweden, and Sabbatsberg Hospital, Stockholm, Sweden). An overview of the included studies, their methods, and design can be seen in Table 6.

Table 6. Overview of the three studies that are included in Paper I-IV

Study	A	B	C
Year	2010-2012	2013-2014	2007-2009
Subjects	200 cases with IBS (Rome III) and 50 controls	75 patients with IBS (Rome III)	86 patients with IBS (Rome II)
Aim	(i) To characterize pathophysiological traits of IBS (ii) to evaluate the effect of a fermented milk product containing probiotics on GI symptoms	To compare the efficacy of a low FODMAP diet compared to traditional IBS diet	To characterize pathophysiological traits of IBS
Design	Case-control study + subgroup continuing with a 14-d parallel, double-blind, randomized controlled trial	Multi-centre, parallel, single-blind randomized controlled trial	Cross-sectional study
Assessments Methods	(i) 4-day food record, IBS-SSS, permeability test, ECG, rectal barostat test, brain MRI, lactulose nutrient challenge test, fecal microbiota profiling, IgE, questionnaires assessing allergy and self-perceived food intolerance (ii) 125 ml of fermented yoghurt with probiotics or a non-fermented control product was consumed twice a day for 14 days	4-day food record, IBS-SSS, questionnaires Study participants received oral and written information about their allocated diet	Rectal barostat test, oro-anal transit time, fecal samples, questionnaires assessing allergy and self-perceived food intolerance, IgE
Included in paper	Paper I (N=122) Paper II (N=114) Paper IV (N=137 cases, 47 controls)	Paper I (N=75) Paper II (N=75) Paper III (N=56)	Paper IV (N=86)

Abbreviations: GI, gastrointestinal; IBS, irritable bowel syndrome; IBS-SSS, IBS severity scoring system; ECG, electrocardiogram; MRI, magnetic resonance imaging; IgE, Immunoglobulin E; FODMAP, fermentable mono-, di-, oligosaccharides and polyols.

All studies were conducted in accordance with the declaration of Helsinki and had received ethical approval from the Regional Ethical Review Board in Gothenburg. Informed written consent was provided from all study participants and they received oral and written information about the study procedures.

Criteria for inclusion were similar in all studies, namely that all participants (except control subjects) were diagnosed with IBS according to the Rome criteria that were currently used, and both women and men between 18 and 65 years of age were eligible (between 18 and 70 years in study B). Subjects were excluded if they had any other GI disorder except IBS explaining their symptoms, or if other illness or severe psychiatric disease, including alcohol abuse, were present. Further, they were not allowed to take probiotic supplements, be pregnant or breastfeeding, or to have abnormal results on standard screening laboratory tests. In study B, only patients with moderate and severe IBS symptoms were enrolled (IBS-SSS ≥175).

Participants were enrolled both from patients being referred primarily from primary care to our specialized GI unit, but also after advertisement in the local newspaper.

To consider: As most IBS patients in Sweden are treated in primary health care, there is a risk of selection bias when studies are performed in a specialized GI unit. These patients might have more severe symptoms and/or more psychological distress compared to patients who are treated in primary health care or compared to those who do not seek healthcare at all. On the other hand, participants in the studies included in this thesis were also recruited through advertisements in the local newspaper, ensuring a more representative group of patients. Furthermore, most of the included patients were referred to our unit from primary care and were normally managed in primary care.

Moreover, studies that focus on diet also are at risk of selection bias as these studies are known to attract individuals who are interested in dietary modifications (study B). Having highly motivated study participants may impact on the results of the interventions, both in terms of internal and external validity.

In all, all studies included both women and men of all subtypes of IBS, and with a broad range of symptom severity and pathophysiological traits – thus, these findings should be transferrable to patients with IBS in general.

Assessment methods and questionnaires

To be able to cover as many aspects of IBS as possible, several validated questionnaires have been used. The main outcome variable in Paper II, III and IV was IBS symptom severity, which in all papers was assessed with IBS-SSS.

To consider: As we lack objective markers of IBS disease and symptom severity, we must rely on subjective and self-reported measures. This applies to most variables used in this thesis, besides objective laboratory measurements and metabolomics analyses.

Most self-reported measures are at risk of response biases of different kinds. Especially in case-control studies assessing past events, as in Paper IV where a history of atopic disease was examined, there is a risk of recall bias. Recall bias is a systematic error caused by difficulties in accurately reporting past events, which might also be influenced by subsequent events.

When several questionnaires are completed in one session, as in the included studies, it might lead to respondent fatigue. This leads to less accurate data as the attention and motivation drops during the answering session. For that reason, the main outcome IBS-SSS has mostly been assessed at separate timepoints from the other questionnaires. Several questionnaires are also web-based, which has the advantage of automatic quality control to ensure that all questions are filled in.

To measure IBS symptom severity in intervention studies, the questionnaire IBS-SSS is widely used. A score reduction of ≥50 is the most used cut-off to define response to treatment, as it is considered to be a clinically relevant reduction in symptom severity, and this facilitates comparisons in outcomes between studies. However, the cut-off is rather harsh and does not consider if individuals have a score reduction of 49 or 51. This might have affected the results in Paper III, as we only used that cut-off to compare metabolomics patterns between groups.

Diet intake has throughout been assessed using a 4-day estimated food record. Records have been kept during four consecutive days, i.e., Wednesday-Saturday. Study participants have received verbal and written instructions on how to perform the diet records, and volumes have been estimated using standard measures or household utensils. Energy and nutrient calculations were performed using the software Dietist XP version 3.1 (Kostdata.se, Stockholm, Sweden) with a FODMAP-database add-on[159].

To consider: As stated previously, all dietary assessment methods have their pros and cons. Food records have the advantage that they do not rely on a person's memory when completed, but there is always a risk that people change their normal dietary habits during recording days in order to make them appear more socially acceptable, e.g., more healthy. This is called a social desirability bias[160], which might have a large impact on the results. In particular, intake of fat is commonly underreported, and intake of healthy foods, such as fish, fruits and vegetables, is commonly overreported[161-163]. The study participants were not aware that FODMAPs was of particular interest for us, but, as the sources of FODMAPs to a large degree consist of vegetables and fruits, there might be a risk that intakes were overestimated.

The software used to calculate energy and nutrient intakes has some limitations. Firstly, we do not have a national Swedish FODMAP database, so the FODMAP database that was used was aggregated from different existing published sources of FODMAPs[159]. This might lead to some systematic biases in intake assessments, as foreign foods might differ in FODMAP content compared to Swedish foods. This mostly concerns the fructose content, because fructose is more widely used abroad as sweetener in soft drinks etc. Still, as we have consistently used data rankings for FODMAP intake instead of absolute amounts in our analyses, this should not have had any major implications for the results in our studies.

Statistical aspects

All four papers included in this thesis are based on data collected in previous studies, so called *post hoc* analyses.

To consider: When data from studies are used for purposes that were not planned initially, problems could arise with the number of subjects included. Usually, the number of subjects included in a study is based on a sufficient power to answer the primary hypotheses of the study. Thus, in *post hoc* analyses, you might lack the power to detect true differences.

In Paper I, we estimated the group mean intake of FODMAPs among women and men with IBS. The number of subjects needed to determine a group mean intake of FODMAPs with high precision, as reported in this thesis frame, was 149 women, which we nearly managed to include. However, the number of men included was underpowered, which had most impact in Paper II – here, we were unable to perform correlation and regression analyses with only 45 men. A strength with the analyses in Paper II is that we knew beforehand what level of precision in FODMAP intake we could achieve, and thus only rankings of FODMAP intake was used.

In Paper III, we performed multivariate analyses on metabolomics samples. Metabolomics is a rather new discipline, which has seen a rapid development during this past decade. Even so, some challenges remain regarding the utilization and standardization of metabolomics samples. These include the application of different analytical techniques, a large diversity in the choice of biofluids/samples and the lack of standardized protocols regarding sample handling and preparation. Luckily, we could take advantage of a research infrastructure with a lot of experience in metabolomics analyses, to ensure that samples were analyzed in the best possible way.

As discussed in Paper III, multivariate analyses are commonly validated using training and test sets. This usually involves splitting your data into two sets, where the first training set comprises of approximately 80% of the data, and later the remaining 20% are projected into the model built on the training set. However, considering the small number of subjects included in the analyses in this thesis and the heterogenous study population, we chose not to split the data and instead only used the cross-validation by default in the SIMCA software.

In Paper IV, we compared the prevalence of atopic disease among patients with IBS and a non-IBS control group. Although the prevalence of atopy was 55% vs. 40% respectively, which could be considered as a clinically relevant difference, this was not statistically significant. This could be due to type 2 error, i.e., when you cannot detect a difference where there actually is one. This can be resolved by increasing the number of participants. When we performed a *post hoc* power calculation based on the findings in our study, i.e., a prevalence of 55% and 40%, we learnt that we would have needed 173 individuals in each group to have sufficient power (80%) to detect true differences between these groups with our two-sided test. However, it is important to stress that this research question was not the main aim of the study; to answer whether atopic disease is more common among patients with IBS compared to non-IBS controls would require well-designed epidemiological studies.

In the same paper, we observed that atopic IBS patients did not exhibit more severe IBS symptoms compared with non-atopic patients. However, data presented in this thesis frame suggest that there might be an increasing symptom burden with increasing number of allergic manifestations. Only six individuals reported having all three allergic manifestations, so again, we might not have had sufficient power to detect differences in symptom severity with increasing number of manifestations.

CONCLUSIONS AND FUTURE PERSPECTIVES

Measuring dietary intake is a cumbersome task, and assessment of FODMAPs makes no exception. The results in Paper I indicated that the variation in FODMAP intake is large within individuals, but even larger between individuals. As a result, it becomes difficult to capture the absolute FODMAP intake at the individual level. This has major implications when attempting to perform correlation or regression analyses, as dietary estimates of FODMAPs will not have sufficient precision at the individual level. To resolve this issue, it would probably require to "think outside the box". Combining different methods for diet intake assessment would likely improve the estimations. For instance, it would be desirable to take advantage of the precision in intake estimates using repeated 24-h recalls or several days of food records, combined with the advantages of food frequency questionnaires to capture foods eaten less frequently. Also, if there are ways to include biomarkers of dietary intake, intake estimates would probably cumulatively improve. Otherwise, it is advised to use data rankings instead.

In paper II, we observed that reported intake of FODMAPs was fairly similar among the different subtypes of IBS, except for a somewhat lower intake of lactose among women with IBS-D. Intake of FODMAPs was related to more severe IBS symptoms, mainly in women with IBS-U. Here, we noted that fructose (in excess of glucose) intake was associated with increased IBS symptom severity. Food sources that contribute to fructose intake are many, and include apples, pears, exotic fruits, honey etc. In as much as we analyzed data in a cross-sectional study design, we cannot say whether it is the fructose *per se* that cause symptoms, or by how the fructose is consumed. A correlation does not imply that there is a causation. Symptoms could for instance be caused by an excessive tea consumption together with honey, or by having an irregular meal pattern and snacking on a lot of fruit in between meals. Also, it is intriguing that this relationship was only seen among women with IBS-U. Do these patients have a higher prevalence of fructose intolerance, or do they have other pathophysiological traits in common that explain this occurrence? Further studies looking into meal patterns and consumption on food instead of nutrient intake could possibly provide more clues. It is important also to remember the complexity of foods, as foods that contain high amounts of FODMAPs might generate symptoms for reasons other than the FODMAP component. For instance, cereal based products contain gluten, which might cause symptoms in susceptible individuals, e.g., with non-celiac gluten sensitivity. Cereals are also rich in insoluble dietary fiber which are difficult to

digest. Further, the new findings that ingestion of milk, soy, and wheat can cause local immune reactions in the gut warrants further studies, as these foods are also high in FODMAPs – but in this case, the protein component of the food and not the carbohydrates are believed to cause the reaction. Mechanistic studies and food challenge tests might be useful to validate these findings and provide with further insights.

Using NMR metabolomics in Paper III, we were able to differentiate metabolite patterns between responders and non-responders to a low FODMAP diet. The metabolites that were identified as driving the separation are abundant in certain foods, such as whole grain cereals and legumes. Some of the metabolites might also be related to a decreased energy or carbohydrate intake. However, it is important to emphasize that metabolites identified using a non-targeted metabolomics approach should be considered as hypotheses-generating. This implies that these findings need to be validated in studies exploring the mechanistic actions and contributing with biological understanding of the pathways involved. Most multivariate models in this paper did exhibit a poor fit with low predictive value, especially those performed within the traditional IBS diet. A small sample size together with a heterogenous group of study participants probably contribute to this. As an individual's metabotype is largely affected by traits such as age, gender, body mass index, use of prescription drugs and dietary intake, by controlling for these differences (using matched controls or by stratifying study subjects into more homogenous subsamples), we should be able to minimize the between-subject variation and hopefully be able to isolate metabolites that relate to the mechanistic action of the treatment/research question. Larger studies are thus warranted.

In Paper IV, we demonstrated that atopic disease is common among patients with IBS, but not necessarily more common than among non-IBS controls. Neither self-reported atopic disease nor IgE-levels in serum were associated with the severity of IBS symptoms. However, symptom severity was related to female gender, somatization and the number of food items one reported adverse reactions to. The degree of somatization and the number of food items causing adverse reactions both could be expressions of an increased level of sensitivity, and it would be interesting to examine if these variables correlate to visceral hypersensitivity as well. Also, as previously mentioned, new findings suggest that local food hypersensitivity reactions can arise in the gut, that are not reflected in serum IgE-levels. The role of local gut immune reaction

needs to be elucidated in order to understand the link between food hypersensitivity and symptom generation in IBS.

In summary, as IBS can manifest in a variety of ways, there are probably several treatment options that may be efficient in reducing symptoms of IBS – the difficult part is to provide the right treatment option for the right patient. It is also likely that individuals who exhibit different pathophysiological traits may benefit from different diets, or even other treatment strategies. We hope that our ongoing randomized controlled trial comparing three different treatment options for IBS (see Appendix) will provide more understanding on predictors of response to treatment, and how we best can tailor treatments for patients with different symptomatology. In the future, we will hopefully be able to give more personalized treatment advice to these patients, taking into consideration not only the subtype of IBS, but also other pathophysiological traits, clinical parameters and metabolic phenotypes.

ACKNOWLEDGEMENTS

During my doctoral studies, I have been privileged to be surrounded by many supportive, intelligent and kind people, and I would like to extend my sincere gratitude and appreciation to all of you involved in the process of writing of this thesis.

Firstly, to all the **study participants** who have volunteered in our studies – without your selfless act, partaking in both time-consuming and intricate procedures, we would not have been able to conduct any research. A huge THANK YOU to each and everyone of you!

Anna Winkvist, I could not have wished for a better main supervisor! Your pedagogical skills during the course in statistics awoke my interest in research in the first place, and I will be forever grateful for that. Besides being an excellent teacher and a great researcher, you have a very warm and including personality. Thank you for all the encouragement, never ending support, and for believing in me.

My co-supervisor **Magnus Simrén**, thank you for introducing me into the world of IBS research and for excellent guidance through it. I am truly thankful for welcoming me into the lab and for demonstrating how excellent translational research works. I admire your endless energy and enthusiasm for conducting new research.

My co-supervisors **Stine Störsrud**, I appreciate all the hands-on practical stuff that you have helped me with throughout these years, and for teaching me about diet in IBS. You are a very hard-working and energetic person, and I am very glad to have you in my team! **Helen Lindqvist**, thank you for helping me understand the field of metabolomics (and for having the patience to answer the same questions over and over again), I could not have done it without you.

Hans Törnblom, co-author of all papers included in my thesis – you are a very humble and knowledgeable gastroenterologist, which both I and (I am sure) all of the patients appreciate very much.

To **all co-authors** contributing with new insights and perspectives – thank you for the collaboration.

To all staff at the NMR-centrum, **Anders**, **Daniel**, **Göran** and **Millie**, who with a lot of experience (and patience!) have assisted with all the practicalities and technicalities regarding the metabolomics analyses – thank you!

Till alla mina nuvarande och gamla kollegor på **Klinisk Nutrition** – ni är underbara och det har varit ett sant nöje att arbeta med er! Tack för att ni gett mig förtroendet att undervisa på dietistprogrammet, och för allt snicksnack i lunchrummet, under fredagsfikat och på alla AW:n. Detsamma gäller alla kollegor på **Magtarmlab** – ni har förgyllt min tid under doktorandstudierna med er humor och ert härliga bemötande. Jag ser fram emot att få arbeta mer er igen!

Lasse Ellegård, trots att det inte blev så mycket metabolomik som det var tänkt från början så är jag tacksam för det stöd jag fick i början av mina doktorandstudier.

Lena Böhn, tack för att du med sådan entusiasm tog över stafettpinnen som dietist i CARIBS när jag gick på föräldraledighet. Bra jobbat!

Jörgen och **Gudmundur,** tack för att i egenskap av chef ha välkomnat mig till avdelningen för klinisk nutrition och för ert stöd under denna tid.

Bodil, tack för att du lärde mig allt jag behövde veta om variation i kostintag. Att den första artikeln kom till är till stor del din förtjänst.

Linnea och **Sofia I,** det är en ynnest att få ha så goda vänner som kollegor. Tack för allt stöd och pepp i stort och smått, både på jobbet och privat (och för allt vin och alla skratt!).

Till alla mina **doktorandkollegor**, tack för allt gött snack – till viss del forskningsrelaterat, men mest om andra viktiga saker här i livet ☺

Till **alla mina vänner**, **Anna**, **Sofia G**, **Kristina, Sofia H** och hela **HLB-gänget** och exakt precis alla ni andra som också finns i mitt liv och påminner mig om att det finns ett liv "på andra sidan". Ni är bäst!

Tack till hela min **stora familj - mina föräldrar** för att ni alltid haft förtroende för mig och för att ni alltid låtit mig fatta mina egna beslut. Till mina syskon **Ari**, **Jani**, **Johanna** och **Kalle**, för att ni hejat på längs vägen.

Min älskade **lilla familj**. Tack **Magnus** för allt ditt stöd, pepp och för alla dagar du vabbat och nattat för att detta skulle bli möjligt. Till min son **Stellan**, för att du påminner mig om det som är viktigt i livet ♥

REFERENCES

1 Oka, P., Parr, H., Barberio, B., Black, C. J., Savarino, E. V. & Ford, A. C. Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *The Lancet Gastroenterology & Hepatology* **5**, 908-917, doi:https://doi.org/10.1016/S2468-1253(20)30217-X (2020).

2 Lovell, R. M. & Ford, A. C. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clinical gastroenterology and hepatology* **10**, 712-721. e714 (2012).

3 Lacy, B. E., Mearin, F., Chang, L., Chey, W. D., Lembo, A. J., Simren, M. & Spiller, R. Bowel Disorders. *Gastroenterology* **150**, 1393-1407.e1395, doi:https://doi.org/10.1053/j.gastro.2016.02.031 (2016).

4 Dunlop, S. P., Jenkins, D. & Spiller, R. C. Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. *The American Journal of Gastroenterology* **98**, 1578-1583, doi:https://doi.org/10.1016/S0002-9270(03)00375-7 (2003).

5 Spiller, R. & Garsed, K. Postinfectious Irritable Bowel Syndrome. *Gastroenterology* **136**, 1979-1988, doi:https://doi.org/10.1053/j.gastro.2009.02.074 (2009).

6 Thabane, M., Kottachchi, D. & Marshall, J. Systematic review and meta-analysis: the incidence and prognosis of post-infectious irritable bowel syndrome. *Alimentary pharmacology & therapeutics* **26**, 535-544 (2007).

7 Tobin, M. C., Moparty, B., Farhadi, A., DeMeo, M. T., Bansal, P. J. & Keshavarzian, A. Atopic irritable bowel syndrome: a novel subgroup of irritable bowel syndrome with allergic manifestations. *Annals of Allergy, Asthma & Immunology* **100**, 49-53 (2008).

8 Thomsen, S. F. Epidemiology and natural history of atopic diseases. *European Clinical Respiratory Journal* **2**, 24642, doi:10.3402/ecrj.v2.24642 (2015).

9 Magnusson, J., Lin, X. P., Dahlman-Höglund, A., Hanson, L. Å., Telemo, E., Magnusson, O., Bengtsson, U. & Ahlstedt, S. Seasonal intestinal inflammation in patients with birch pollen allergy. *Journal of allergy and clinical immunology* **112**, 45-51 (2003).

10 Powell, N., Huntley, B., Beech, T., Knight, W., Knight, H. & Corrigan, C. J. Increased prevalence of gastrointestinal symptoms in patients with allergic disease. *Postgraduate medical journal* **83**, 182-186 (2007).

11 Polster, A. V., Palsson, O. S., Törnblom, H., Öhman, L., Sperber, A. D., Whitehead, W. E. & Simrén, M. Subgroups of IBS patients are characterized by specific, reproducible profiles of GI and non-GI symptoms and report differences in healthcare utilization: A population-based study. *Neurogastroenterology & Motility* **31**, e13483 (2019).

12 Kennedy, P. J., Cryan, J. F., Dinan, T. G. & Clarke, G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? *World journal of gastroenterology: WJG* **20**, 14105 (2014).

13 Mearin, F., Baró, E., Roset, M., Badía, X., Zárate, N. & Pérez, I. Clinical patterns over time in irritable bowel syndrome: symptom instability and severity variability. *American Journal of Gastroenterology* **99**, 113-121 (2004).

14 Fond, G. *et al.* Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis. *European archives of psychiatry and clinical neuroscience* **264**, 651-660 (2014).

15 Cole, J. A., Rothman, K. J., Cabral, H. J., Zhang, Y. & Farraye, F. A. Migraine, fibromyalgia, and depression among people with IBS: a prevalence study. *BMC gastroenterology* **6**, 1-8 (2006).

16 Cremonini, F. & Talley, N. the overlap between functional dyspepsia and irritable bowel syndrome—a tale of one or two disorders? *Alimentary pharmacology & therapeutics* **20**, 40-49 (2004).

17 Lackner, J. M. *et al.* Type, Rather Than Number, of Mental and Physical Comorbidities Increases the Severity of Symptoms in Patients With Irritable Bowel Syndrome. *Clinical Gastroenterology and Hepatology* **11**, 1147-1157, doi:https://doi.org/10.1016/j.cgh.2013.03.011 (2013).

18 Lacy, B. E. & Patel, N. K. Rome criteria and a diagnostic approach to irritable bowel syndrome. *Journal of clinical medicine* **6**, 99 (2017).

19 Kanazawa, M., Hongo, M. & Fukudo, S. Visceral hypersensitivity in irritable bowel syndrome. *Journal of Gastroenterology and Hepatology* **26**, 119-121, doi:https://doi.org/10.1111/j.1440-1746.2011.06640.x (2011).

20 Mertz, H., Naliboff, B., Munakata, J., Niazi, N. & Mayer, E. A. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. *Gastroenterology* **109**, 40-52 (1995).

21 Simrén, M., Törnblom, H., Palsson, O. S., van Tilburg, M. A. L., Van Oudenhove, L., Tack, J. & Whitehead, W. E. Visceral hypersensitivity is associated with GI symptom severity in functional GI disorders: consistent findings from five different patient cohorts. *Gut* **67**, 255-262, doi:10.1136/gutjnl-2016-312361 (2018).

22 Major, G., Pritchard, S., Murray, K., Alappadan, J. P., Hoad, C. L., Marciani, L., Gowland, P. & Spiller, R. Colon Hypersensitivity to Distension, Rather Than Excessive Gas Production, Produces Carbohydrate-Related Symptoms in Individuals With Irritable Bowel Syndrome. *Gastroenterology* **152**, 124-133.e122, doi:https://doi.org/10.1053/j.gastro.2016.09.062 (2017).

23 Barbara, G. *et al.* Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* **126**, 693-702, doi:10.1053/j.gastro.2003.11.055 (2004).

24 Park, C. H., Joo, Y. E., Choi, S. K., Rew, J. S., Kim, S. J. & Lee, M. C. Activated mast cells infiltrate in close proximity to enteric nerves in diarrhea-predominant irritable bowel syndrome. *J Korean Med Sci* **18**, 204-210 (2003).

25 Uranga, J. A., Martínez, V. & Abalo, R. Mast Cell Regulation and Irritable Bowel Syndrome: Effects of Food Components with Potential Nutraceutical Use. *Molecules* **25**, 4314 (2020).

26 Santos, J., Saperas, E., Nogueiras, C., Mourelle, M., Antolín, M., Cadahia, A. & Malagelada, J. R. Release of mast cell mediators into the jejunum by cold pain stress in humans. *Gastroenterology* **114**, 640-648, doi:10.1016/S0016-5085(98)70577-3 (1998).

27 Vivinus-Nébot, M. *et al.* Combination of allergic factors can worsen diarrheic irritable bowel syndrome: role of barrier defects and mast cells. *Am J Gastroenterol* **107**, 75-81, doi:10.1038/ajg.2011.315 (2012).

28 Aguilera-Lizarraga, J. *et al.* Local immune response to food antigens drives meal-induced abdominal pain. *Nature* **590**, 151-156, doi:10.1038/s41586-020-03118-2 (2021).

29 Barbara, G. Mucosal Barrier Defects in Irritable Bowel Syndrome. Who Left the Door Open? *Official journal of the American College of Gastroenterology | ACG* **101**, 1295-1298 (2006).

30 Zhou, Q., Zhang, B. & Nicholas Verne, G. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *PAIN* **146**, 41-46, doi:https://doi.org/10.1016/j.pain.2009.06.017 (2009).

31 Spiller, R., Jenkins, D., Thornley, J., Hebden, J., Wright, T., Skinner, M. & Neal, K. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acuteCampylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* **47**, 804-811 (2000).

32 Chadwick, V. S., Chen, W., Shu, D., Paulus, B., Bethwaite, P., Tie, A. & Wilson, I. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* **122**, 1778-1783 (2002).

33 Bertiaux-Vandaële, N. *et al.* The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *American journal of gastroenterology* **106**, 2165-2173 (2011).

34 Piche, T. Tight junctions and IBS-the link between epithelial permeability, low-grade inflammation, and symptom generation? *Neurogastroenterology & Motility* **26**, 296-302 (2014).

35 Schroeder, B. O. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. *Gastroenterology Report* **7**, 3-12, doi:10.1093/gastro/goy052 (2019).

36 König, J., Wells, J., Cani, P. D., García-Ródenas, C. L., MacDonald, T., Mercenier, A., Whyte, J., Troost, F. & Brummer, R.-J. Human intestinal barrier function in health and disease. *Clinical and translational gastroenterology* **7**, e196 (2016).

37 Shi, N., Li, N., Duan, X. & Niu, H. Interaction between the gut microbiome and mucosal immune system. *Military Medical Research* **4**, 1-7 (2017).

38 Jakobsson, H. E. *et al.* The composition of the gut microbiota shapes the colon mucus barrier. *EMBO reports* **16**, 164-177 (2015).

39 Koh, A., De Vadder, F., Kovatcheva-Datchary, P. & Bäckhed, F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165**, 1332-1345 (2016).

40 Donia, M. S. & Fischbach, M. A. Small molecules from the human microbiota. *Science* **349** (2015).

41 Hamer, H. M., Jonkers, D. M. A. E., Bast, A., Vanhoutvin, S. A. L. W., Fischer, M. A. J. G., Kodde, A., Troost, F. J., Venema, K. & Brummer, R.-J. M. Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. *Clinical Nutrition* **28**, 88-93, doi:https://doi.org/10.1016/j.clnu.2008.11.002 (2009).

42 Oran, M., Tulubas, F., Mete, R., Aydin, M., Sarikaya, H. G. & Gurel, A. Evaluation of paraoxonase and arylesterase activities in patients with irritable bowel syndrome. *J Pak Med Assoc* **64**, 820-822 (2014).

43 Nielsen, D. S. G., Jensen, B. B., Theil, P. K., Nielsen, T. S., Knudsen, K. E. B. & Purup, S. Effect of butyrate and fermentation products on epithelial integrity in a mucus-secreting human colon cell line. *Journal of Functional Foods* **40**, 9-17, doi:https://doi.org/10.1016/j.jff.2017.10.023 (2018).

44 Karczewski, J., Troost, F. J., Konings, I., Dekker, J., Kleerebezem, M., Brummer, R.-J. M. & Wells, J. M. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **298**, G851-G859 (2010).

45 Chapman, M. A., Grahn, M. F., Boyle, M. A., Hutton, M., Rogers, J. & Williams, N. S. Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. *Gut* **35**, 73-76, doi:10.1136/gut.35.1.73 (1994).

46 Törnblom, H., Van Oudenhove, L., Sadik, R., Abrahamsson, H., Tack, J. & Simrén, M. Colonic transit time and IBS symptoms: what's the link? *American Journal of Gastroenterology* **107**, 754-760 (2012).

47 Simrén, M., Törnblom, H., Palsson, O. S., Van Oudenhove, L., Whitehead, W. E. & Tack, J. Cumulative effects of psychologic distress, visceral hypersensitivity, and abnormal transit on patient-reported outcomes in irritable bowel syndrome. *Gastroenterology* **157**, 391-402. e392 (2019).

48 DuPont, A. W., Jiang, Z.-D., Harold, S. A., Snyder, N., Galler, G. W., Garcia-Torres, F. & DuPont, H. L. Motility abnormalities in irritable bowel syndrome. *Digestion* **89**, 119-123 (2014).

49 Kellow, J. E. & Phillips, S. F. Altered small bowel motility in irritable bowel syndrome is correlated with symptoms. *Gastroenterology* **92**, 1885-1893 (1987).

50 Simrén, M., Castedal, M., Svedlund, J., Abrahamsson, H. & Bjõrnsson, E. Abnormal propagation pattern of duodenal pressure waves in the irritable bowel syndrome (IBS). *Digestive diseases and sciences* **45**, 2151-2161 (2000).

51 Simrén, M., Månsson, A., Langkilde, A. M., Svedlund, J., Abrahamsson, H., Bengtsson, U. & Björnsson, E. S. Food-related gastrointestinal symptoms in the irritable bowel syndrome. *Digestion* **63**, 108-115 (2001).

52 Hayes, P., Corish, C., O'mahony, E. & Quigley, E. A dietary survey of patients with irritable bowel syndrome. *Journal of human nutrition and dietetics* **27**, 36-47 (2014).

53 Brown, S., Cann, P. & Read, N. Effect of coffee on distal colon function. *Gut* **31**, 450-453 (1990).

54 Rao, S., Welcher, K., Zimmerman, B. & Stumbo, P. Is coffee a colonic stimulant? *European journal of gastroenterology & hepatology* **10**, 113-118 (1998).

55 Papa, A. *et al.* Effect of moderate and heavy alcohol consumption on intestinal transit time. *Panminerva medica* **40**, 183-185 (1998).

56 Chiba, T. & Phillips, S. F. Alcohol-related diarrhea. *Addiction biology* **5**, 117-125 (2000).

57 Böhn, L., Störsrud, S., Törnblom, H., Bengtsson, U. & Simrén, M. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. *American Journal of Gastroenterology* **108**, 634-641 (2013).

58 Akbar, A., Yiangou, Y., Facer, P., Walters, J. R., Anand, P. & Ghosh, S. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. *Gut* **57**, 923-929 (2008).

59 Maggi, C. A., Theodorsson, E., Santicioli, P., Patacchini, R., Barbanti, G., Turini, D., Renzi, D. & Giachetti, A. Motor response of the human isolated colon to capsaicin and its relationship to release of vasoactive intestinal polypeptide. *Neuroscience* **39**, 833-841, doi:https://doi.org/10.1016/0306-4522(90)90266-7 (1990).

60 Rao, S. S. C., Kavelock, R., Beaty, J., Ackerson, K. & Stumbo, P. Effects of fat and carbohydrate meals on colonic motor response. *Gut* **46**, 205-211 (2000).

61 Simrén, M., Abrahamsson, H. & Björnsson, E. S. An exaggerated sensory component of the gastrocolonic response in patients with irritable bowel syndrome. *Gut* **48**, 20-27, doi:10.1136/gut.48.1.20 (2001).

62 Serra, J., Salvioli, B., Azpiroz, F. & Malagelada, J. R. Lipid-induced intestinal gas retention in irritable bowel syndrome. *Gastroenterology* **123**, 700-706 (2002).

63 Nanda, R., James, R., Smith, H., Dudley, C. & Jewell, D. Food intolerance and the irritable bowel syndrome. *Gut* **30**, 1099-1104 (1989).

64 Monsbakken, K., Vandvik, P. & Farup, P. Perceived food intolerance in subjects with irritable bowel syndrome–etiology, prevalence and consequences. *European journal of clinical nutrition* **60**, 667-672 (2006).

65 Dainese, R., Galliani, E. A., De Lazzari, F., Di Leo, V. & Naccarato, R. Discrepancies between reported food intolerance and sensitization test findings in irritable bowel syndrome patients. *The American Journal of*

Gastroenterology **94**, 1892-1897, doi:https://doi.org/10.1016/S0002-9270(99)00282-8 (1999).

66 Alun Jones, V., Shorthouse, M., McLaughlan, P., Workman, E. & Hunter, J. O. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. *The Lancet* **320**, 1115-1117, doi:https://doi.org/10.1016/S0140-6736(82)92782-9 (1982).

67 Lee, H. J. *et al.* Self-reported Food Intolerance in Korean Patients With Irritable Bowel Syndrome. *J Neurogastroenterol Motil* **25**, 222-232, doi:10.5056/jnm18125 (2019).

68 Vickery, B. P., Chin, S. & Burks, A. W. Pathophysiology of food allergy. *Pediatric Clinics* **58**, 363-376 (2011).

69 Schäfer, T., Böhler, E., Ruhdorfer, S., Weigl, L., Wessner, D., Heinrich, J., Filipiak, B., Wichmann, H. E. & Ring, J. Epidemiology of food allergy/food intolerance in adults: associations with other manifestations of atopy. *Allergy* **56**, 1172-1179 (2001).

70 Bischoff, S. & Crowe, S. E. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. *Gastroenterology* **128**, 1089-1113 (2005).

71 Rentzos, G., Johanson, L., Goksör, E., Telemo, E., Lundbäck, B. & Ekerljung, L. Prevalence of food hypersensitivity in relation to IgE sensitisation to common food allergens among the general adult population in West Sweden. *Clinical and translational allergy* **9**, 22 (2019).

72 Misselwitz, B., Pohl, D., Frühauf, H., Fried, M., Vavricka, S. R. & Fox, M. Lactose malabsorption and intolerance: pathogenesis, diagnosis and treatment. *United European gastroenterology journal* **1**, 151-159 (2013).

73 Misselwitz, B., Butter, M., Verbeke, K. & Fox, M. R. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management. *Gut* **68**, 2080-2091, doi:10.1136/gutjnl-2019-318404 (2019).

74 Varjú, P. *et al.* Lactose intolerance but not lactose maldigestion is more frequent in patients with irritable bowel syndrome than in healthy controls: A meta-analysis. *Neurogastroenterology & Motility* **31**, e13527 (2019).

75 Cancarevic, I., Rehman, M., Iskander, B., Lalani, S. & Malik, B. H. Is there a correlation between irritable bowel syndrome and lactose intolerance? *Cureus* **12** (2020).

76 Yang, J. *et al.* Prevalence and presentation of lactose intolerance and effects on dairy product intake in healthy subjects and patients with irritable bowel syndrome. *Clinical gastroenterology and hepatology* **11**, 262-268. e261 (2013).

77 Spiller, R. *et al.* Guidelines on the irritable bowel syndrome: mechanisms and practical management. *Gut* **56**, 1770-1798, doi:10.1136/gut.2007.119446 (2007).

78 Mai, F. Somatization disorder: a practical review. *The Canadian Journal of Psychiatry* **49**, 652-662 (2004).

79 Radu, M., Moldovan, R., Pintea, S., Băban, A. & Dumitrașcu, D. Predictors of outcome in cognitive and behavioural interventions for irritable bowel

syndrome. A meta-analysis. *Journal of Gastrointestinal & Liver Diseases* **27** (2018).

80 Windgassen, S., Moss-Morris, R., Goldsmith, K. & Chalder, T. Key mechanisms of cognitive behavioural therapy in irritable bowel syndrome: The importance of gastrointestinal related cognitions, behaviours and general anxiety. *J Psychosom Res* **118**, 73-82, doi:10.1016/j.jpsychores.2018.11.013 (2019).

81 Lövdahl, J., Ringström, G., Agerforz, P., Törnblom, H. & Simrén, M. Nurse-Administered, Gut-Directed Hypnotherapy in IBS: Efficacy and Factors Predicting a Positive Response. *American Journal of Clinical Hypnosis* **58**, 100-114, doi:10.1080/00029157.2015.1030492 (2015).

82 Peters, S., Yao, C., Philpott, H., Yelland, G., Muir, J. & Gibson, P. Randomised clinical trial: the efficacy of gut-directed hypnotherapy is similar to that of the low FODMAP diet for the treatment of irritable bowel syndrome. *Alimentary pharmacology & therapeutics* **44**, 447-459 (2016).

83 D'Silva, A., MacQueen, G., Nasser, Y., Taylor, L. M., Vallance, J. K. & Raman, M. Yoga as a Therapy for Irritable Bowel Syndrome. *Digestive Diseases and Sciences* **65**, 2503-2514, doi:10.1007/s10620-019-05989-6 (2020).

84 Simrén, M., Törnblom, H., Palsson, O. S. & Whitehead, W. E. Management of the multiple symptoms of irritable bowel syndrome. *The Lancet Gastroenterology & Hepatology* **2**, 112-122 (2017).

85 Jarrett, M., Visser, R. & Heitkemper, M. Diet triggers symptoms in women with irritable bowel syndrome: The patient's perspective. *Gastroenterology Nursing* **24**, 246-252 (2001).

86 Hookway, C., Buckner, S., Crosland, P. & Longson, D. Irritable bowel syndrome in adults in primary care: summary of updated NICE guidance. *Bmj* **350** (2015).

87 McKenzie, Y. A. *et al.* British Dietetic Association systematic review and evidence-based practice guidelines for the dietary management of irritable bowel syndrome in adults (2016 update). *Journal of Human Nutrition and Dietetics* **29**, 549-575, doi:https://doi.org/10.1111/jhn.12385 (2016).

88 Böhn, L., Störsrud, S., Liljebo, T., Collin, L., Lindfors, P., Törnblom, H. & Simrén, M. Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial. *Gastroenterology* **149**, 1399-1407. e1392 (2015).

89 Eswaran, S. L., Chey, W. D., Han-Markey, T., Ball, S. & Jackson, K. A randomized controlled trial comparing the low FODMAP diet vs. modified NICE guidelines in US adults with IBS-D. *American Journal of Gastroenterology* **111**, 1824-1832 (2016).

90 Varney, J., Barrett, J., Scarlata, K., Catsos, P., Gibson, P. R. & Muir, J. G. FODMAPs: food composition, defining cutoff values and international application. *Journal of gastroenterology and hepatology* **32**, 53-61 (2017).

91 Sitrin, M. D. in *The gastrointestinal system* 137-158 (Springer, 2014).

92 Roberfroid, M. & Slavin, J. Nondigestible Oligosaccharides. *Critical Reviews in Food Science and Nutrition* **40**, 461-480, doi:10.1080/10408690091189239 (2000).
93 Beyer, P. L., Caviar, E. M. & McCallum, R. W. Fructose intake at current levels in the United States may cause gastrointestinal distress in normal adults. *Journal of the American Dietetic Association* **105**, 1559-1566 (2005).
94 Yao, C. K., Tan, H. L., Van Langenberg, D., Barrett, J. S., Rose, R., Liels, K., Gibson, P. R. & Muir, J. G. Dietary sorbitol and mannitol: food content and distinct absorption patterns between healthy individuals and patients with irritable bowel syndrome. *Journal of Human Nutrition and Dietetics* **27**, 263-275 (2014).
95 Jones, H. F., Butler, R. N. & Brooks, D. A. Intestinal fructose transport and malabsorption in humans. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **300**, G202-G206 (2011).
96 Gibson, P. R., Newnham, E., Barrett, J. S., Shepherd, S. & Muir, J. G. Fructose malabsorption and the bigger picture. *Alimentary pharmacology & therapeutics* **25**, 349-363 (2007).
97 Beaugerie, L., Flourié, B., Marteau, P., Pellier, P., Franchisseur, C. & Rambaud, J.-C. Digestion and absorption in the human intestine of three sugar alcohols. *Gastroenterology* **99**, 717-723 (1990).
98 Grabitske, H. A. & Slavin, J. L. Gastrointestinal Effects of Low-Digestible Carbohydrates. *Critical Reviews in Food Science and Nutrition* **49**, 327-360, doi:10.1080/10408390802067126 (2009).
99 Wang, X. J., Camilleri, M., Vanner, S. & Tuck, C. Biological Mechanisms for Symptom Causation by Individual FODMAP Subgroups-The Case for a More Personalised Approach to Dietary Restriction. *Alimentary pharmacology & therapeutics* **50**, 517-529 (2019).
100 Gibson, P. R. & Shepherd, S. J. Evidence-based dietary management of functional gastrointestinal symptoms: the FODMAP approach. *Journal of gastroenterology and hepatology* **25**, 252-258 (2010).
101 Whelan, K., Martin, L. D., Staudacher, H. M. & Lomer, M. C. E. The low FODMAP diet in the management of irritable bowel syndrome: an evidence-based review of FODMAP restriction, reintroduction and personalisation in clinical practice. *Journal of Human Nutrition and Dietetics* **31**, 239-255, doi:https://doi.org/10.1111/jhn.12530 (2018).
102 Wilson, B. & Whelan, K. Prebiotic inulin-type fructans and galacto-oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders. *Journal of gastroenterology and hepatology* **32**, 64-68 (2017).
103 Staudacher, H. M., Ralph, F. S. E., Irving, P. M., Whelan, K. & Lomer, M. C. E. Nutrient Intake, Diet Quality, and Diet Diversity in Irritable Bowel Syndrome and the Impact of the Low FODMAP Diet. *Journal of the Academy of Nutrition and Dietetics* **120**, 535-547, doi:https://doi.org/10.1016/j.jand.2019.01.017 (2020).

104 O'Keeffe, M., Jansen, C., Martin, L., Williams, M., Seamark, L., Staudacher, H. M., Irving, P. M., Whelan, K. & Lomer, M. C. Long-term impact of the low-FODMAP diet on gastrointestinal symptoms, dietary intake, patient acceptability, and healthcare utilization in irritable bowel syndrome. *Neurogastroenterology & Motility* **30**, e13154, doi:https://doi.org/10.1111/nmo.13154 (2018).

105 Harer, K. N. Irritable bowel syndrome, disordered eating, and eating disorders. *Gastroenterology & hepatology* **15**, 280 (2019).

106 Melchior, C., Desprez, C., Riachi, G., Leroi, A.-M., Déchelotte, P., Achamrah, N., Ducrotté, P., Tavolacci, M.-P. & Gourcerol, G. Anxiety and Depression Profile Is Associated With Eating Disorders in Patients With Irritable Bowel Syndrome. *Frontiers in Psychiatry* **10**, doi:10.3389/fpsyt.2019.00928 (2020).

107 Mari, A., Hosadurg, D., Martin, L., Zarate-Lopez, N., Passananti, V. & Emmanuel, A. Adherence with a low-FODMAP diet in irritable bowel syndrome: are eating disorders the missing link? *European Journal of Gastroenterology & Hepatology* **31**, 178-182, doi:10.1097/meg.0000000000001317 (2019).

108 Halmos, E. P. & Gibson, P. R. Controversies and reality of the FODMAP diet for patients with irritable bowel syndrome. *Journal of gastroenterology and hepatology* **34**, 1134-1142 (2019).

109 Gibson, G. R. *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature reviews Gastroenterology & hepatology* **14**, 491 (2017).

110 Macfarlane, S. & Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society* **62**, 67-72 (2003).

111 Staudacher, H. M., Scholz, M., Lomer, M. C., Ralph, F. S., Irving, P. M., Lindsay, J. O., Fava, F., Tuohy, K. & Whelan, K. Gut microbiota associations with diet in irritable bowel syndrome and the effect of low FODMAP diet and probiotics. *Clinical Nutrition* (2020).

112 Bennet, S. M. P., Böhn, L., Störsrud, S., Liljebo, T., Collin, L., Lindfors, P., Törnblom, H., Öhman, L. & Simrén, M. Multivariate modelling of faecal bacterial profiles of patients with IBS predicts responsiveness to a diet low in FODMAPs. *Gut* **67**, 872-881, doi:10.1136/gutjnl-2016-313128 (2018).

113 Staudacher, H. M., Lomer, M. C., Anderson, J. L., Barrett, J. S., Muir, J. G., Irving, P. M. & Whelan, K. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *The Journal of nutrition* **142**, 1510-1518 (2012).

114 Halmos, E. P., Christophersen, C. T., Bird, A. R., Shepherd, S. J., Gibson, P. R. & Muir, J. G. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut* **64**, 93-100, doi:10.1136/gutjnl-2014-307264 (2015).

115 Poslusna, K., Ruprich, J., de Vries, J. H., Jakubikova, M. & van't Veer, P. Misreporting of energy and micronutrient intake estimated by food records

and 24 hour recalls, control and adjustment methods in practice. *British Journal of Nutrition* **101**, S73-S85 (2009).

116 Naska, A., Lagiou, A. & Lagiou, P. Dietary assessment methods in epidemiological research: current state of the art and future prospects. *F1000Research* **6** (2017).

117 Tucker, K. L. Assessment of usual dietary intake in population studies of gene–diet interaction. *Nutrition, metabolism and cardiovascular diseases* **17**, 74-81 (2007).

118 Thompson, F. E. & Subar, A. F. Dietary assessment methodology. *Nutrition in the Prevention and Treatment of Disease*, 5-48 (2017).

119 Conway, J. M., Ingwersen, L. A., Vinyard, B. T. & Moshfegh, A. J. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. *The American Journal of Clinical Nutrition* **77**, 1171-1178, doi:10.1093/ajcn/77.5.1171 (2003).

120 Schatzkin, A., Kipnis, V., Carroll, R. J., Midthune, D., Subar, A. F., Bingham, S., Schoeller, D. A., Troiano, R. P. & Freedman, L. S. A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. *International journal of epidemiology* **32**, 1054-1062 (2003).

121 Kipnis, V. *et al.* Structure of dietary measurement error: results of the OPEN biomarker study. *American journal of epidemiology* **158**, 14-21 (2003).

122 Basiotis, P. P., Thomas, R. G., Kelsay, J. L. & Mertz, W. Sources of variation in energy intake by men and women as determined from one year's daily dietary records. *The American journal of clinical nutrition* **50**, 448-453 (1989).

123 Beaton, G. H. *et al.* Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *The American journal of clinical nutrition* **32**, 2546-2559 (1979).

124 Gibson, R. S. *Principles of nutritional assessment*. (Oxford university press, USA, 2005).

125 Beaton, G. H., Milner, J., McGuire, V., Feather, T. E. & Little, J. A. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. *The American journal of clinical nutrition* **37**, 986-995 (1983).

126 Cole, T. & Black, A. Statistical aspects in the design of dietary surveys. *The Dietary Assessment of Populations. MRC Environmental Epidemiology Unit Report*, 5-7 (1984).

127 Sempos, C., Looker, A., Johnson, C. & Woteki, C. in *Monitoring dietary intakes* 99-109 (Springer, 1991).

128 Nelson, M., Black, A. E., Morris, J. A. & Cole, T. J. Between-and within-subject variation in nutrient intake from infancy to old age: estimating the

number of days required to rank dietary intakes with desired precision. *The American journal of clinical nutrition* **50**, 155-167 (1989).
129 Marr, J. & Heady, J. Within-and between-person variation in dietary surveys: number of days needed to classify individuals. *Human nutrition. Applied nutrition* **40**, 347-364 (1986).
130 Willett, W. *Nutritional epidemiology*. (Oxford university press, 2012).
131 Black, A., Cole, T., Wiles, S. & White, F. Daily variation in food intake of infants from 2 to 18 months. *Human nutrition. Applied nutrition* **37**, 448-458 (1983).
132 Tarasuk, V. & Beaton, G. H. The nature and individuality of within-subject variation in energy intake. *The American journal of clinical nutrition* **54**, 464-470 (1991).
133 Beaton, G. H. *et al.* Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *The American Journal of Clinical Nutrition* **32**, 2546-2559, doi:10.1093/ajcn/32.12.2546 (1979).
134 Williams, E. A., Nai, X. & Corfe, B. M. Dietary intakes in people with irritable bowel syndrome. *BMC gastroenterology* **11**, 1-7 (2011).
135 Torres, M. J., Sabate, J.-M., Bouchoucha, M., Buscail, C., Hercberg, S. & Julia, C. Food consumption and dietary intakes in 36,448 adults and their association with irritable bowel syndrome: Nutrinet-Sante study. *Therapeutic advances in gastroenterology* **11**, 1756283X17746625 (2018).
136 Tigchelaar, E. F. *et al.* Habitual diet and diet quality in Irritable Bowel Syndrome: A case-control study. *Neurogastroenterol Motil* **29**, doi:10.1111/nmo.13151 (2017).
137 Mazzawi, T., Hausken, T., Gundersen, D. & El-Salhy, M. Effects of dietary guidance on the symptoms, quality of life and habitual dietary intake of patients with irritable bowel syndrome. *Molecular medicine reports* **8**, 845-852 (2013).
138 Ligaarden, S. C., Lydersen, S. & Farup, P. G. Diet in subjects with irritable bowel syndrome: a cross-sectional study in the general population. *BMC gastroenterology* **12**, 1-8 (2012).
139 Khayyatzadeh, S., Esmaillzadeh, A., Saneei, P., Keshteli, A. & Adibi, P. Dietary patterns and prevalence of irritable bowel syndrome in Iranian adults. *Neurogastroenterology & Motility* **28**, 1921-1933 (2016).
140 Böhn, L., Störsrud, S. & Simrén, M. Nutrient intake in patients with irritable bowel syndrome compared with the general population. *Neurogastroenterology & motility* **25**, 23-e21 (2013).
141 Nilholm, C., Larsson, E., Sonestedt, E., Roth, B. & Ohlsson, B. Assessment of a 4-Week Starch-and Sucrose-Reduced Diet and Its Effects on Gastrointestinal Symptoms and Inflammatory Parameters among Patients with Irritable Bowel Syndrome. *Nutrients* **13**, 416 (2021).
142 Saito, Y. A., Locke III, G. R., Weaver, A. L., Zinsmeister, A. R. & Talley, N. J. Diet and functional gastrointestinal disorders: a population-based case–

control study. *American Journal of Gastroenterology* **100**, 2743-2748 (2005).
143 Buscail, C., Sabate, J.-M., Bouchoucha, M., Kesse-Guyot, E., Hercberg, S., Benamouzig, R. & Julia, C. Western Dietary Pattern Is Associated with Irritable Bowel Syndrome in the French NutriNet Cohort. *Nutrients* **9**, 986 (2017).
144 Staudacher, H. M., Ralph, F. S., Irving, P. M., Whelan, K. & Lomer, M. C. Nutrient intake, diet quality, and diet diversity in irritable bowel syndrome and the impact of the low FODMAP diet. *Journal of the Academy of Nutrition and Dietetics* **120**, 535-547 (2020).
145 Sahi, T. Genetics and epidemiology of adult-type hypolactasia. *Scandinavian Journal of Gastroenterology* **29**, 7-20 (1994).
146 Miranda Gómez, J., Vázquez Polo, M., Pérez Junkera, G., Fernández Gil, M. d. P., Bustamante Gallego, M. Á., Navarro Santamaría, V., Simón Magro, E. & Martínez González, O. FODMAP Intake in Spanish Population: Open Approach for Risk Assessment. (2020).
147 Potischman, N. & Freudenheim, J. L. Biomarkers of nutritional exposure and nutritional status: an overview. *The Journal of nutrition* **133**, 873S-874S (2003).
148 Bingham, S. A. Biomarkers in nutritional epidemiology. *Public health nutrition* **5**, 821-827 (2002).
149 Jenab, M., Slimani, N., Bictash, M., Ferrari, P. & Bingham, S. A. Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Human genetics* **125**, 507-525 (2009).
150 Kubinec, R. *et al.* Simultaneous analysis of carbohydrates, polyols and amines in urine samples using chemical ionization gas chromatography with tandem mass spectrometry. *Journal of separation science* **41**, 449-458 (2018).
151 Wamelink, M., Smith, D., Jakobs, C. & Verhoeven, N. Analysis of polyols in urine by liquid chromatography–tandem mass spectrometry: a useful tool for recognition of inborn errors affecting polyol metabolism. *Journal of inherited metabolic disease* **28**, 951-963 (2005).
152 Lindon, J. C., Nicholson, J. K., Holmes, E. & Everett, J. R. Metabonomics: metabolic processes studied by NMR spectroscopy of biofluids. *Concepts in Magnetic Resonance: An Educational Journal* **12**, 289-320 (2000).
153 Wishart, D. S. Metabolomics: applications to food science and nutrition research. *Trends in Food Science & Technology* **19**, 482-493, doi:https://doi.org/10.1016/j.tifs.2008.03.003 (2008).
154 Worley, B. & Powers, R. Multivariate analysis in metabolomics. *Current Metabolomics* **1**, 92-107 (2013).
155 Ponnusamy, K., Choi, J. N., Kim, J., Lee, S.-Y. & Lee, C. H. Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *J Med Microbiol* **60**, 817-827, doi:10.1099/jmm.0.028126-0 (2011).
156 Yamamoto, M., Pinto-Sanchez, M. I., Bercik, P. & Britz-McKibbin, P. Metabolomics reveals elevated urinary excretion of collagen degradation

and epithelial cell turnover products in irritable bowel syndrome patients. *Metabolomics* **15**, 82, doi:10.1007/s11306-019-1543-0 (2019).

157 Baranska, A. *et al.* Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. *Alimentary Pharmacology & Therapeutics* **44**, 45-56, doi:https://doi.org/10.1111/apt.13654 (2016).

158 Segers, K., Declerck, S., Mangelings, D., Heyden, Y. V. & Eeckhaut, A. V. Analytical techniques for metabolomic studies: a review. *Bioanalysis* **11**, 2297-2318, doi:10.4155/bio-2019-0014 (2019).

159 Liljebo, T., Störsrud, S. & Andreasson, A. Presence of Fermentable Oligo-, Di-, Monosaccharides, and Polyols (FODMAPs) in commonly eaten foods: extension of a database to indicate dietary FODMAP content and calculation of intake in the general population from food diary data. *BMC nutrition* **6**, 1-10 (2020).

160 Hebert, J. R., Clemow, L., Pbert, L., Ockene, I. S. & Ockene, J. K. Social desirability bias in dietary self-report may compromise the validity of dietary intake measures. *Int J Epidemiol* **24**, 389-398, doi:10.1093/ije/24.2.389 (1995).

161 Heitmann, B. L. & Lissner, L. Dietary underreporting by obese individuals--is it specific or non-specific? *Bmj* **311**, 986-989 (1995).

162 Bingham, S. *et al.* Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *British Journal of Nutrition* **73**, 531-550 (1995).

163 Lafay, L., Mennen, L., Basdevant, A., Charles, M., Borys, J., Eschwege, E. & Romon, M. Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Sante (FLVS) study. *International journal of obesity* **24**, 1500-1506 (2000).

APPENDIX

The role of CARbohydrates in Irritable Bowel Syndrome (CARIBS): Protocol for a randomized controlled trial comparing three different treatment options

Sanna Nybacka[1], Hans Törnblom[2], Magnus Simrén[2, 3], Stine Störsrud[2]

1) Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden

2) Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

3) Center for Functional Gastrointestinal and Motility Disorders, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

ABSTRACT

Background Although it is widely acknowledged that food intake can worsen symptoms in patients with irritable bowel syndrome (IBS), there is a lack of efficient treatments that are applicable to all patients and subtypes of IBS. As patients exhibit diverging symptom profiles and symptomatology, it is likely that the most successful treatment option will differ among patients; therefore, this large, randomized controlled trial comparing three different treatment options for patients with IBS is highly warranted. **Methods** A randomized controlled trial will be conducted evaluating the effectiveness of three different treatment options for patients with IBS, with 100 patients receiving each treatment; a diet with low total carbohydrate content (LCD); a diet combining low fermentable oligo-, di-, monosaccharides and polyols (FODMAP) and traditional dietary advice (LFTD); optimized medical treatment (OMT). The study consists of a 10-day screening period, 28-days of intervention, and a 6-month follow up for patients receiving dietary treatment. Questionnaires assessing both gastrointestinal and extra-intestinal symptoms will be used as endpoints, as well as metabolomics, microbiota profiling and immunological markers. Further, qualitative methods will be used to evaluate the patient's experiences regarding diet treatments. **Discussion** By collecting a wide range of data before, during and after treatment in a large group of patients with IBS with diverging bowel habits and symptomatology, we will gain new insights in predictors of response to treatment. That information can in the future be used to personalize treatments for the patient, based on the individual's phenotype and IBS symptoms. Also, long-term effects of two different dietary treatments will be evaluated regarding impact on gut microbiota and clinical laboratory tests, but also to ensure that they are safe, effective, and applicable to patients with IBS.
Trial registration This trial has been registered in ClinicalTrials.gov ID NCT02970591

BACKGROUND

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by abdominal pain together with altered bowel habits and/or stool form[1]. Some of the pathophysiological traits in IBS include disturbed gut-brain interactions, GI motility abnormalities, visceral hypersensitivity, or altered gut microenvironment, including unfavorable gut microbial composition[2-4]. However, available treatment options are limited which leads to unsatisfactory symptom relief among many patients. This leads to reduction in quality of life and impaired working ability, with substantial costs for both the individual and the society[5].

A majority of IBS patients report that their GI symptoms are diet-related[5-7]. Different dietary approaches have been proposed to reduce symptoms in IBS[8]. Traditional dietary advice based on the NICE guidelines focuses on limiting foods that are recognized to provoke symptoms, and emphasizes regular meal intake and portion size control[9]. A diet consisting of a low amount of fermentable carbohydrates, the low FODMAP diet (fermentable oligo-, di-, monosaccharides and polyols) is nowadays also used as a treatment option to reduce GI symptoms in patients with IBS, most frequently as a second-line treatment option[10]. FODMAPs are found in a wide range of legumes, certain vegetables and fruits, cereals (wheat, rye, and barley), dairy products and food items containing sweeteners. Intake of FODMAPs is believed to cause symptoms through bacterial fermentation and increased gas production. This, in combination with increased water retention through osmosis, distends the colon and can cause pain and other IBS-related symptoms in susceptible individuals, e.g. with visceral hypersensitivity[11,12]. This dietary strategy has been tested in several randomized trials and seems to be effective in 50-80% of patients with IBS[13]. To our knowledge, no trial has tested the combined effect of traditional dietary advice together with a low FODMAP content.

It has been acknowledged that patients who adhere to a low carbohydrate, high fat diet (LCHF) for weight loss purposes, or to lower their blood glucose levels, have reported less GI symptoms when following this diet[14]. One pilot study has investigated the effect of a diet with low carbohydrate content in patients with IBS, and although the sample size was small, the effect was very promising[15]. However, most patients that seek help for their IBS complaints will most likely see a primary care physician who frequently offers various prescription drugs. As to date, no study has compared the effect of a strategy where pharmacological treatment options are used, with a diet low in total carbohydrate content, or a low FODMAP diet in combination with traditional dietary advice in patients with IBS.

Since IBS is a heterogeneous disease with different predominant symptom patterns as well as underlying pathophysiological traits, personalized treatment options are needed. A better understanding of predictors of a favorable outcome with different treatment options can facilitate this and reduce the overall symptom burden in this large patient group, which in turn could reduce costs for the society and the individual. A large, randomized trial comparing the effects of these three different treatment strategies for patients with IBS thorough examination of factors predicting a favorable outcome is therefore warranted.

Research objectives and hypotheses

The overall aim of this study is to compare three different treatment options for patients with IBS; a diet with low total carbohydrate content (LCD); a diet combining low FODMAP and traditional dietary advice (LFTD); optimized medical treatment (OMT).

Specific aims:

- In a 4-week randomized controlled trial compare the response to three different treatment options for IBS, defined as a reduction in IBS symptom severity score by >50, using the validated IBS severity scoring system (IBS-SSS)[16].

- To study the effects of these treatment options on quality of life, anxiety and depression, fatigue, extra-intestinal symptoms, metabolic factors, gut microbiota, and immunological markers.

- During a six-month post-intervention period, study to what extent participants choose to maintain their allocated diet, and if it is possible to reintroduce certain amounts of FODMAPs successfully, and to assess the long-term effects on global IBS symptoms, metabolic factors and gut microbiota.

- To identify pre-treatment factors, such as sociodemographic factors, IBS symptom severity, predominant IBS symptom, extra-intestinal symptoms, psychological factors, microbiota composition, and metabolic fingerprints that can predict response to the treatments.

- To evaluate the participant's subjective experiences related to dietary treatment using qualitative methods.

The primary hypothesis is that a LFTD diet would improve GI symptoms in a larger proportion of patients than a LCD and OMT. Secondary hypotheses to be tested are: 1) the reduction in GI symptoms is dependent on the compliance to the allocated intervention; 2) it is possible to reintroduce FODMAPs in a systematic manner after a 4-week elimination with sustained effect on GI symptoms; 3) the degree to which the patients maintain their allocated diet during the six month follow-up depend on how large the symptom reduction was during the intervention period; 4) a treatment aimed to reduce GI symptoms will also favorably affect extra-intestinal symptoms, microbiota composition, quality of life, work productivity, and psychological factors; 7) A combination of microbiota composition, metabolic fingerprint and GI symptom pattern can predict treatment outcome.

METHODS

Study design

This study is a single-center, single-blinded, randomized controlled trial with three treatment arms, with 100 participants receiving each treatment.

Study setting

The study is carried out in an outpatient clinic specialized in functional GI disorders at the Sahlgrenska University Hospital, Gothenburg, Sweden, beginning in January 2017.

Eligibility criteria

Patients eligible for enrolment must fulfill all the inclusion criteria and none of the exclusion criteria seen in **Table 1.**

Table 1. Inclusion and exclusion criteria for participation in the CARIBS trial.

Inclusion criteria	Exclusion criteria
IBS according to Rome IV	Any other serious disease or illness
≥18 years and older	Other GI disease, including celiac disease
IBS-SSS ≥175	Other disease that may affect GI function, including bariatric surgery
Gothenburg region citizen	Allergy or food hypersensitivity (other than lactose intolerance)
	Any (major) dietary restrictions
	Pregnant or breast feeding
	BMI ≤18 or ≥35 kg/m^2
	Unable to communicate in Swedish
	Previously been treated with any of the intervention arms, including having tested all of the pharmacological treatment options of relevance for the symptom profile of the patient.

Abbreviations: IBS, irritable bowel syndrome; IBS-SSS, IBS severity scoring system; BMI, body mass index; GI, gastrointestinal

Participant timeline

The study consists of a 10-day screening period, followed by a 4-week intervention and a 6-month follow-up period. An overview of enrolment, allocation and intervention is shown in **Figure 1.**

	SCREENING		STUDY PERIOD						
	Enrolment	Screening	Allocation	Post-allocation				Follow-up	
TIMEPOINT	$-t_{10d}$	$-t_{10}$ -- $-t_{1}$	0	t_{1w}	t_{2w}	t_{3w}	t_{4w}	t_{3month}	t_{6month}
VISIT	1		2				3	4	5
Eligibility screen by physician	X								
Informed consent	X								
Demographics questionnaire	X								
Allocation			X						
INTERVENTIONS:									
Low carbohydrate diet				◆	—	—	—	—	◆
Low FODMAP + traditional IBS diet				◆	—	—	—	—	◆
Optimized medical treatment				◆	—	—	◆		
ASSESSMENTS:									
Food record		X						X	X
Questionnaires: *IBS-SSS, BSF, GSRS-IBS*		X		X	X	X	X	X	X
Questionnaires: *HAD, VSI, MFI-20, IBS-QoL, PHQ-15, WPAI-IBS*			X				X	X	X
Questionnaires: *HSP, CSI*			X						
Anthropometry: *weight, height*	X						X	X	X
Blood samples*			X						
Blood samples §			X				X	X	X
Microbiota: *fecal sample*			X				X	X	X
Metabolomics: *serum, urine and fecal samples*			X				X	X	X
Immunologic markers: *serum*			X				X		
Treatment satisfaction questionnaire							X	X	X
Qualitative interview *(subgroup of n=20)*								X	

Figure 1. A schematic overview of screening, allcation, intervention, and follow-up.
*Tissue transglutaminase/ IgA, hemoglobin, white blood cells, platelets, sodium, potassium, creatinine, calcium, c-reactive protein, thyroid stimulating hormone, free thyroxine, aspartate aminotransferase, alanine aminotransferase, albumin. § P-glucose, glycated hemoglobin, cholesterol, high density lipoprotein, low density lipoprotein, triglycerides. Abbreviations: BSF, bristol stool form; CSI, central sensitization inventory; FODMAP, fermentable oligo-, di-, monosaccharides and polyols; GSRS-IBS, gastrointestinal symptom rating scale-IBS; HAD, hospital anxiety and depression scale; HSP, highly sensitive person scale; IBS, irritable bowel syndrome; IBS-QoL, IBS quality of life; IBS-SSS, IBS severity scoring system; MFI-20, multidimentional fatigue inventory-20; VSI, visceral sensitivity index; WPAI-IBS, work productivity and activity impairment questionnaire.

In short, at the first visit, after receiving verbal and written information about the study, all patients provide their written informed consent. The diagnosis of IBS is confirmed by a physician, who perform a physical examination and ensure that the patient fulfil the Rome IV criteria for IBS[1]. Thereafter a 10-day screening period begins, where a daily stool diary based on the Bristol stool form (BSF) scale[17] is completed and a 4-day food record is kept. At the return visit, the patient complete IBS-SSS to assess the severity of their IBS symptoms during the 10-day screening period. Patients who score ≥175 on IBS-SSS (i.e., moderate to severe IBS symptoms) are eligible to be randomized into one of the treatment arms. Patients with IBS-SSS <175 will be excluded from further participation and is offered a regular visit to a dietitian or physician at the clinic.

Questionnaires assessing GI, extra-intestinal, and psychological symptoms, fatigue, quality of life and work productivity are completed after allocation before starting the intervention, at the end of the intervention period, and during follow-up. A 4-day food record is repeated twice during the follow-up period. During the intervention period, participants are instructed to complete a daily stool diary (BSF), as well as to note any deviation from their allocated diet intervention. The patients also complete questionnaires on a weekly basis to assess the severity of their GI symptoms (IBS-SSS and GI Symptom Rating Scale-IBS (GSRS-IBS).

Interventions

Upon fulfilling all inclusion criteria and none of the exclusion criteria, patients will be randomized and receive either LCD, LFTD or OMT. Dietary treatment will be administered by a dietitian and pharmacological treatment by a physician. All patients are informed that both diets and medical treatments are aiming to relieve symptoms, and that none of the treatments are believed to cure IBS.

Overview of dietary treatments

Patients assigned to dietary interventions receive oral and written information about the diet. No detailed information about the composition or the name of the diets are given. All foods included in the intervention will be delivered to the patient once a week using a conventional grocery supplier with home delivery service. A booklet with practical considerations, detailed meal plans, recipes and lists with foods that are allowed and not allowed during the intervention is provided. The recipes are based on a standardized energy level, regardless of energy requirement, thus patients are instructed to either eat less or to add extra foods if needed (according to the lists provided), to maintain weight stability. If patients need to deviate from their detailed meal plan, all deviations from their diet will have to be reported in a daily symptom diary. After two weeks of intervention, patients are contacted by e-mail to check for compliance and to record any adverse events.

A) Low carbohydrate diet - LCD

The LCD consists mainly of starch-free vegetables, fish, poultry, beef, dairy products, eggs, nuts, seeds, berries, and fruits that are low in carbohydrates (i.e., kiwi, pomegranate and grapefruit). The diet consists of approximately 10 E% carbohydrates,

25 E% protein and 65 E% fat. Detailed information about the nutritional composition can be seen in **Table 2**. Patients allocated to this diet are informed to avoid starchy and sugary foods, such as pasta, rice, potatoes, bread, fruit and confectionaries. Because of the reduction in total carbohydrates, and thus the sources to dietary fiber, nuts and seeds are included providing with approximately 25 grams of dietary fiber/day. For those participants who normally consume a lactose free diet, the LCD can be offered without lactose as well.

B) Low FODMAP diet + traditional dietary advice - LFTD

Patients are advised to eat small meals on regular basis, with three main meals and three snacks each day. All meals should be eaten slowly, and the food should be chewed properly; vegetables and fruits are recommended to be peeled or boiled; food triggers such as coffee, alcohol, fizzy drinks, sweeteners, fatty and spicy foods are limited. Intake of dietary fibers is approximately 30 grams/day and mainly consists of soluble fibers from oats, gluten free bread, chia seeds, vegetables, and fruits. This diet is also low in FODMAPs, and therefore do not contain any lactose, onions, legumes, wheat-based products, and high-FODMAP fruits and vegetables. Detailed information about the nutritional composition can be seen in Table 2.

Table 2. Energy and nutrient composition of diet A and B

	LCD	**LFTD**
Energy (kcal)	2353	2380
Carbohydrate (g)	48	278
Fat (g)	178	89
Protein (g)	133	99
Dietary fiber (g)	25.4	30.2
Vit C (mg)	169	210
Iron (mg)	13.6	12.2
Total FODMAPs (g)	16.6	3.4
Lactose (g)	6.3	0.2
Fructose in excess of glucose (g)	2.0	0.7
Fructan (g)	3.3	1.7
Galacto-oligosaccharides (g)	1.1	0.3
Polyols (g)	3.7	0.4
Carbohydrate (E%)	10	50
Fat (E%)	67	33
Protein (E%)	23	17
Saturated fat (E%)	25	11
Monounsaturated fat (E%)	25	11
Polyunsaturated fat (E%)	9	9
Total weight of food (g/day)	1368	1850
Proportion of animal foods* (%)	50	30

*meat, poultry, fish, shellfish, egg, dairy. LCD, low carbohydrate diet; LFTD, low FODMAP + traditional IBS dietary advice

C) Optimized medical treatment - OMT

Patients assigned to receive optimized medical treatment meet the physician for an assessment of their predominant GI symptom and medications tested previously. After that, a choice of evidence-based medical treatment options based on their predominant symptom is made, using the list shown in **Table 3**, with preference for the first-line options if these have not been tried before (e.g., bulking agent or osmotic laxative for constipation, and loperamide for diarrhea). Medications are tested for 4 weeks, and only one medication is allocated per patient. After 2 weeks, the physician contacts the patient for a telephone follow up to check for compliance to treatment and to adjust dosage if necessary. If the medication is terminated due to e.g., side effects by the patient before 4 weeks, no alternative medication is given during the intervention period.

Table 3. Pharmacological treatment options based on predominant symptom with the starting dose used.

Constipation	Diarrhea	Abdominal pain
Bulking agent (Sterculia) 4g q.d.	Loperamide 2mg b.i.d.	*Chronic / frequent pain:* Amitriptyline 25 mg q.h.s.
Osmotic laxative (Macrogol) 13.125g q.d.	Cholestyramine 4g q.d.	*Episodic pain:* Hyocyamin 0.2mg prn.
Linaclotide 290µg q.d.	Ondansetron 4mg q.d.	*Pain with diarrhea:* Amitriptyline 25mg q.h.s
		Pain with constipation: Linaclotide 290µg q.d.

Abbreviations: q.d, once a day; b.i.d, twice a day; q.h.s, before bed; prn, as needed

Follow-up
After the third visit, participants in the medical treatment arm will end their formal participation in the trial and will be offered a regular visit to a dietitian if wanted. However, they will be contacted by mail after ≥six months with questions regarding the severity of symptoms and current treatments used. They will be financially compensated for the cost of the medication during their intervention. Patients in the dietary intervention arms are informed that during the next 6 months they may or may not continue with their allocated diet, and structured follow-up visits with repeated 4-day food records are scheduled at three and six months after end of the intervention.

Systematic follow-up on LFTD treatment
Patients in the LFTD arm will be given a structured re-challenge schedule to be able to test whether individual FODMAPs are tolerated or not. The schedule consists of a

comprehensive list of food items within each FODMAP category, and selected foods that are suitable for re-challenge tests. The preference of the participant decides which category to test first, and only one FODMAP category is re-challenged at a time. The amount of the selected food is increased for three days to evaluate tolerance and tolerance levels. Then, after a wash out-period of four days when all FODMAPs are limited, the next FODMAP category can be re-challenged. When individual tolerance to FODMAPs have been evaluated, patients are encouraged to re-introduce FODMAPs to the diet in amounts that can be well tolerated.

Outcomes

The primary outcome measure is the proportion of patients that respond favorably to the treatment regarding the severity of IBS symptoms. Responders will be defined as subjects with a reduction in IBS-SSS ≥50 (i.e., calculated as the change in IBS-SSS between randomization visit and end of intervention period, with a total score ranging from 0-500), which is considered to be a clinically relevant symptom reduction[16], and a cut-off that is commonly recommended for use in clinical studies to define responders.

Secondary outcomes:

- The absolute and percent change in IBS-SSS and GSRS-IBS from randomization visit to end of intervention period (within groups and between groups).
- Compliance to the treatment in relation to treatment outcome.
- Predictors of response to treatment (demographics questionnaire data, microbiota, metabolites, immunology) through comparisons between responders and non-responders.
- Determinants for GI symptoms assessed with GSRS-IBS and IBS-SSS.
- Maintained adherence to dietary intervention during follow-up and predictors for long-term adherence.
- Change in extra-intestinal symptoms, quality of life, work productivity and psychological factors in relation to treatment.

Other outcomes:

- Change in metabolite profiles during intervention and follow-up.
- Change in microbiota composition during intervention and follow-up.
- Change in immunological markers during intervention.
- Patient's subjective experiences related to the dietary intervention described by qualitative methods.

Sample size

The power calculation is based on the primary outcome where the expected response rate is set to 40% (LCD), 65% (LFTD), and 40% (OMT) in the treatment arms. With 80% power to detect differences between groups, and α=0.05, 83 patients are needed in each group. To account for a 15% drop-out rate we will assign 100 patients to each treatment arm.

Recruitment
Patients will be recruited by referral either to a dietitian or physician at a specialized gastrointestinal unit at the Sahlgrenska Hospital, Gothenburg, Sweden, or by advertising. Patients referred to the unit from e.g., primary care are sent an invitation letter with a proposal to participate in the study, and upon approval they are scheduled for a screening visit at the clinic. Patients will also be recruited by advertisement in the local newspaper or in social media platforms.

Allocation
Patients are randomly assigned to receive either one of the three treatments in a ratio of 1:1:1. For allocation of the patients, an external web-randomization program is used. One of the study dietitians log in on the webpage and enter the initials of the patient, whereby the patient gets assigned to a treatment. No stratification is made before randomization.

Blinding
Patients allocated to the dietary treatment options will be given detailed information about the foods included in the diet, but will not be given any specified name of the diet (e.g., low FODMAP or low carbohydrate, high fat (LCHF) diet). Medical treatment is open label. Data entry and analyses will be performed by persons blinded to group assignment.

Data collection methods

Food records: Participants will record all foods and drinks consumed during four consecutive days before the allocation visit and twice during the follow-up period, which means that random weekdays and weekend days will be combined. Verbal and written instructions in how to complete the food record are given, and patients are encouraged to maintain their regular diet during the recording days. All records are kept in a booklet that is provided, and detailed information about the consumed foods and drinks are required. Quantities are estimated using household utensils or standard measures, and food labels, cooking methods etc. are noted. Trained dietitians will enter all diet records into the nutrient calculation software Dietist XP version 3.1 (Kostdata.se, Stockholm, Sweden) and a Swedish database of FODMAP contents in foods[18].

Questionnaires:
IBS-SSS (IBS-Severity Scoring System)[16], evaluates the severity of IBS symptoms (score range 0-500) and is filled in at day -1, day 7, day 14, day 21, day 28, and 3 and 6 months after completion of intervention.

GSRS-IBS (GI Symptom Rating Scale-IBS)[19], evaluates IBS-specific GI symptoms and is filled in at day -1, day 7, day 14, day 21, day 28, and 3 months and 6 months after completion of intervention.

BSF (Bristol Stool Form) scale[17] is used as the basis for a stool diary that is filled in during 10 days of screening and during the 28 days of intervention. In this diary, the patient records each stool and the stool consistency.

HAD (Hospital Anxiety and Depression scale)[20], is used to determine the severity of anxiety and depression, and is filled in at day 0, 29 and 3 and 6 months after the intervention.

VSI (Visceral Sensitivity Index)[21] measures GI-specific anxiety, i.e. anxiety originates from a fear of GI symptoms, related to the unpredictable symptom pattern commonly found in IBS. The form is filled in at day 0, 29 and 3 and 6 months after completion of intervention.

PHQ-15 (Patient Health Questionnaire)[22] measures the severity of somatic symptoms. Excluding the three GI symptoms in the questionnaire yields a measure of non-GI somatic symptom severity, PHQ-12[23]. The form is filled in at day 0, 29 and 3 and 6 months after completion of intervention.

MFI-20 (Multidimensional Fatigue Inventory-20)[24] measures general fatigue, physical fatigue, decreased activity, reduced motivation and mental fatigue. The form is filled in at day 0, 29 and 3 and 6 months after completion of intervention.

WPAI-IBS (Work Productivity and Activity Impairment Questionnaire: Irritable Bowel Syndrome)[25] measures whether IBS symptoms affect the ability to work and to perform everyday activities, with four different variables, absenteeism, presenteeism, overall work impairment, and activity impairment. The form is filled in at day 0, 29 and 3 and 6 months after completion of intervention.

IBS-QoL (IBS-Quality of Life)[26] Is an IBS-specific QoL questionnaire that measure ten domains found to be relevant to patients with IBS; emotional health, mental health, health belief, sleep, energy, physical functioning, diet, social role, physical role, and sexual relations. The form is filled in at day 0, 29 and 3 and 6 months after completion of intervention.

HSP (Highly Sensitive Person scale)[27] is a questionnaire that assesses the degree of environmental sensitivity and personality traits, that categorize individuals into low, medium-, and highly sensitive person. The form is filled in at day 0.

CSI (Central Sensitization Inventory)[28] focuses on sensitivity for pain and symptoms associated with central sensitization. It comprises 25 health-related symptoms common to central sensitization. The form is filled in at day 0.

The following questionnaires are web-based; HAD, VSI, MFI-20, IBS-QoL, PHQ-15, WPAI-IBS, HSP, and CSI. The platform on which these questionnaires are entered provide automated score calculations and since it is not possible to skip questions there will be no missing data. The IBS-SSS, BSF, and GSRS-IBS during the intervention period is completed on paper questionnaires.

Biological samples: Fasting blood samples are taken and sent to the Department of clinical chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden, for analysis of transglutaminase, IgA antibodies, total IgA-levels, hemoglobin, white cell count, platelets, sodium, potassium, creatinine, calcium, C-reactive protein, thyroid stimulating hormone, free thyroxine, aspartate aminotransferase, alanine aminotransferase, albumin, glucose, HbA1c and blood lipids (cholesterol, HDL, LDL, triglycerides) at visit 2. At visit 3, 4, and 5, glucose, HbA1c and blood lipids are analyzed. At visit 2, 3, 4, and 5, a 4 ml serum sample is taken, stored in 4°C in 30 min before being centrifuged in 4°C at 4000 rpm for 10 minutes, whereby 1 ml serum is frozen in -80°C within 2 hours for later analysis of metabolomics as well as other analyses. Urine samples (3 ml) are centrifuged in 4°C at 4000 rpm for 10 minutes, and frozen in -80°C within 2 hours for later analysis of metabolomics. Serum, fecal and urinary samples will be analyzed with nuclear magnetic resonance (NMR) for metabolomic profile, and microbial composition and function will be analyzed using whole-genome sequencing.
At visit 2 and 3 - 3 ml serum and 4 ml heparin plasma are frozen in -80°C for later immunological analyses. Biological samples that are not immediately analyzed are stored at -80°C in a biobank at our unit.

Energy and nutrient calculations
Energy and nutrient intakes will be calculated using the software Dietist XP 3.1 (kostdata.se, Stockholm, Sweden) linked to a Swedish food composition table provided by the National Food Agency, including a FODMAP database add-on[18]. The FODMAP database is aggregated from published sources of analyzed FODMAP content and include fructose, fructan, lactose, galacto-oligosaccharide (GOS), and polyol content (g/100 g). As only fructose in excess of glucose counts as a FODMAP, excess fructose will be calculated by subtracting intake of fructose (g)-glucose (g) for each separate meal. If the glucose content is higher than the fructose content, a value of 0 will be denoted for excess fructose. Intakes of nutrients are first summarized for each meal, and thereafter summarized into intakes per day, and finally, presented as the mean intake of all four days. Cut offs for reliable habitual energy intakes will be set for energy levels ≤800 kcal/day or ≥4500 kcal/day.

Measure of compliance
During the intervention period, patients receiving dietary treatment should note any deviations from their allocated diet in a daily symptom diary. Beside the recepies and foods that follow each intervention, there are specified lists with foods that are allowed and forbidden during the intervention period. As long as the study participant comply to the foods that are on the "allowed" list, the participant is considered to be compliant. Compliance will be calculated for each week, with a score ranging from 0-10, where 10 is full compliance. Any deviation that do not adhere with the intervention diet will result in a score reduction (1 for each deviation). Compliance to medical treatment will be examined during telephone check-up and at visit 3, but will not be scored.

Statistical methods
Normally distributed variables will be presented as mean±standard deviation (SD) and non-normally distributed variables as median (range). Differences in means between the three intervention groups will be analyzed with analysis of variance (ANOVA), using Bonferroni correction for multiple testing. For the main outcome, i.e., to compare the proportion of responders in each treatment group, the chi-square test will be applied. For the absolute change in IBS-SSS, the percentage change will be calculated and analyzed with paired t-test within groups (baseline vs. end of intervention) and using ANOVA for comparisons between groups. For comparing the change at multiple timepoints (during follow-up), mixed model analyses will be used.
Logistic regression will be applied to evaluate predictors of response for the various treatments (binary outcome responders/non-responders), and determinants for GI symptoms (IBS-SSS, as continuous variable) will be assessed using bivariate and multivariable linear regression analysis. Further, multivariate analysis and bioinformatics will be applied to metabolomics, microbiota and immunological markers.

Research ethics approval
The project has ethical approval from the Regional Ethics Committee in Gothenburg (Dnr 278-16).

Consent
Written informed consent will be provided from all study participants before entering the study. All patients are informed that the consent may be withdrawn at any moment if they wish to, without any further notice.

Confidentiality
All clinical examinations and visits are recorded in the medical record according to the clinical routine of the hospital. All results will be presented at the group level and no personal data will be reported, i.e., the patient confidentiality is protected.

Ancillary and post-trial care
After the last study visit, patients can be referred to receive additional treatment in regular hospital care if there is a need for it.

Dissemination policy
Results of this study will be published in peer-reviewed scientific journals.

Summary

The Car-IBS study is a large, randomized controlled study of high quality, which is of importance when results are interpreted for implementation in the treatment of patients with IBS. As large quantities of data will be collected, exploring both the efficacy of the intervention during a 4-week period, but also the effectiveness during follow-up, this study will bring us new insights in the management of IBS symptoms.

As we do not know the long-term effects of maintaining a LCD or LFTD, which both are exclusion diets, we will carefully monitor the two groups receiving dietary treatment for 6 months. Studies have demonstrated that a lack of fermentable carbohydrates in the diet can alter the composition and diversity of the gut microbiota, which could potentially lead to adverse health outcomes in the long run [29]. Also, studies have shown that restrictive diets can impact the total energy intake, which could lead to unwanted weight loss and lack in nutrients[30,31]. Therefore, we have added a six-month follow up in the study protocol, to be able to study the effects on nutrient intake as well as the gut microbiota composition.

We anticipate that this randomized controlled trial will provide with more understanding on predictors of response to treatment, and how we best can tailor treatments for IBS patients with different symptomatology.

REFERENCES

1 Lacy, B. E., Mearin, F., Chang, L., Chey, W. D., Lembo, A. J., Simren, M. & Spiller, R. Bowel Disorders. *Gastroenterology* **150**, 1393-1407.e1395, doi:https://doi.org/10.1053/j.gastro.2016.02.031 (2016).

2 Kanazawa, M., Hongo, M. & Fukudo, S. Visceral hypersensitivity in irritable bowel syndrome. *Journal of Gastroenterology and Hepatology* **26**, 119-121, doi:https://doi.org/10.1111/j.1440-1746.2011.06640.x (2011).

3 Zhou, Q., Zhang, B. & Nicholas Verne, G. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *PAIN* **146**, 41-46, doi:https://doi.org/10.1016/j.pain.2009.06.017 (2009).

4 Piche, T. Tight junctions and IBS-the link between epithelial permeability, low-grade inflammation, and symptom generation? *Neurogastroenterology & Motility* **26**, 296-302 (2014).

5 Black, C. J. & Ford, A. C. Global burden of irritable bowel syndrome: trends, predictions and risk factors. *Nature Reviews Gastroenterology & Hepatology* **17**, 473-486 (2020).

6 Böhn, L., Störsrud, S., Törnblom, H., Bengtsson, U. & Simrén, M. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. *American Journal of Gastroenterology* **108**, 634-641 (2013).

7 Hayes, P., Corish, C., O'mahony, E. & Quigley, E. A dietary survey of patients with irritable bowel syndrome. *Journal of human nutrition and dietetics* **27**, 36-47 (2014).

8 Rej, A., Aziz, I., Tornblom, H., Sanders, D. & Simrén, M. The role of diet in irritable bowel syndrome: implications for dietary advice. *Journal of internal medicine* **286**, 490-502 (2019).

9 Hookway, C., Buckner, S., Crosland, P. & Longson, D. Irritable bowel syndrome in adults in primary care: summary of updated NICE guidance. *Bmj* **350** (2015).

10 McKenzie, Y. A. *et al.* British Dietetic Association systematic review and evidence-based practice guidelines for the dietary management of irritable bowel syndrome in adults (2016 update). *Journal of Human Nutrition and Dietetics* **29**, 549-575, doi:https://doi.org/10.1111/jhn.12385 (2016).
11 Moayyedi, P., Simrén, M. & Bercik, P. Evidence-based and mechanistic insights into exclusion diets for IBS. *Nature Reviews Gastroenterology & Hepatology* **17**, 406-413 (2020).
12 Staudacher, H. M. & Whelan, K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. *Gut* **66**, 1517-1527 (2017).
13 van Lanen, A.-S., de Bree, A. & Greyling, A. Efficacy of a low-FODMAP diet in adult irritable bowel syndrome: a systematic review and meta-analysis. *European Journal of Nutrition*, 1-18 (2021).
14 McClernon, F. J., Yancy Jr, W. S., Eberstein, J. A., Atkins, R. C. & Westman, E. C. The effects of a low-carbohydrate ketogenic diet and a low-fat diet on mood, hunger, and other self-reported symptoms. *Obesity* **15**, 182-182 (2007).
15 Austin, G. L., Dalton, C. B., Hu, Y., Morris, C. B., Hankins, J., Weinland, S. R., Westman, E. C., Yancy, W. S. & Drossman, D. A. A Very Low-Carbohydrate Diet Improves Symptoms and Quality of Life in Diarrhea-Predominant Irritable Bowel Syndrome. *Clinical Gastroenterology and Hepatology* **7**, 706-708.e701, doi:https://doi.org/10.1016/j.cgh.2009.02.023 (2009).
16 Francis, C. Y., Morris, J. & Whorwell, P. J. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Alimentary pharmacology & therapeutics* **11**, 395-402 (1997).
17 Blake, M., Raker, J. & Whelan, K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. *Alimentary pharmacology & therapeutics* **44**, 693-703 (2016).
18 Liljebo, T., Störsrud, S. & Andreasson, A. Presence of Fermentable Oligo-, Di-, Monosaccharides, and Polyols (FODMAPs) in commonly eaten foods: extension of a database to indicate dietary FODMAP content and calculation of intake in the general population from food diary data. *BMC nutrition* **6**, 1-10 (2020).
19 Wiklund, I., Fullerton, S., Hawkey, C., Jones, R., Longstreth, G., Mayer, E., Peacock, R., Wilson, I. & Naesdal, J. An irritable bowel syndrome-specific symptom questionnaire: development and validation. *Scandinavian journal of gastroenterology* **38**, 947-954 (2003).
20 Bjelland, I., Dahl, A. A., Haug, T. T. & Neckelmann, D. The validity of the Hospital Anxiety and Depression Scale: an updated literature review. *Journal of psychosomatic research* **52**, 69-77 (2002).
21 Labus, J., Bolus, R., Chang, L., Wiklund, I., Naesdal, J., Mayer, E. & Naliboff, B. The Visceral Sensitivity Index: development and validation of a

gastrointestinal symptom-specific anxiety scale. *Alimentary pharmacology & therapeutics* **20**, 89-97 (2004).
22 Kroenke, K., Spitzer, R. L. & Williams, J. B. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. *Psychosomatic medicine* **64**, 258-266 (2002).
23 Spiller, R., Humes, D., Campbell, E., Hastings, M., Neal, K., Dukes, G. & Whorwell, P. The PHQ-12 Somatic Symptom scale as a predictor of symptom severity and consulting behaviour in patients with irritable bowel syndrome and symptomatic diverticular disease. *Alimentary Pharmacology and Therapeutics* **32**, 811 (2010).
24 Smets, E., Garssen, B., Bonke, B. d. & De Haes, J. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *Journal of psychosomatic research* **39**, 315-325 (1995).
25 Reilly, M., Bracco, A., Ricci, J. F., Santoro, J. & Stevens, T. The validity and accuracy of the Work Productivity and Activity Impairment questionnaire–irritable bowel syndrome version (WPAI: IBS). *Alimentary pharmacology & therapeutics* **20**, 459-467 (2004).
26 Hahn, B., Kirchdoerfer, L., Fullerton, S. & Mayer, E. Evaluation of a new quality of life questionnaire for patients with irritable bowel syndrome. *Alimentary pharmacology & therapeutics* **11**, 547-552 (1997).
27 Lionetti, F., Aron, A., Aron, E. N., Burns, G. L., Jagiellowicz, J. & Pluess, M. Dandelions, tulips and orchids: Evidence for the existence of low-sensitive, medium-sensitive and high-sensitive individuals. *Translational psychiatry* **8**, 1-11 (2018).
28 Neblett, R., Cohen, H., Choi, Y., Hartzell, M. M., Williams, M., Mayer, T. G. & Gatchel, R. J. The Central Sensitization Inventory (CSI): establishing clinically significant values for identifying central sensitivity syndromes in an outpatient chronic pain sample. *The Journal of Pain* **14**, 438-445 (2013).
29 Bennet, S. M. P., Bohn, L., Storsrud, S., Liljebo, T., Collin, L., Lindfors, P., Tornblom, H., Ohman, L. & Simren, M. Multivariate modelling of faecal bacterial profiles of patients with IBS predicts responsiveness to a diet low in FODMAPs. *Gut*, doi:10.1136/gutjnl-2016-313128 (2017).
30 Bohn, L., Storsrud, S., Liljebo, T., Collin, L., Lindfors, P., Tornblom, H. & Simren, M. Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial. *Gastroenterology* **149**, 1399-1407.e1392, doi:10.1053/j.gastro.2015.07.054 (2015).
31 Eswaran, S. L., Chey, W. D., Han-Markey, T., Ball, S. & Jackson, K. A randomized controlled trial comparing the low FODMAP diet vs. modified NICE guidelines in US adults with IBS-D. *American Journal of Gastroenterology* **111**, 1824-1832 (2016).