

De rol van FODMAPs in bovenste gastro-intestinale effecten en signalisatie tussen maagdarmsstelsel en hersenen

(The role of FODMAPs in upper GI effects and gut-brain signaling at the behavioral level)

Masterproef voorgedragen tot het behalen van de graad van Master in de biomedische wetenschappen door
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Preface

Nine months ago I started this Master's thesis project at TARGID. I was excited, motivated and ready to give it my all to bring it to a good end and to learn a lot of new things. However, I would never have imagined to come as far as I did. Therefore, I would like to take the opportunity to thank a few people who were indispensable in order to make this happen.

First of all, I would like to thank my promotor, Prof. Dr. Jan Tack, for giving me the opportunity to work and grow in his research team and for the helpful recommendations and opinions. Next, I would really like to express my gratitude to my supervisor, Dr. Jess Biesiekierski, for all the help, good advice and support throughout the year. You gave me the chance to learn and achieve more than I ever could have wished for. Thank you for the pleasant collaboration! Also a big thank-you for Prof. Dr. Lukas Van Oudenhove for the statistical help and advice and to all other colleagues from TARGID who were in any way involved in my project this year.

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Last but not least, I would like to thank my boyfriend: Aaron, thanks for your patience and for all your loving support and advice!

Summary

Increasing evidence suggests that Fermentable Oligo-, Di- and Monosaccharides and Polyols (FODMAPs) can trigger symptoms in functional gastrointestinal disorders (FGIDs), including irritable bowel syndrome (IBS). Furthermore, dysfunction of the brain-gut axis (BGA) is involved in the pathophysiology of FGIDs. In this Master's thesis, the role of FODMAPs in upper gastrointestinal (GI) response, symptom generation and BGA was assessed. Four solutions (high fructan, high fructose, FODMAP mix and glucose) were administered to 15 healthy volunteers, three days to two weeks apart in a single-blinded, randomized order. Six IBS patients were challenged with fructans and glucose under the same conditions. Intra-gastric pressure (IGP) was measured during gastric infusion, as a measure of gastric accommodation (GA), and for the following three hours. Symptom and psychological questionnaires were filled out at predetermined time points. Fructan infusion induced a significantly decreased GA compared to the other solutions in healthy volunteers. Paradoxically, the same infusion significantly increased accommodation in patients. In healthy controls, cramps and flatulence were increased following fructose and FODMAP mix. All symptoms were increased in the IBS population following both drinks, and cramps were significantly higher following fructans compared to glucose. In patients, vigor decreased significantly from baseline and was significantly lower compared to healthy controls. Tension, depression and negative affect were higher in patients compared to healthy volunteers. In conclusion, FODMAPs, especially fructans, exert significant effects on upper GI physiology and symptoms in health and IBS. Unraveling the sensory, neural and/or hormonal pathways involved in the effects on gastric physiology in the healthy state and in IBS and mechanisms involved in changes in the BGA following FODMAP intake requires further investigation.

List of abbreviations

5-HT	Serotonin
ACh	Acetylcholine
ANOVA	Analysis of variance
ANS	Autonomic nervous system
ATD	Acute tryptophan depletion
BGA	Brain-gut axis
CCK	Cholecystokinin
cGMP	Guanosine 3',5'-cyclic monophosphate
CH ₄	Methane
CNS	Central nervous system
ENS	Enteric nervous system
FD	Functional dyspepsia
FGID	Functional gastrointestinal disorders
FM	Fructose malabsorption
FODMAPs	Fermentable Oligo-, Di- and Mono-saccharides and Polyols
FOS	Fructo-oligosaccharides
GA	Gastric accommodation
GHSR	Growth hormone secretagogue receptor
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GOS	Galacto-oligosaccharides
GPCR	G-protein-coupled receptor
H ₂	Hydrogen
HFD	High FODMAP diet
HRM	High-resolution solid-state manometer
HV	Healthy volunteer
IBS	Irritable bowel syndrome
IBS-C	Constipation predominant IBS
IBS-D	Diarrhea predominant IBS
IGP	Intragastric pressure
LES	Lower esophageal sphincter
LFD	Low FODMAP diet
MMC	Migrating motor complex
NA	Negative affect
NO	Nitric oxide
NOS	Nitric oxide synthase

NSAIDs	Non-steroidal anti-inflammatory drugs
PA	Positive affect
PANAS	Positive and Negative Affect Scale
POMS	Profile of Mood States
SAM	Self-Assessment Manikin
SEM	Standard error of the mean
SSRI	Selective Serotonin Reuptake Inhibitor
STAI	State-Trait Anxiety Inventory
TRP	Tryptophan
VAS	Visual analogue scale

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1 Introduction

Functional gastrointestinal disorders (FGIDs) are the most frequent conditions patients present with in gastroenterology clinical practice, and they also comprise a major portion of primary care (1). FGIDs are defined as conditions in which a variable combination of chronic or recurrent gastrointestinal (GI) symptoms are present but cannot be explained by structural or biochemical abnormalities (2, 3). Irritable bowel syndrome (IBS) and functional dyspepsia (FD) are the two most prevalent types of FGIDs (3). IBS constitutes a major health problem affecting 10-20% of the general population, mostly women (4). This functional disorder is characterized by chronically returning unexplained abdominal pain or discomfort, bloating and altered bowel habits. Although it is a highly prevalent condition, the pathophysiology of IBS remains poorly understood and is most likely multifactorial (5, 6). The same is true for FD, the upper GI counterpart of IBS. FD is a clinical disorder characterized by chronic or recurrent upper abdominal symptoms without identifiable structural or biochemical cause by conventional diagnostic techniques (7). Symptoms typically associated with this disorder are epigastric pain, early satiety, postprandial fullness, bloating, anorexia, belching, nausea, and vomiting. These symptoms can originate from gastroduodenal dysmotility, visceral hypersensitivity, or disturbances in the brain-gut axis. However, also for this FGID, the pathophysiology remains largely unknown (7).

It is now commonly accepted that the diet plays a role in the pathogenesis of FGIDs, including IBS and FD (8, 9). Furthermore, it has been reported that fasting or excluding certain food products from the diet improves GI symptoms (9, 10). Recently it has been demonstrated that poorly absorbed, short chain carbohydrates, called FODMAPs, can trigger symptoms in FGID patients (11, 12). FODMAP is an acronym for Fermentable Oligo-, Di- and Mono-saccharides and Polyols. This group of carbohydrates and sugar alcohols comprises fructose, lactose, fructo- and galacto-oligosaccharides (FOS and GOS) and polyols, all found naturally in foods. These FODMAPs are poorly or non-absorbed and are osmotically active, increasing the water delivery in the intestinal lumen (13, 14). Furthermore, these components are fermented by microflora in the gut, leading to an augmented gas production (15). Together these two mechanisms can lead to luminal distention, which translates to GI symptoms like bloating and abdominal pain in IBS patients (16).

Besides typical GI symptoms, non-GI symptoms such as headache, fatigue, anxiety and depression are also commonly reported in FGIDs (17, 18). This indicates the presence of a communication pathway between the brain and the gut, which is called the brain-gut axis (BGA). In addition to psychosocial factors such as stress and emotions, affecting the function of the GI tract, gastric afferents also influence specific brain regions involved in mood and behavior (19, 20). Recently, it has been demonstrated that a subliminal intragastric infusion of fatty acids can attenuate neural and behavioral responses to sad emotion (21). However, the exact mechanisms underlying this interaction are not fully understood. Furthermore, a study of Ong *et al.* suggests that

there is a relationship between a high FODMAP diet (HFD) on the one hand and fatigue and lethargy on the other hand. However, the effect of a HFD on fatigue has never been formally studied (15).

To the best of our knowledge, no extensive studies on the effect of FODMAPs on intragastric pressure (IGP) and on emotions and mood have been conducted to date. In this Master's thesis, we aimed to acquire more insight into the role of FODMAPs in the upper GI response and symptom development in patients with IBS. Furthermore, we aimed to investigate whether FODMAPs may induce changes in emotion and mood, and how this differs in health and IBS.

2 Overview of the literature

2.1 Physiology of the digestive system

2.1.1 The gastrointestinal tract

The gastrointestinal (GI) tract plays a vital role in the normal functioning of the body. It consists of the mouth, esophagus, stomach, duodenum, jejunum, ileum and colon and functions to modify ingested food to provide energy and nutrition. Through the mouth and the esophagus food is delivered to the stomach (22). The stomach is a central, complex organ composed of neurons of the enteric nervous system (ENS) and smooth muscles, in which contractions are controlled and regulated by gastric myo-electrical activity (23, 24). Anatomically, the stomach consists of three regions: the fundus, the corpus or the body and the antrum (Figure 1). In regards to motor function, the stomach can be divided into two regions: the proximal stomach, which comprises the fundus and the proximal part of the corpus, and the distal stomach consisting of the distal part of the corpus and the antrum (25).

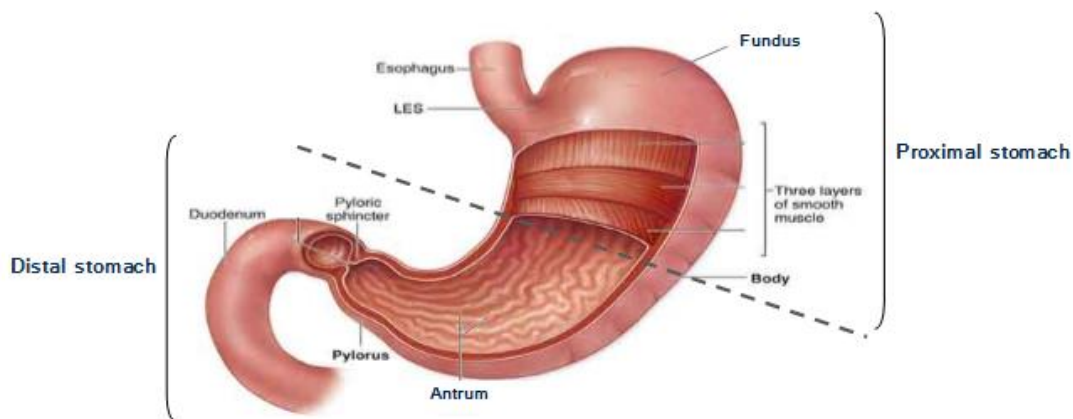


Figure 1: Anatomical regions of the stomach. The stomach can be divided into three anatomical regions: the fundus, the corpus or body and the antrum. The fundus and proximal part of the corpus belong to the proximal stomach. The distal stomach comprises the distal part of the corpus and the antrum. Contractions of the smooth muscles are controlled by myo-electrical activity. Figure adapted from (26).

When food is ingested, the proximal stomach accommodates to temporarily store the meal. During this storage phase, initiation of the digestion takes place by grinding of the food and by the secretion of proteases and acids (22). Hereafter, the stomach delivers the food to the duodenum at the correct time and speed (23). The small intestine, consisting of the duodenum, jejunum and ileum, plays an important role in further digestion of the food and the absorption of nutrients. From the small intestine the food arrives into the colon. The colon is the site where water and electrolytes are absorbed and the fecal matter is stored before it leaves the body (22).

2.1.2 Gastric motility

2.1.2.1 Fasted state and the migrating motor complex

The electrical properties of the stomach change upon food intake. In the interdigestive state, the muscle tone in the proximal stomach is high, due to a constant cholinergic input by the vagal nerve and the myo-electrical properties of the fundus. The distal stomach and the small intestine on the other hand, display a recurrent contraction pattern called the migrating motor complex (MMC) (23, 27). The MMC is thought to play a role in the clearance of secretions, debris and microbes from the stomach during fasting (25). More recently, the MMC has also been implicated in the control of hunger and food intake (28, 29). The MMC can be divided into four phases. In the first quiescent phase no contractions occur. This is followed by a second phase characterized by low-amplitude random contractions. Phase III is a period of peak mechanical and electrical activity where contractions are regular, with three contractions per minute, and of maximal amplitude and duration. 50% of the phase III contractions originate in the antrum and travel all the way to the ileum. The other 50% originates in the duodenum. Phase IV is characterized by rapid decreasing activity and merges into the next quiescent phase I. It takes approximately 90 to 120 minutes for these four phases to occur (22, 27, 30, 31). A study of Luiking *et al.* suggested that the duration of the MMC depends on the origin of phase III. After a phase III of antral origin, the MMC cycle duration and phase III in the duodenum are longer than after a duodenal phase III (27).

The MMC is controlled by the ENS, humoral factors such as motilin, and extrinsic innervation (22). It can be disrupted by several factors including the ingestion of food, certain hormones (for example cholecystokinin) and the administration of certain drugs (for example atropine, an antimuscarinic drug, and hexamethonium, a ganglionic blocker) (31, 32). The duration of disruption of the MMC after food intake depends on the type, volume and composition and caloric content of the meal (33). For instance, a fat rich meal is more effective in disrupting the MMC than proteins or sugars (22, 34)

2.1.2.2 Fed state and gastric accommodation

Ingestion of a meal induces a drop in muscle tone of the fundic smooth muscle, keeping IGP low during food intake and increasing gastric storage capacity (Figure 2). This is called the gastric accommodation (GA) reflex (23). GA is mediated by parasympathetic and sympathetic pathways which cause a decrease in cholinergic and adrenergic output and activate the release of nitric oxide (NO) from intrinsic nerves. NO is synthesized by nitric oxide synthase (NOS) in gastric inhibitory neurons. Upon release, it diffuses into the smooth muscle cells of the GI tract through passive transport pathways. Once in its target cells, NO stimulates the synthesis of guanosine 3',5'-cyclic monophosphate (cGMP), which in turn has an inhibitory effect on smooth muscle tone causing relaxation of the stomach (35, 36).

Another factor playing a role in the regulation of GA is serotonin (5-HT). Serotonin is a neurotransmitter of which about 95% is found in the GI tract, where it is produced by enterochromaffin cells and enteric neurons (37). Evidence for the role of 5-HT in GA has been supported by pharmacological studies. A study of Tack *et al.* showed that administration of Selective Serotonin Reuptake Inhibitors (SSRI's), leading to an increase in available 5-HT, is associated with an enhanced GA (38). When 5-HT activates nitrenergic neurons, NO is released, inducing relaxation of the gastric muscles. It is now commonly accepted that 5-HT has various other functions in the GI tract. For example, activation of 5-HT₄ receptors on cholinergic neurons releases acetylcholine (ACh), which leads to contraction of the gastric muscles (39). Furthermore, when 5-HT binds to its 5-HT₃ receptor on extrinsic afferent neurons, sensations such as nausea and discomfort are conveyed to the central nervous system (CNS) (37). Other important functions of serotonin are control of secretion and visceral perception (40).

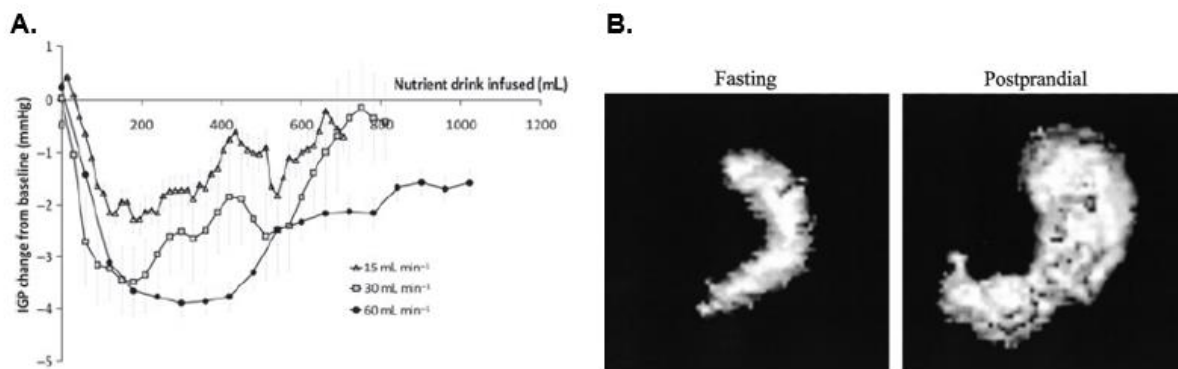


Figure 2: Gastric accommodation reflex. (A) After infusion of the nutrient drink, there's a drop in intragastric pressure. There is a difference in amplitude of the drop between the different infusion rates (35) (B) visualisation of postprandial relaxation of the proximal part of the stomach (41).

The gastric relaxation reflex plays an important role in the modulation of satiation. Gastric relaxation allows food to temporarily reside in the stomach before it is delivered to the duodenum, but the accompanying distention may result in a number of sensations, including fullness and abdominal pressure. GA is not only elicited by normal food ingestion, but can also be observed during intra-gastric or intra-duodenal infusion of nutrients (7, 35, 42). Impaired postprandial GA is a prevalent finding in patients with functional dyspepsia (FD) and other related disorders, such as diabetic gastropathy and post-fundoplication syndrome (36).

After this initial accommodation, tonic peristaltic contractions of the proximal stomach occur, which move the food towards the antrum. In addition, segmental powerful, non-propulsive contractions in the distal stomach serve to grind and mix the stomach contents into smaller particles that can then be delivered to the small intestine (22, 25).

2.2 Clinical problems – Functional gastrointestinal disorders

Functional gastrointestinal disorders (FGIDs) are very common and are characterized by a variable combination of chronic or recurrent GI symptoms in the absence of structural or tissue abnormalities (2, 43). The most common FGIDs are functional dyspepsia (FD) and irritable bowel syndrome (IBS) (44). The diagnosis of these functional diseases is based on symptoms, such as pain, nausea, vomiting, bloating, diarrhea, constipation, difficult passage of food or feces, or any combination (45) and not on demonstrable structural or physiological abnormalities in the GI tract (2). Patients with FGIDs also often suffer from psychological problems (46). It remains to be determined whether the psychological comorbidities play a causal role in FGID pathogenesis, are a consequence of FGID symptoms or are another manifestation of a common predisposition or a common pathophysiological process. Nevertheless, psychological comorbidity can certainly contribute to symptom severity by affecting gut physiology, symptom experience and illness behavior (1, 44). As a consequence of GI and non-GI symptoms, FGIDs are associated with significant work absenteeism, impaired quality of life and increased medical costs (46).

2.2.1 Functional dyspepsia

2.2.1.1 Background

Functional dyspepsia (FD) is a common clinical GI syndrome characterized by chronic or recurrent symptoms in the upper abdominal region in the absence of organic, systemic or metabolic disease (7, 47). The most frequent symptoms of FD include postprandial fullness, early satiation, epigastric pain and burning, upper abdominal bloating, weight loss, nausea and vomiting and belching. These symptoms lead to a considerable decrease in quality of life. Despite the great amount of work conducted in this area, the cause of FD remains unknown (7, 48). However, it is now accepted that FD is a heterogeneous disease in which several mechanisms play a role.

2.2.1.2 Diagnosis

The Rome Committee for the Classification of Functional Gastrointestinal Disorders has defined FD on the basis of the presence of specific symptoms. Rome III diagnostic criteria for FD (Table 1) comprise the presence of one or more of the following symptoms: (i) bothersome postprandial fullness, (ii) early satiation, (iii) epigastric pain, (iv) epigastric burning. Furthermore, there may not be evidence for any structural disease that can explain the symptoms. These criteria must be fulfilled for the previous three months with symptom onset at least six months prior to diagnosis (43). In practice, when patients present with recurrent or chronic upper GI symptoms, a limited set of investigative tests (especially endoscopy) are performed to identify structural or biochemical abnormalities that may explain their symptoms. When no organic or metabolic abnormalities can be found, the patient is diagnosed with FD (49).

Table 1: Rome III criteria for functional dyspepsia and irritable bowel syndrome (43).

Functional dyspepsia	Irritable bowel syndrome
1. One or more of the following: a. Bothersome postprandial fullness b. Early satiation c. Epigastric pain d. Epigastric burning AND 2. No evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms <i>* Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis</i>	Recurrent abdominal pain or discomfort** at least 3 days/month in the last 3 months associated with two or more of the following: 1. Improvement with defecation 2. Onset associated with a change in frequency of stool 3. Onset associated with a change in form (appearance) of stool <i>* Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis</i> <i>** "Discomfort" means an uncomfortable sensation not described as pain.</i> <i>In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days a week during screening evaluation is recommended for subject eligibility</i>

2.2.1.3 Pathophysiological mechanisms

Impaired gastric accommodation

It has been suggested that impaired GA might contribute to the pathogenesis of symptoms in patients with FD and other GI disorders (36, 50). The first evidence for the association between reduced GA and FD came from gastric imaging studies with ultrasound or scintigraphy (36, 51), and has since been confirmed by several studies. Tack *et al.* measured GA using the barostat and reported a 40% prevalence of impaired GA in FD patients (36). Furthermore, they found an association between impaired GA and symptoms of early satiation and weight loss. Due to the decreased relaxation of the stomach, the gastric storage capacity (reservoir) is limited. Food intake in FD patients will therefore cause a less pronounced drop in postprandial IGP compared to healthy people. This way impaired GA can lead to dyspeptic symptoms such as early satiation (7). These findings have been confirmed in preliminary studies using IGP to measure GA (52). In another study, conducted by Troncon *et al.*, GA was assessed by measuring IGP with an intragastric polyethylene bag during fasting and after ingestion of a liquid mixed nutrient meal composed of carbohydrates (12.0%), proteins (4.0%) and lipid (4.0%) (53). They found that the gastric tone in FD patients was significantly higher compared to healthy volunteers, both during fasting and postprandially. This abnormal rise in proximal gastric tone, together with increased sensitivity to distention found in FD patients, suggest that impaired gastric relaxation may play a role in symptom development (53). The underlying pathogenesis of impaired GA is not fully understood and several contributing factors have been suggested, including impaired vagovagal reflex signaling, defective intrinsic inhibitory innervation of the smooth muscle in the proximal stomach, and psychological factors, such as anxiety (36).

Delayed gastric emptying

Another mechanism reported in 30 to 70% of patients with FD is delayed gastric emptying (23, 54, 55). A meta-analysis of 17 studies found that the mean time of gastric emptying is 1.5 times slower in patients with FD compared to healthy controls (56, 57). However, not all FD patients show this change in gastric emptying time, which has led to subgrouping FD patients with and without aberrant gastric emptying (56). In 2002, a study from the Department of Gastroenterology in Leuven revealed an association between delayed gastric emptying and postprandial fullness, nausea and vomiting (55). These symptoms were significantly more present in patients with delayed gastric emptying than in patients without delayed emptying (55). With this study, they confirmed and expanded on earlier results of the study of Stanghellini *et al.*, which found a relationship between delayed emptying and fullness and severe vomiting (57). Furthermore, a positive correlation between the improvement of gastric emptying and the improvement of symptoms had previously been observed (58). A more recent study of Vanheel *et al.* found that fullness, bloating and belching were associated with the presence of food in the stomach, which is extended with delayed gastric emptying. Moreover, they provided evidence for the gastric origin of these symptoms by showing that their severity decreases when food progresses towards the small intestine (59).

These results are contradicted by American studies, which were not able to find a relationship between a specific symptom profiles and delayed gastric emptying. Two studies conducted by Talley *et al.* found that there was an association between symptoms and impaired quality of life, but that the decrease in quality of life could not be explained by delayed gastric emptying. These results highlight that delayed gastric emptying is only one contributing factor to triggering upper GI symptoms. Further research is required to fulfil our understanding of the association between delayed gastric emptying and specific symptom profiles in patients with FD.

Evidence for food as a trigger in FD

The role of dietary components in symptom induction is complex and under-investigated, regardless many patients report that FD symptoms mainly occur after the intake of food (60-63). Bisschops *et al.* showed in 218 patients with FD that the intensity of different symptoms, namely epigastric pain, bloating, postprandial fullness, early satiation, nausea, vomiting, belching and epigastric burning, increased rapidly after food intake to gradually decrease after reaching a peak (60). The intensity level remained elevated throughout the four hour measurement period of the experiment. Whereas fullness and bloating displayed an early peak, the latest peak was seen for epigastric pain and burning (60). Also a Belgian study, investigating whether specific symptoms originate in the stomach or in the intestine, demonstrated an early peak for fullness, bloating and belching, implying a gastric symptom origin. For epigastric pain and epigastric burning, on the other hand, the intensity stayed elevated in the gastric and intestinal phase (59). These observations show that the time to reach maximal intensity varies for different symptoms, and that a distinction can be made between symptoms during the gastric phase and during the intestinal phase of food.

In addition, studies assessing nutritional behavior in FD patients show that they tolerate significantly smaller quantities of food. They consume significantly less meals per day compared to healthy controls and have a longer overnight fast, thought to be due to an early dinner (61, 63). These studies found no significant difference in carbohydrate, protein or alcohol intakes (61), but the study of Carvahlo *et al.* showed a slightly decreased intake of fat (63). Pilichiewicz *et al.* found only small differences in eating pattern and composition, but a remarkable difference in symptom generation between healthy controls and FD patients. The authors reported no less than 612 symptoms in total, or 26 per patient, of which 64% of these symptoms were associated with food intake (61).

Other studies have tried to identify specific food items that are linked to the induction of GI symptoms in FD. An association of FD symptoms with ingestion of fatty foods, carbohydrates, coffee, carbonated drinks, and some fruits and vegetables is commonly reported by patients with FGIDs (62, 63). The study of Carvalho *et al.* also showed that milk and wheat-containing products induced symptoms in respectively 44% and more than 33% of the FD patients (63).

Based on these available studies, it remains difficult to establish the exact role of food composition in symptom generation in FD. A limited number of studies have been conducted on this topic, and the ones that have been, are often contradictory (63, 64). Furthermore, dietary habits, such as eating pattern and composition, differ between individual populations, which makes it difficult to conduct large dietary studies (64). Additionally, to perform dietary studies in a patient population, patients are exposed to foods that they believe provoke their symptoms, making it unattractive for them to participate, and risking nocebo effects (65, 66). Nevertheless, more research is needed to enhance our understanding of the role of food in symptom induction and how it can be used in the current treatment of FD.

2.2.2 Irritable bowel syndrome

2.2.2.1 Background

Irritable bowel syndrome (IBS) is, similar to FD, a common FGID affecting 10 – 15% of the population, with female predominance (5, 67, 68). It accounts for a major proportion of GI care worldwide and has an impact on the quality of life. Furthermore, IBS produces considerable costs for the patients and for society, in terms of work absenteeism and decreased productivity, consultations and unnecessary tests. (68, 69). This GI disorder is characterized by recurring abdominal pain or discomfort and altered bowel habits in the absence of an organic cause of the symptoms (5). Additional symptoms typical of IBS are diarrhea and/or constipation, bloating, flatulence, stool urgency and the feeling of incomplete evacuation (6). Three major clinical variants of IBS can be distinguished: diarrhea predominant IBS (IBS-D), where diarrhea is often painless, but occurs at a chronic-intermittent fashion; constipation predominant IBS (IBS-C) with abdominal pain; and a variant of IBS characterized by chronic abdominal pain associated with constipation or alternating bowel habits (IBS-mixed or IBS-unspecified) (70).

2.2.2.2 Diagnosis

The Rome III criteria (Table 1) are the most widely applied criteria for the diagnosis of IBS (68). These criteria recommend that the diagnosis of IBS is based on the presence of recurrent abdominal pain or discomfort at least three days per month in the previous three months. Discomfort is defined as an uncomfortable sensation not described as pain. This abdominal pain or discomfort has to be associated with at least two of the following criteria: (i) improvement of the symptoms with defecation, (ii) onset associated with a change in frequency or with a change in appearance (iii) of stool. These criteria must be fulfilled for the previous 3 months with symptom onset at least 6 months before the diagnosis (43).

2.2.2.3 Pathophysiological mechanisms

Despite the large amount of research conducted on the pathophysiology of IBS, it is not yet fully understood, and multiple factors are likely to contribute.

Abnormal gastrointestinal motility

Already in the 60s it was discovered that patients with IBS display abnormal GI motility (71), which is generally thought of as a colonic motor dysfunction, but available evidence suggests that abnormalities in other parts of the GI tract are also involved (72). Kellow *et al.* showed that motor abnormalities in the small intestine were often accompanied by abdominal symptoms, providing evidence that not only dysfunction of the colon, but also abnormalities in the small intestine are involved in symptom development (73). Furthermore, the study of Cann *et al.* also reported a change in transit time through the small intestine in patients with IBS, again demonstrating that abnormalities of the small intestine also play a role in the pathophysiology of IBS (72).

Visceral hypersensitivity

Another key factor in the mechanism of IBS is visceral hypersensitivity, an excessive perception of response to gut stimuli (68, 74). Ritchie was the first to report an increased sensitivity to colonic balloon distention in IBS patients compared to controls (75). Additional studies have pointed out that a subset of patients display an increased sensitivity to distention of the small intestine or the colon (68). This increased visceral sensation can be caused by a change in sensitivity of receptors in the GI wall or by altered modulation of GI signals in the CNS (68). The significant role of the CNS was further highlighted in a study of Whitehead *et al.* where the authors concluded that psychological factors, rather than biological differences between patients and healthy volunteers, determine the pain perception (76).

Psychological factors

Psychosocial factors also contribute to symptom generation, although it is unlikely that they are the cause of the symptoms (77). The link between psychological factors and FGIDs is based mostly on epidemiological studies and the relationship between life events and GI symptoms. Furthermore it has been demonstrated that behavioral therapy and drugs that modify psychological well-being, such as antidepressants, can improve IBS symptoms (68).

IBS is often accompanied with comorbid affective disorders, such as anxiety, hostility and phobia (77). Moreover, a large part of the patients have a history of emotional, physical or sexual abuse (77-80). Another important factor that can affect symptom generation or intensity is stress. Stress can alter the motor function of the intestines and change visceral perception (77). IBS patients often report an association between a stressful event, for example divorce, death of a family member or separation from a spouse, and the onset or exacerbation of their symptoms (68). A study of Drossman *et al.* reported that stress altered stool pattern in 73% of the IBS patients, compared to 54% of the control group. Furthermore 84% of the IBS patients and 68% of the controls in this study reported an association between stress and abdominal pain (81). Further evidence of the link between symptoms in IBS and psychological factors is delivered by the observation that IBS patients who seek medical care have significantly more psychological problems than asymptomatic IBS patients (68).

Food intolerance

It has long been shown that food intolerance may play a role in the pathogenesis of IBS (82). Jones *et al.* reported that putting patients with IBS on a very restricted diet cleared their symptoms, and that specific foods such as wheat, corn, dairy products, coffee, tea and citrus fruits were the most common culprits in provoking symptoms (82). This and other studies have shown that food sensitivity and intolerance is a crucial factor in the pathogenesis of IBS (82-85). Moreover, a Norwegian population study found that 70% of 84 patients with IBS developed symptoms after food intake (9). Changes in food choice have recently become a well-accepted and evidence-based method to suppress symptoms and increase quality of life for patients with IBS (9).

2.3 Dietary triggers of GI symptoms

The association between ingestion of food and induction of symptoms in FGIDs is commonly accepted. Moreover, one of the approaches used to improve symptoms is the exclusion of certain food products from the diet. However, finding the exact foods or food components that trigger symptoms has been difficult to prove (9). More recently, an increasing amount of research investigating the link between diet and FGIDs has focused on poorly absorbed, small dietary components, called FODMAPs, which have been shown to trigger GI symptoms (11).

2.3.1 Fermentable Oligo-saccharides, Di-saccharides, Mono-saccharides and polyols (FODMAPs)

FODMAPs is an acronym for Fermentable Oligo-saccharides, Di-saccharides, Mono-saccharides and polyols. They are a group of dietary short-chain carbohydrates that are poorly absorbed in the small intestine and more specifically include fructose, lactose, fructans or fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and polyols such as sorbitol and mannitol (13). FODMAPs are found naturally in a range of foods, highlighted in Table 2.

2.3.1.1 Fructose

Fructose is a hexose that is present in a variety of dietary products and can be found in the diet as free fructose, in sucrose or as polymer structure in fructans. It is taken up through glucose-transporters in the small intestine. Absorption varies and occurs more rapidly in the presence of glucose than for free fructose because glucose cotransport is involved in the uptake of fructose (86). Therefore, when fructose is in excess of glucose, it is regarded as a FODMAP. Foods high in excess fructose include honey, apples, pears and watermelon. Fructose malabsorption is rather a normal physiologic state, occurring in 40% of the population and is therefore a poor predictor for FGIDs (69). However, a combination of visceral hypersensitivity and intestinal distention, triggered by fructose malabsorption is still able to trigger GI symptoms in patients (11, 69, 87)

2.3.1.2 Lactose

Lactose is a disaccharide consisting of glucose and galactose, and is present in high amounts in milk, ice cream, and soft cheeses. When ingested, lactose is hydrolyzed into its two composing monosaccharides in the proximal small intestine by an enzyme called lactase (88). Malabsorption of lactose is a common phenomenon, occurring in 15-100% of the population, depending on a range of factors including ethnicity (89), and is caused by an impaired activity or reduced expression of lactase (88). When lactose is not absorbed in the small intestine, it is fermented by colonic bacteria. Consequently, short-chain fatty acids and gases including hydrogen (H₂) and methane (CH₄) are produced, which can trigger GI symptoms such as bloating and diarrhea (88). The intensity of symptoms caused by lactose malabsorption depends on the severity of the hypolactasia and on the bowel's response to luminal distention (11).

2.3.1.3 Oligosaccharides

FOS and GOS are oligosaccharides containing fructose chains and galactose chains, respectively. Fructo-oligosaccharides, also called fructans, are often added to dietary products including yoghurt, bread and pasta as an additional fiber source (11). The highest sources of FOS include large amounts of wheat, onion, garlic, watermelon and legumes. Beans, cabbage, and sprouts are dietary products rich in GOS. Since the human body lacks hydrolases to break down these saccharides, both FOS and GOS are poorly absorbed molecules in everybody (11, 69, 87).

2.3.1.4 Polyols

Mannitol, sorbitol, maltitol, xylitol and isomalt are molecules belonging to the polyol group. Foods rich in polyols include apples, pears, peaches, cherries and nectarines. Furthermore, these sugar alcohols are often used as artificial sweeteners to replace sugar, and are only partly digested and absorbed in the small intestine. The absorption of polyols worsens when ingested together with fructose, leading to an additive effect on symptoms induced (11). Additionally, polyols often have a laxative effect, where by water follows unabsorbed polyols to the colon to be eliminated through the feces, making the latter more soft. In other words, the higher the polyol intake, the more (unabsorbed) polyols will be transported to the colon and the more water will be retracted to the colon (11, 90).

Table 2: Overview of common food sources of FODMAPs (9, 11, 13, 91).

Type of food	Excess fructose	Lactose	Fructans	GOS	Polyols
Fruit	Apple, pear, watermelon, mango		Peach, watermelon		Apple, pear, peach, cherries
Vegetables and legumes	Asparagus, artichokes		Onion, garlic, asparagus, artichokes	Beans, lentils, chickpeas, Brussel sprouts, cabbage	Cauliflower, mushrooms, broccoli
Grains and cereals			Wheat, rye based bread, pasta or cereal		
Milk and milk products		Milk, yoghurt, soft cheese, ice cream			
Other	Honey		Cashews nuts, pistachio nuts		Artificial sweeteners

2.3.2 Mechanism of FODMAPs in symptom induction

Increasing evidence indicates that FODMAPs contribute to symptom development in patients with FD, IBS or other FGIDs (16, 92). Nevertheless, they are unlikely to be the cause of the disease (13, 14). There are a few mechanisms that can explain why intake of FODMAPs leads to symptom generation. First, when ingested, FODMAPs are poorly absorbed and are transferred from the small intestine to the colon, where they are available for fermentation by gut bacteria. Bacterial fermentation results in the production of hydrogen (H₂) and methane (CH₄) gasses (Figure 3) (14). Patients with FGIDs are commonly hypersensitive to gas production or show an increased production of certain gases resulting in luminal distention (16, 87). These processes contribute to certain GI symptoms such as flatulence, bloating and abdominal pain or discomfort (14). In some cases, bacterial overgrowth can be present. Bacterial overgrowth is a process that is characterized

by the movement of bacteria from the colon up to the small intestine, where they can ferment FODMAPs. The production of H₂ and CH₄ gas through this fermentation results in distention of the small intestine, which again leads to symptoms such as abdominal pain or discomfort (87).

In addition to changes in sensitivity of the bowel and gas production, other characteristics of FODMAPs also play a role in symptom development. These saccharides have an osmotic effect in the small intestine, increasing the delivery of water to the bowel (Figure 3). This water is excreted with the feces, making the feces less solid. This may induce a laxative effect and can lead to diarrhea in some patients (13, 14). The extent to which each FODMAP exerts osmotic or fermentation effects depends on the chain length of the saccharide and degree of malabsorption (9).

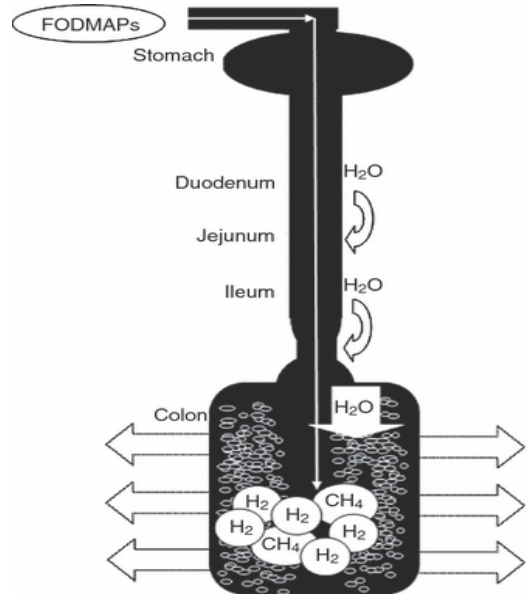


Figure 3: Mode of action of FODMAPs. Ingested poorly absorbed FODMAPs are fermented by colonic bacteria. This leads to the production of gasses. Through the osmotic effect of FODMAPs water is extracted to the bowel and excreted through the feces. Together these processes lead to luminal distention (14).

It has been established in several studies that FODMAPs are involved in the development of GI symptoms. Ong *et al.* showed that a high FODMAP diet (HFD) significantly worsened all observed symptoms (abdominal pain or discomfort, bloating, nausea, wind, heartburn and lethargy) in patients with IBS in contrast to minimal or no symptoms in healthy volunteers. Interestingly, these symptoms developed during the first day of the HFD and were associated with a greater gas production (15). Several other key studies support the role of FODMAPs in the induction of GI symptoms (13, 93, 94). The majority of studies investigating FODMAPs and symptom development have been conducted in IBS patients (15, 95), whereas data about the role of FODMAPs in symptom development in FD patients is limited.

2.3.3 The low FODMAP approach

A dietary approach restricting FODMAP intake can relieve symptoms in patients with IBS (15, 91, 96), suggesting that FODMAPs offer a way to reduce symptoms and to improve the quality of life. A study by Shepherd *et al.*, where a diet restricting all FODMAPs was provided to patients with IBS and fructose malabsorption (FM), showed sustained improvement of gut symptoms in 74% of participants (69). In a second part of the study, a re-challenge with fructans, fructose or a mix of the two was performed, showing that symptom induction is dose-dependent (69). Furthermore, a prospective observational study by de Roest *et al.* investigating the effect of a low FODMAP diet

(LFD) on GI symptoms found a significant improvement of almost all symptoms in the majority of patients due to a decrease in luminal distention (97).

In addition, Staudacher *et al.* compared LFD instruction to the standard national UK dietary approach, and revealed that symptom improvement was significantly higher for the low FODMAP group compared to the standard group. More specifically, bloating, abdominal pain and flatulence significantly improved more with the low FODMAP diet, and patients reported an improvement in diarrhea, an increase in energy levels and a decrease in nausea (98).

2.4 Brain-gut axis

2.4.1 Extra-intestinal factors

Many patients diagnosed with FD or IBS also exhibit extra-intestinal and psychological symptoms. Common extra-intestinal symptoms associated with FGIDs are depression, anxiety, somatization and neuroticism (17). Growing evidence suggests that psychiatric comorbidities are more common in FGID patients than in controls, where 50-90% of IBS patients have at least one psychiatric disorder, such as mood or anxiety disorders (19). Moreover, it is often the combination of GI and extra-intestinal symptoms that drives patients to seek medical care more than the GI symptoms themselves (99). Subsequently, remarkably more patients who seek medical care show elevated levels of mental or psychological problems compared to non-consulters (17). Furthermore, physical or sexual abuse is reported in 40% of FGID patients (79). An important system involved in this link between FGIDs and psychological disorders is the serotonin system. Besides its role in the gut, as previously described, 5-HT is also a key neurotransmitter in the brain (100). Serotonergic neurons are involved in the central modulation of pain and in the regulation of mood and appetite (37). Abnormalities in the function of the 5-HT system are involved both in the pathophysiology of mood and anxiety disorders and in FGIDs (40, 100).

Other frequently reported extra-intestinal symptoms in FD patients include postprandial headache and drowsiness. Drowsiness is a rather late postprandial symptom and has been found to be related to antral distention, which is greater in patients with FD, and to delayed gastric emptying (18, 101). Studies indicate that psychological disorders may worsen FGID symptoms and vice versa (19). The fact that GI and extra-intestinal symptoms both occur in patients with functional gut problems, indicates that these disorders are not restricted to abnormalities in the GI tract, but that they are multi-systemic (70).

2.4.2 Involvement of the brain-gut axis

2.4.2.1 Background

There is an increasing awareness that a dysfunction of the pathways between the brain and the gut, also called the brain-gut axis (BGA), plays a role in symptom development in FGIDs (19). As the name states, the BGA is a bidirectional connection between the brain and the gut, which comprises neural, hormonal and immune pathways (102). A network of afferent fibers from the GI tract to CNS structures and efferent fibers from the brain to smooth muscles in the GI wall functions as the main signaling pathway (103). Psychosocial factors including stress and emotions can affect the function of the GI tract, illness behavior and symptom perception. Conversely, visceral pain can influence central pain perception, mood and behavior (19, 104, 105). This complex bidirectional communication pathway is involved in the regulation of digestive processes, such as food intake and appetite, and also in the coordination of the physical and emotional state. Moreover, FGIDs are commonly presented together with central symptoms such as loss of appetite, changes in mood and anxiety. Therefore it has been suggested that dysregulation of the BGA is involved in symptom development of FGID and other eating disorders (18, 105).

2.4.2.2 Intrinsic gastrointestinal innervation

Dysregulation of the BGA can occur at different levels. Visceral hypersensitivity for instance can be caused by changes in the neurons innervating the gut, or by a change on the level of the CNS (19). The part of the BGA that is embedded in the GI wall is called the enteric nervous system (ENS). The ENS, also referred to as the mini-brain or the little brain in the gut, is a complex neural network with intrinsic activity, independent of extrinsic neural input. It is involved in controlling different functions of the GI tract, such as motility, absorption and secretion. When changes occur in the structure and function of the ENS neurons, for instance by local inflammation, this can affect functions of the GI tract and may lead to GI disorders (19, 104). However, isolated disturbances in the ENS cannot explain the association between stress, psychologically traumatic or emotional experiences with the development and persistence of FGIDs (106). Therefore, it is highly likely that FGIDs involve other systems, including the autonomic nervous system (ANS) (106) and the limbic system (104).

2.4.2.3 Extrinsic gastrointestinal innervation

An important region in the brain involved in gut control is the limbic system. This is a group of neural structures involved in internal and external homeostasis of the organism. Besides gut control, it is also involved in the generation of emotions, indicating that there is a link between gut problems and emotional state (104, 107). Evidence for this relationship is also delivered by brain imaging studies, showing an overlap between regions in the brain which process visceral sensations and regions involved in emotional regulation (19).

The ANS is considered the primary pathway of the BGA. It regulates the absorption, secretion, blood flow and motility of the GI tract (108). Visceral stimuli can elicit systemic responses including pain and discomfort through the ANS and alterations in the ANS may be involved in symptom development (106). The ANS consists of a sympathetic and parasympathetic component. The overall effect of the sympathetic nervous system on the GI tract is inhibitory: it innervates postganglionic vasoconstrictor neurons, secretion inhibitory neurons and motility inhibiting neurons. The parasympathetic innervation on the other hand leads to gastric acid secretion, stimulation of motility and of enteroendocrine and enterochromaffin cells. The prolonged duration of many emotional states like anxiety or depression are translated into prolonged changes in ANS output to the GI tract (105). As a result, alterations in target cells may also occur, which in turn may lead to chronically changed signaling from the gut to the brain and possibly to remodeling of brain regions that receive this information (105).

A study of Aggarwal *et al.* showed that various disturbances are present in a subgroup of IBS patients. Furthermore, this study found that constipation was associated with increased sympathetic activity, whereas diarrhea was associated with an increase in parasympathetic activity (109). Although there are some studies providing evidence of the involvement of the ANS in FGIDs, more studies are needed for a better understanding of the exact mechanisms by which it plays a role in symptom development.

2.4.2.4 Gut-brain signaling

Sensory information from the GI tract is carried to the brainstem and the spinal cord via vagal and spinal afferents. Both pathways are thought to transmit different aspects of sensory information. Physiological information including motor activity or composition of the luminal content in the upper GI tract is mainly relayed by vagal neurons, which are activated by mechanoreceptors and chemoreceptors (110, 111). In contrast, the spinal afferents process noxious pathophysiological information, for example intense mechanical or chemical stimuli arising from tissue injury, ischemia or inflammation. However, it is suggested that there is some overlap and interplay between these two pathways. All of this sensory information is processed in the spinal cord and brain stem and conveyed to higher brain areas like the hypothalamus (110).

Besides this neural signaling, hormonal signaling also plays an important role in transferring information from the GI tract to the CNS. Throughout the GI tract there are several types of enteroendocrine cells which secrete specific hormones such as ghrelin and GLP-1. These hormones exert their functions through the vagus nerve or by acting directly on specific brain areas. Neural and endocrine signals are integrated in higher brain centers to regulate food intake and energy expenditure.

2.4.3 Limitations in current knowledge

IBS is a well-studied syndrome with respect to the involvement of the BGA (105). Much less is known about the role of the BGA in symptom development in FD patients (112). Although in previous decades a lot of progress has been made in our understanding of the role of the BGA in FGIDs, the exact mechanisms of symptom development and the interplay of different food components remains largely incomplete (112). A recent study of Van Oudenhove *et al.* described the attenuation of neural and behavioral responses to sad emotions. However, the underlying mechanisms of the association between food intake and the BGA needs to be further elucidated to better understand the central effect of food in FGIDs (21). This thesis will address the common dietary triggers of IBS symptoms (FODMAPs) and their role in FGID, specifically the generation of upper GI symptoms, and how this may putatively interplay with the BGA.

2.5 Aim and hypothesis

In the context of FD we will examine how FODMAPs, alone or in combination, alter the upper GI tract response to carbohydrate infusion by using high resolution manometry and symptom questionnaires. Furthermore, we aim to gain more insight in the effect of these FODMAPs on mood and emotional state using psychological questionnaires, and how this differs in healthy volunteers and patients with IBS. We hypothesize that fructans will induce higher levels of symptoms intensity as a consequence of general complete malabsorption and that symptom generation and emotional effects will be elevated in patients compared to healthy volunteers following FODMAP infusion.

3 Experimental work

3.1 Materials and methods

3.1.1 Study population

The study was conducted in 15 healthy, adult volunteers and a pilot group of 6 IBS patients, all aged between 18 and 65 years. Healthy volunteers were included if they had no symptoms or history of GI disease, other significant diseases, psychological disorders or drug allergies and were not pregnant, taking any medication or had a drug history. They were recruited from a healthy volunteer database and through oral advertising. All IBS patients met the Rome III criteria for IBS, which was verified by a screening questionnaire (see Appendix 1) and under supervision of a gastroenterologist, and had no other significant diseases, psychological disorders or drug allergies. Other exclusion criteria for the patients were pregnancy, major GI surgeries and the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids or other immunosuppressive drugs in the preceding 6 months. Drugs potentially affecting GI motility or sensitivity had to be discontinued at least 1 week before the study. The patients were recruited through an advert in '*Metro*', a free Belgian newspaper.

All participants followed a diet low in fiber and fermentable carbohydrates the day before each test to ensure a low baseline symptom level and low microbial gut activity. Furthermore, participants were asked to refrain from alcohol, tea and coffee and intense physical activity for at least 12h before participation. Written informed consent was obtained before the start of the study. The study was approved by the Medical Ethics Committee of the University Hospitals Leuven (S57061) and was performed in accordance with the Declaration of Helsinki.

3.1.2 High resolution manometry

Manometric studies were carried out with the high-resolution solid-state manometer (HRM) system ManoScan 360 (Sierra Scientific Instruments, Los Angeles, USA) after an overnight fast. The manometry probe, ManoScan ESO catheter (Given Imaging, Duluth, US) with 36 circumferential channels spaced at 1cm intervals, was inserted through the nose into the stomach after calibration of the probe. This calibration was performed before each experiment. Furthermore, reference values to compensate for differences in temperature were set before the insertion of the probe and immediately after the probe was removed from the participant by holding the probe in the air. The placement of the probe was as far as possible towards or into the duodenum. Moreover, at least one of the 36 sensors was positioned in the lower esophageal sphincter (LES) (Figure 4).

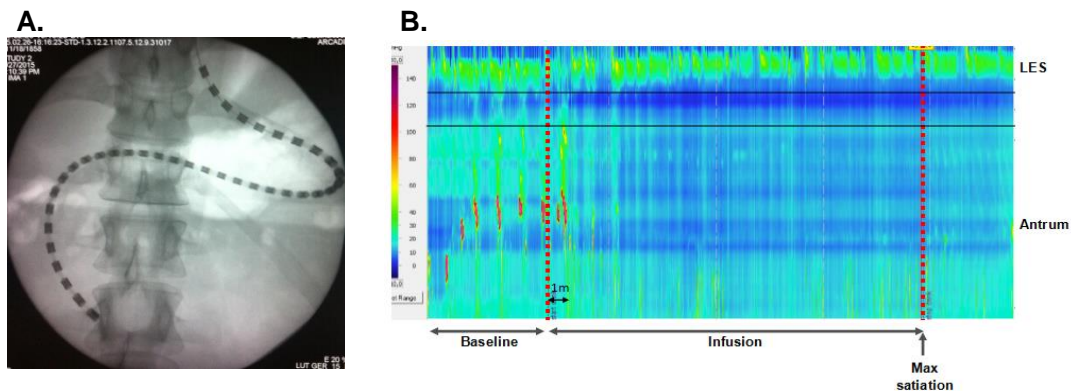


Figure 4: Example of positioning of the manometry probe and manoview output. (A) A manometry probe was placed through the nose into the stomach and as possible towards or into the duodenum. At least one of the 36 sensors was positioned in the lower esophageal sphincter (LES). (B) Pressure changes are visualized by a change in color in the Manoview Analysis program. After a baseline recording and when the participant was in late phase II of the MMC, intragastric infusion was started. Infusion stopped when maximal satiety was reached.

To facilitate the insertion of the probe, Xylocaine® 10% local anesthetic throat spray (NV AstraZeneca SA, Brussels, Belgium) and endogel (TELIC, SA, Bigues, Spain) were used. Fluoroscopic images were taken to confirm the position of the probe. The images were taken at the lowest dose of radiation while the participant's lower abdomen and thyroid gland were covered with lead protection clothing. All the personnel in the room wore a lead jacket and thyroid gland protection. After confirming that the probe was correctly inserted, it was fixed to the participant's nose with adhesive tape to prevent movement of the probe. Intragastric pressure (IGP) was measured during the infusion of the test or control solution and for three hours thereafter to assess the recovery of IGP and the late postprandial effects of the FODMAPs on IGP (Figure 5).

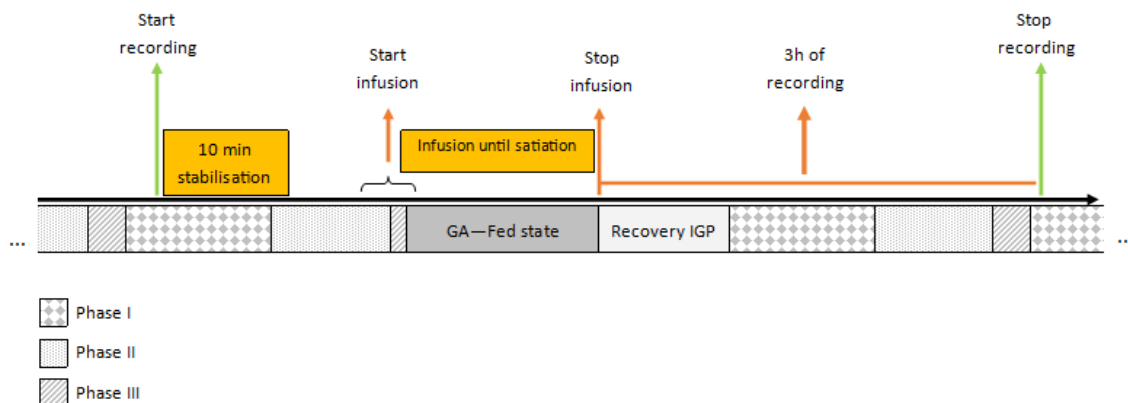


Figure 5: Schematic presentation of the study protocol. After the manometry probe and infusion tube were placed through the nose into the stomach and their position was confirmed, the recording started. After a 10 minute stabilization period and when the participant was in a late phase II or a phase III of the hunger cycle (MMC), the FODMAP or control solution was administered to the participant until he/she was fully satiated. Recording was continued for 3h post infusion to assess the recovery of the IGP and the late postprandial effects of the FODMAPs on IGP.

3.1.3 Test solutions

Four different solutions differing in FODMAP content (Table 3) were administered to the healthy volunteers. These four solutions were tested on different occasions, two days to two weeks apart, in a randomized order, which was determined by a computer-generated list. Based on GA results of the healthy volunteers, it was decided to only administer the fructan and control solution to the IBS patients on two different occasions in a randomized order, with the option for a third visit to test the fructose solution. All participants were blinded to the identification of the solution being infused.

Table 3: Characteristics of the four different solutions.

Solution	FODMAP	Content (g) *	Osmolarity (mOsmol/kg)	Energy content (kcal)
Fructans	Fructo-oligosaccharides	19	71,66	28,50
Fructose	Fructose	50	589,00	200,00
FODMAP mix	Fructo-oligosaccharides	5		7,50
	Fructose	15		60,00
	Galacto-oligosaccharides	10		17,00
	Sorbitol	5		12,05
	Mannitol	5		12,00
	Total	40	358,30	108,55
Control	Glucose	50	528,30	185,00

* Doses were chosen on the basis of previous study designs (69, 113) and were per 500ml water.

After a stabilization period of at least 10 minutes and when the participant was in late phase II, during which irregular contractions occur, or phase III, characterized by high amplitude regular contractions in the stomach (three contractions per minute) or duodenum (12 contractions per minute), of the hunger cycle (MMC), the infusion of the FODMAP or control solution was started. The solutions were administered through an infusion tube which was placed through the nose into the upper stomach. The position of the infusion tube was confirmed fluoroscopically or based on the numbers on the tube, where after it was also fixed to the participant's nose. All solutions were administered at a speed of 60ml/min (35) using a Infusomat® SpaceP pump set (B Braun, Melsungen, Germany) until satiation.

3.1.4 Questionnaires

Throughout the experiment, which took approximately 4h in total, the participants were asked to fill out different questionnaires to assess their satiation, GI symptom levels, emotional state and mood. Questionnaires used in this study were the following: satiation questionnaire, GI symptom questionnaire (Appendix 2), Profile Of Mood States (POMS)-fatigue subscale (Appendix 3) (114), Self-Assessment Manikin (SAM) (Appendix 4) (115), the validated Dutch version of the Positive and Negative Affect Scale (PANAS) (Appendix 5) (116) and the 20-item version of the State-Trait Anxiety Inventory (STAI) version DY1 (Appendix 6) (117). Figure 6 gives an overview of time points at which the different questionnaires were filled out.

3.1.4.1 Satiating questionnaire

The satiation questionnaire was filled out every minute during infusion of the carbohydrate or control solution, starting at time point 0. The participants scored their satiation on a scale ranging from 0 to 5 (0 = no feeling, 5 = uncomfortable feeling). When they were fully satiated (score 5), the infusion was stopped.

3.1.4.2 GI symptom questionnaire

To assess the effect of the different solution compositions on satiety and intensity of epigastric symptoms (such as nausea, fullness, cramps and pain) and GI symptoms (such as abdominal pain, bloating and wind), each participant filled out the symptom questionnaire immediately before infusion, every five minutes during infusion and every 15 minutes after infusion. The format of the questionnaire was a 10 cm visual analogue scale (VAS).

3.1.4.3 Psychological questionnaires

To assess the emotional state, SAM and POMS questionnaires were filled out immediately before the infusion and every 15 minutes until the end of the experiment. SAM uses graphic characters to score valence, arousal and dominance or control on a nine-point scale. Using the short, validated Dutch version of the POMS questionnaire, five different emotional states, namely fatigue, vigor, anger, anxiety and depression were assessed. These five items were scored on a 10 cm VAS at the above mentioned predefined time points.

Before, immediately following and one, two and three hours after infusion, the participants were asked to fill out the PANAS and STAI questionnaires. The PANAS questionnaire comprises 20 different emotions and mood states, of which 10 items measure positive affect (PA), such as interested, proud, inspired, etc., and 10 items measure negative affect (NA), such as nervous, restless, upset, etc. These mood states were scored by the volunteers on a five-point scale ranging from not at all or very slightly (1) to extremely (5), to assess how they feel at that moment (118). The DY1 version of the STAI-questionnaire is composed of 20 different statements about emotion and mood such as 'I feel safe' or 'I feel nervous'. These 20 items were scored on a scale from 1 (totally not) to 4 (extremely).

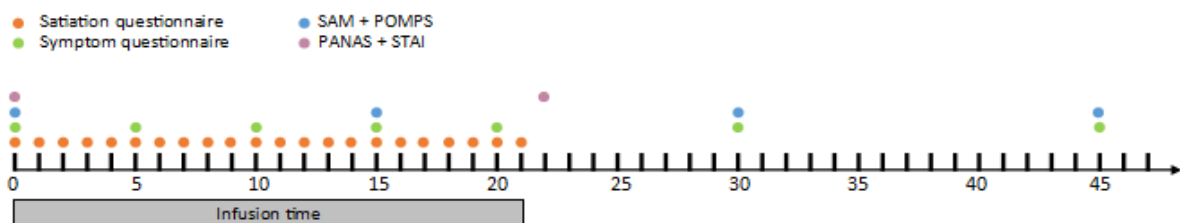


Figure 6: Time points when questionnaires were completed. Participants needed to complete six different questionnaires. All questionnaires were filled out before the infusion started for a baseline scoring. The satiation questionnaire was filled out every minute during infusion. The symptom questionnaire was filled out every five minutes during infusion and every 15 minutes after infusion, together with the SAM and POMS questionnaires, which were filled out every 15 minutes during the entire experiment. The PANAS and STAI questionnaires were filled out before, immediately after and 1, 2 and 3 hours after the infusion.

3.1.5 Data analysis

3.1.5.1 Manometry analysis

Manometric measurements were analyzed with ManoView Analysis 2.0.1 software (Sierra Scientific Instruments). Using the two set references, before insertion and after removal of probe, thermal compensation and an interpolated thermal compensation were applied. Next, the data was converted into a text file and further analyzed in excel with the help of a template designed by TARGID and used in previous studies (35). To assess changes in IGP the average pressure of the first five measuring channels under the LES were considered. Data was presented as change in IGP from baseline (mean \pm SEM).

3.1.5.2 Questionnaires

The symptom questionnaire was analyzed by measuring the score of each symptom on a scale of 100. The same analysis approach was used for the POMS questionnaire. For the PANAS questionnaire, the scores on the 10 items reflecting PA were added up and the scores on the 10 items reflecting NA were added. For both the PA and NA, total score ranges between 10 and 50. Higher scores reflect higher levels of PA or NA. The STAI questionnaire was analyzed using a template which automatically calculates the state-trait anxiety score. For the SAM questionnaire, scores on a 1 to 9 range scale were compared within and between groups.

3.1.6 Statistical analysis

All data were statistically analyzed with GraphPad Prism Version 5.00. Baseline demographic data were compared between the control group and the patient group with an unpaired t-test. Data from IGP measurements and the different questionnaires were tested for statistical significance within and between groups using paired or unpaired t-tests, respectively, or with repeated measures analysis of variance (ANOVA), with subsequent post-test for multiple comparisons. Data obtained from the questionnaires were analyzed in Prism to detect differences in mean values over all time points, and in SAS, where a more complex mixed model analysis was performed to correct for the different time points the questionnaires were filled out. The low sample size of the patient population did not allow proper analysis in SAS regarding the comparison between patients and healthy volunteers. Therefore, the comparison of mean scores over all time points between patients and healthy volunteers was conducted in Prism. Data were considered significantly different at $p < 0.05$, whereas $0.05 < p > 0.1$ was considered a trend, taking into account the n-values. Results are presented as mean \pm SEM.

3.2 Results

3.2.1 Study Population

The study was performed in 15 healthy volunteers (7 men and 8 women) and 6 IBS patients (2 men and 4 women). Only two healthy volunteers and two patients had previous experience with gastrointestinal catheter studies. Table 4 summarizes the demographics of the study population. The mean age and BMI of the healthy volunteers was 22.4 (19-32) and 22.99 (18.38-30.24) respectively. Four of them had a BMI between 25 and 30, classified as overweight. All healthy participants met the inclusion/exclusion criteria. The mean age of the IBS patients was 37 (22-55), which was significantly older than the healthy volunteers. The two male patients had a BMI above 30, classified as obese, whereas the females' BMI was normal. All patients were non-smokers. Only one of the patients completed the optional third visit with fructose challenge. Therefore, patient data concerning fructose are not shown in this Master's thesis.

Table 4: Demographics of the study population. Data are shown as mean (range). Significant p-values are marked in bold.

Parameter	HV	IBS	p-value
Age	22.4 (19-32)	37 (22-55)	0.0064
Height	1.74 (1.56-1.85)	1.71 (1.6-1.82)	0.3900
Weight	69.67 (54-98)	73.33 (48-105)	0.5850
BMI	22.99 (18.38-30.24)	24.74 (18.75-31.7)	0.5100

3.2.2 Time and volume of infusion

For the healthy volunteers, the drinking time and correspondent volume infused was largest for the fructan solution and least for fructose (Table 5). However, there was no significant difference in drinking time and volume infused between the four solutions. Patients had similar drinking time and volume for fructans and glucose (not significantly different). Furthermore, drinking time and volume infused did not significantly differ between patients and healthy controls (Table 5).

Table 5: Mean drinking time and volume infused of healthy volunteers (HV) and patients (IBS).

Solution	HV		IBS		p-value
	Time	Volume	Time	Volume	
Fructans	22.93 ± 4.14	1376 ± 248.3	19.17 ± 3.3	1150 ± 198	0.5937
Fructose	18.00 ± 2.46	1080 ± 147.3			
FODMAP mix	20.87 ± 2.57	1252 ± 154.4			
Glucose	18.20 ± 2.01	1092 ± 125.7	19.00 ± 5.15	1140 ± 308.7	0.8635

3.2.3 Intra-gastric pressure measurements

3.2.3.1 Gastric accommodation during infusion

Healthy volunteers

Figure 7A shows the change in IGP from baseline during the infusion of the solutions in healthy volunteers. Cut off for analysis of the GA was taken at the point where data was available for 50% of the participants. The mean GA in healthy volunteers is significantly less pronounced after fructan infusion compared to the other solutions (fructose: $p = 0.0003$; FODMAP mix: $p = 0.0002$; glucose: $p = 0.0002$). The mean change in IGP during the infusion of fructose, FODMAP mix and glucose did not differ significantly (Table 6). Nadir IGP (defined as lowest IGP during accommodation) was not significantly different between the four solutions (Table 6). Only a trend to lower nadir IGP was found for fructans compared to glucose. Time to nadir IGP was likewise not significantly different.

Patient data and comparison with healthy volunteers

During fructan infusion, patients show a significantly greater drop in IGP compared to the glucose infusion ($p < 0.0001$) (Figure 7B). Nadir IGP was significantly lower for fructans compared to glucose ($p = 0.0313$) (Table 6). Time to nadir was not significantly different between the two solutions. When comparing GA between healthy volunteers and patients, a significant greater GA during fructan infusion was found in patients ($p < 0.0001$). Furthermore, nadir IGP during fructan infusion was significantly lower in IBS patients ($p = 0.0016$). Mean IGP change during glucose infusion was also significantly lower in patients ($p = 0.0193$), but correspondent nadir IGP was not. Time to nadir IGP was not significantly different between patients and healthy controls.

Table 6: Mean values of IGP and nadir IGP for healthy volunteers (HV) and patients (IBS). Significant p -values are marked in bold

	Solution	IGP		NADIR IGP	
		Mean \pm SEM	p -value	Mean \pm SEM	p -value
HV	Fructans vs.	-2,07 \pm 0,15		-3.27 \pm 0.43	
	Fructose	-2.83 \pm 0.24	0.0003	-4.21 \pm 0.80	0.2565
	FODMAP mix	-2.70 \pm 0.21	0.0002	-4.20 \pm 0.64	0.3224
	Glucose	-2.85 \pm 0.21	0.0002	-4.26 \pm 0.50	0.0742
	Fructose vs.				
	FODMAP mix		0.8498		0.9954
	Glucose		0.3684		0.9330
	FODMAP mix vs.				
	Glucose		0.9306		0.9472
IBS	Fructans vs.	-5.44 \pm 0.41		-7.13 \pm 0.59	
	Glucose	-3.66 \pm 0.26	< 0.0001	-4.38 \pm 0.56	0.0313
HV vs. IBS	Fructans		< 0.0001		0.0016
	Glucose		0.0193		0.6685

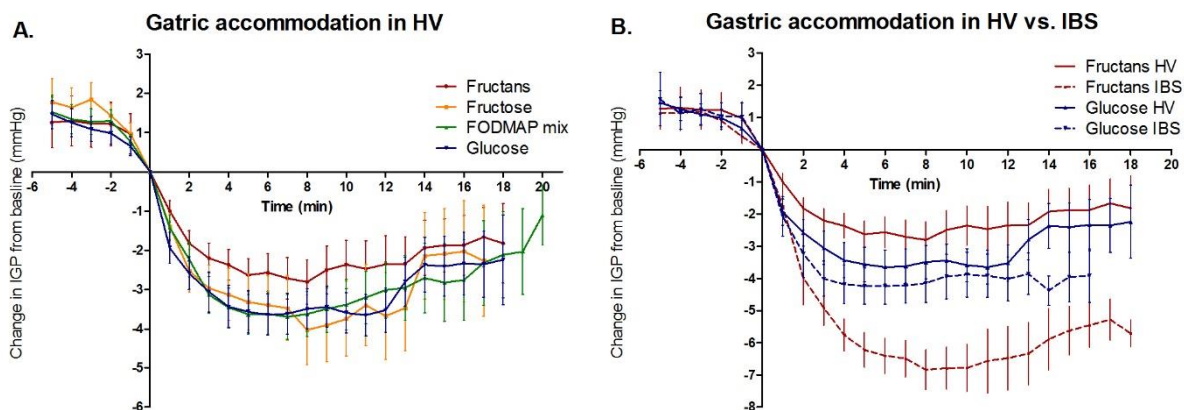


Figure 7: Change in IGP from baseline during infusion of the different solutions. (A) A significantly less pronounced change in IGP was observed during fructan infusion in healthy volunteers (HV) (fructose: $p = 0.0003$; FODMAP mix: $p = 0.0002$; glucose: $p = 0.0002$). The other solutions (fructose, FODMAP mix and glucose) did not induce significantly different changes in IGP. (B) Fructan infusion in patients (IBS) induced a significantly greater drop in IGP compared to glucose in patients ($p < 0.0001$) and to fructans in HV ($p = 0.0003$). Mean IGP during glucose infusion is significantly lower in patients than in HV ($p = 0.0193$).

3.2.3.2 Intra-gastric pressure during four hour experiment

Healthy volunteers

IGP was measured for three hours after the infusion was stopped. When considering the IGP change from baseline of healthy volunteers during the whole duration of the experiment (Figure 8A), fructans were associated with an elevated IGP throughout the entire measurement. Similarly, a statistically significant difference in overall IGP change between the fructan solution and the three other solutions was found (p -values < 0.0001), with a greater change in IGP for fructans. Furthermore, the mean change in IGP following the fructose solution was significantly higher than following the FODMAP mix and glucose solution ($p < 0.0001$). The only two solutions that did not induce a significant difference in IGP change during the entire experiment were the FODMAP mix and the glucose solution ($p = 0.0948$). Cut off for the analysis of IGP during the whole duration of the experiment was taken at 180 minutes, which was when data were available for 100% of the patients, allowing for more accurate analysis of the data.

Patient data and comparison with healthy volunteers.

Although fructans induced a greater drop in IGP during infusion, this difference with glucose disappeared after recovery of IGP (Figure 8B). IGP following fructan and glucose infusion remained at similar levels until the end of the experiment. Notably, both solutions induced an increase in IGP at the end of the measurement. When comparing mean IGP of the patient population, no significant difference could be found between fructans and glucose.

Mean IGP following fructan infusion differed significantly between the patient population and the healthy controls ($p < 0.0001$), with lower IGP in the patient population. In addition, a significant difference was found between the mean IGP following glucose infusion between the patients and healthy volunteers ($p = 0.0004$), with higher IGP levels in patients.

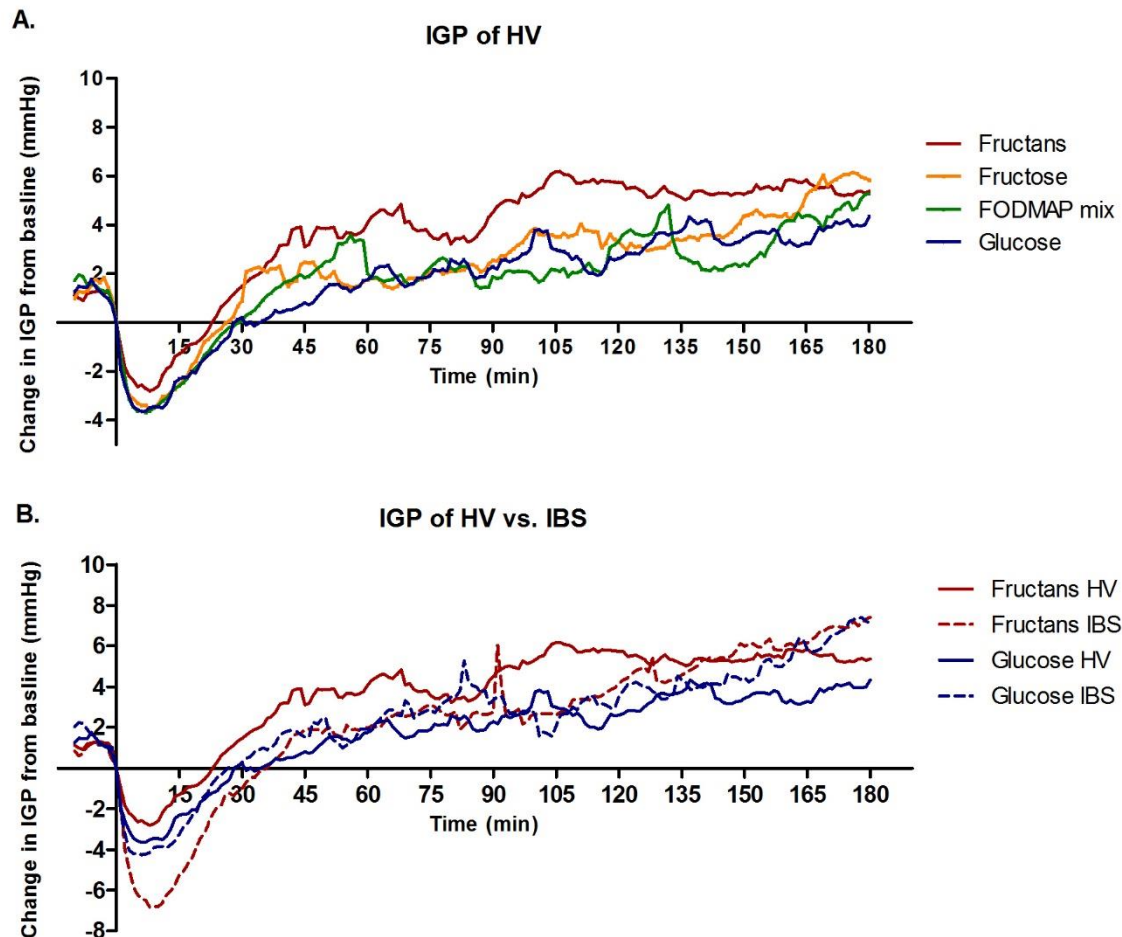


Figure 8: Change in IGP from baseline in healthy volunteers (HV) and patients (IBS). (A) Significantly elevated pressures were measured in HV during and after fructan infusion compared to the other three solutions ($p < 0.0001$). Fructose was associated with a significantly higher mean IGP change compared to the FODMAP mix and glucose ($p < 0.0001$). (B) Mean IGP during and following infusion differed significantly between the patient population and the healthy controls for fructans ($p < 0.0001$) and glucose ($p = 0.0005$).

3.2.4 Symptoms

Healthy volunteers

Symptom scores at the different time points and for the four solutions are shown in Figure 9. After the start of the infusion, hunger scores initially decreased significantly ($p < 0.0001$) for all solutions to recover thereafter (Figure 9A). None of the three FODMAP solutions differed significantly in hunger scores from glucose after correcting for the different time points. However, a significant difference was found between the mean hunger scores over all time points following fructan solution compared to the FODMAP mix ($p = 0.0024$) and between the fructose solution and

FODMAP mix ($p = 0.0027$), with lower hunger scores following the FODMAP infusion. A similar decrease and recovery in scores were seen for the expected amount to eat (data not shown). Satiation and fullness scores showed the opposite pattern (Figure 9B and 9C), increasing significantly from baseline during and partly after the infusion of the solutions (satiation: not analyzed because of too limited variance between solutions; fullness: fructans: $p < 0.0001$; fructose: $p = 0.0046$; FODMAP mix: $p < 0.0001$; glucose: $p < 0.0001$) and declining after reaching a peak. There was no significant difference in sensation of fullness between glucose and the three FODMAPs after correction for the different time points.

All four solutions caused an early rise in bloating scores, increasing already during the infusion of the solutions (Figure 9D). However, the change in bloating from baseline was only significant following fructans ($p = 0.0254$). A trend was found for fructose and glucose ($p = 0.0506$ and $p = 0.0767$, respectively). When mean scores over all time points were compared, fructose (15.91 ± 1.87 VAS) induced significantly more bloating than the three other solutions (fructans: 10.30 ± 1.59 VAS, $p = 0.0003$; FODMAP mix: 12.39 ± 1.05 VAS, $p = 0.0140$; glucose: 7.75 ± 1.46 VAS, $p < 0.0001$). Furthermore, mean scores were significantly higher for fructans and FODMAP mix compared to glucose ($p = 0.0055$ and $p < 0.0001$, respectively). However, after correcting for the different time points, none of the FODMAP solutions induced significantly more bloating than glucose.

Nausea (Figure 9E), belching (Figure 9F) and pain (data not shown) were also assessed using the symptom questionnaire. Scores for these three symptoms stayed very low throughout the experiment and variance between the four solutions is limited. Therefore, these values do not allow proper analysis with correction for the different time points.

Flatulence was frequently reported by healthy volunteers for all solutions. As shown in Figure 9G, flatulence increased shortly after the infusion of the solutions and was least induced by the control solution. All four solutions induced a significant change from baseline in flatulence scores (fructans: $p = 0.0057$, fructose: $p = 0.0045$, FODMAP mix: $p = 0.0008$, glucose: $p = 0.0102$). After reaching a peak, a gradual decline in flatulence following glucose was observed, whereas scores stayed elevated throughout the experiment for the three FODMAP solutions. Mean values for flatulence over all time points were significantly different for the three FODMAP solutions compared to glucose (fructans: 12.24 ± 1.33 VAS, $p = 0.0003$; fructose: 14.59 ± 1.50 VAS, $p < 0.0001$; FODMAP mix: 15.51 ± 1.53 VAS, $p = 0.0005$; glucose: 6.54 ± 0.96). Furthermore, significantly higher mean values were found for fructose and FODMAP mix compared to fructans (fructose: $p = 0.0073$, FODMAP mix: $p = 0.0046$). However, after correcting for the different time points, only a trend for higher flatulence scores following FODMAP mix ($p = 0.0995$) and no significant differences for the other solutions compared to glucose were found.

Elevated scores for cramps were reported after the fructose solution and the FODMAP mix compared to fructans and glucose. Moreover, only fructose and FODMAP mix induced a significant change in cramps from baseline (fructose: $p = 0.0393$, FODMAP mix: $p = 0.0039$). When comparing mean values over all time points, scores were significantly higher for fructose and the FODMAP mix compared to the other two carbohydrates (p -values < 0.0001 ; fructans: 5.25 ± 0.61 VAS, fructose: 13.57 ± 1.44 VAS, FODMAP mix: 13.80 ± 1.58 VAS, glucose: 4.67 ± 0.63 VAS). However, when adjusting for the different time points, these significances became undone and only a trend was found for the FODMAP mix compared to glucose ($p = 0.0800$).

Diarrhea was a frequently reported symptom after FODMAP challenge in the healthy population, but was not assessed by one of the questionnaires. Table 7 summarizes the proportion of healthy volunteers reporting diarrhea.

Table 7: Proportion of healthy participants reporting diarrhea.

	Number of subject with symptom score			
	None	Mild	Moderate	Severe
Fructans	4/15	1/15	4/15	6/15
Fructose	4/15	0/15	4/15	7/15
FODMAP mix	1/15	4/15	3/15	7/15
Glucose	11/15	3/15	1/15	0/15

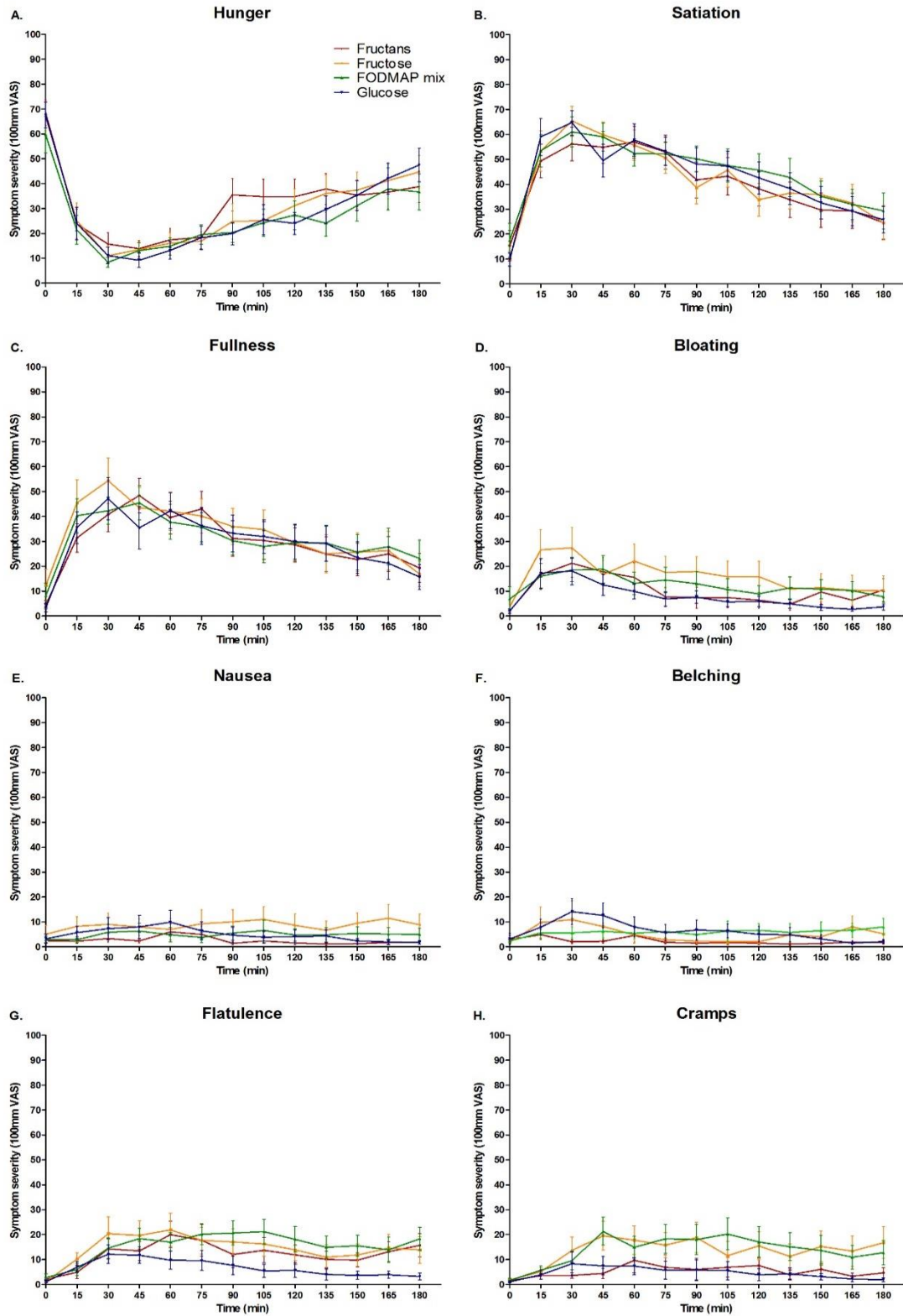


Figure 9: Overview of symptom scores at different time points in healthy volunteers. Hunger, expected amount to eat, satiation, bloating, fullness, nausea, belching, wind, cramps and pain were assessed on a 100mm VAS, where 0 = none and 100 = worst. Scores associated with fructan challenge are shown in red. Scores associated with fructose challenge are shown in orange. Scores associated with FODMAP mix challenge are shown in green and scores associated with glucose challenge are shown in blue.

Patient data and comparison with healthy volunteers

Similar to the healthy volunteers, a significant drop in hunger was observed in the IBS patients (fructans: $p < 0.0032$, glucose: $p = 0.0104$). Hunger scores were not significantly different between the fructan and the glucose solution (data not shown). Comparable results were obtained for expected amount to eat and satiation (data not shown). Both fructans and glucose induced a significant increase in fullness (fructans: $p = 0.001$; glucose: $p = 0.0063$), but again no significant difference between the two solutions was detected (data not shown). Regarding bloating, only a trend towards increased scores from baseline was found following fructan infusion ($p = 0.0504$). Glucose did not induce a significant increase in bloating scores. No significant difference was found in bloating between fructans and glucose after correcting for the different time points (Figure 10A). However, when comparing mean scores over all time points, bloating was significantly greater following fructan infusion compared to the control solution (fructans: 33.65 ± 2.52 VAS, glucose: 23.42 ± 1.68 VAS, $p = 0.0024$). Scores for nausea (Figure 10B), belching (Figure 10C) and pain (Figure 10F) were not significantly different between fructans and glucose after correcting for the different time points. However, mean scores for nausea and pain over all time points were significantly higher after fructan infusion (nausea: fructans: 18.06 ± 1.98 VAS, glucose: 13.88 ± 1.23 VAS, $p = 0.0199$; pain: fructans: 19.91 ± 2.32 VAS, glucose: 13.47 ± 1.24 VAS, $p = 0.0191$). Flatulence and cramps increased considerably from baseline following the fructan infusion (flatulence: $p = 0.0032$, cramps: $p = 0.0027$) (Figure 10D and 10E). Furthermore, scores for cramps after correcting for the different time points were significantly higher following fructan infusion than following glucose infusion ($p = 0.0246$), whereas a trend for increased flatulence was found following fructans ($p = 0.0553$).

Mean scores over all time points for hunger, expected amount to eat, satiation and fullness did not differ significantly neither for fructans nor for glucose between healthy volunteers and patients. For all other symptoms (bloating, nausea, belching, flatulence, cramps and pain), mean scores were significantly greater in IBS patients than in healthy controls, both for fructans and glucose (p -values < 0.0001).

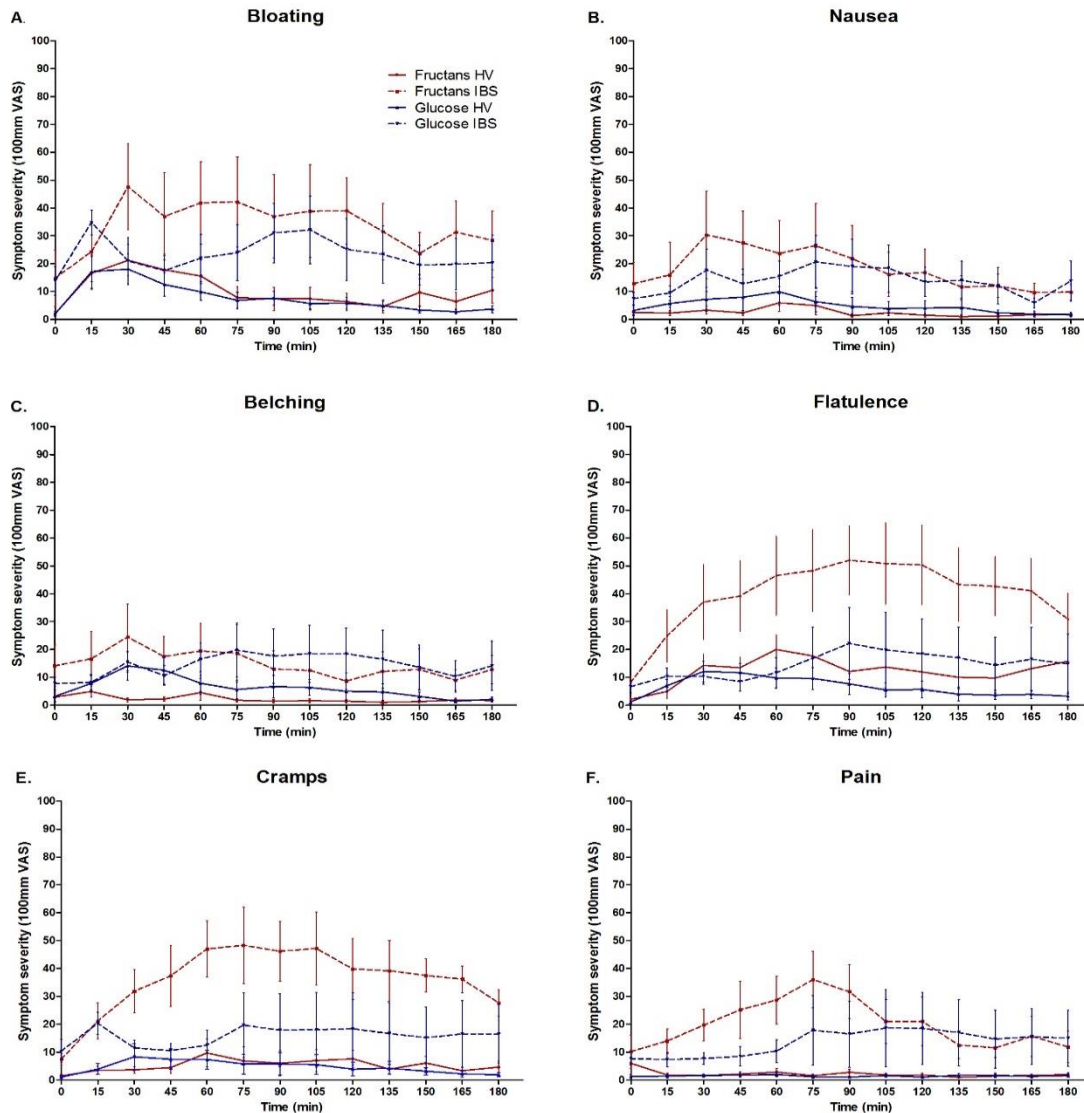


Figure 10: Overview of symptom scores following fructans and glucose in healthy volunteers (HV) and patients (IBS). Significant elevated levels of bloating, nausea, belching, wind, cramps and pain were found in patients compared to healthy volunteers ($p < 0.0001$). In patients, cramps were significantly higher after fructan infusion than after glucose infusion ($p = 0.0246$). A trend to increased wind was found following fructan infusion ($p = 0.0553$).

3.2.5 Questionnaires

3.2.5.1 POMS

Healthy volunteers

Analysis of the POMS questionnaire showed that mean fatigue scores over all time points were significantly lower for the fructan solution (VAS score: 23.49 ± 1.049) than for the fructose solution (35.31 ± 1.3 VAS, $p < 0.0001$), FODMAP mix (34.82 ± 1.15 VAS, $p < 0.0001$) and glucose solution (33 ± 1.8 VAS, $p = 0.001$) (Figure 11A). However, after correction for the different time points, no significant differences could be found. Significant differences for vigor (defined as physical strength

and good health), were found for the fructan and glucose solution compared to fructose ($p = 0.0002$) and FODMAP mix (fructans: $p < 0.0001$, glucose: $p = 0.0005$) over all time points, but these significances were reversed after correction for the different time points (Figure 11B). Anger, tension and depression were also assessed by the POMS questionnaire. In healthy volunteers, scores for these emotions and moods were too low and showed too limited variations to allow proper analysis. The maximum scores for anger, tension and depression did not exceed three, nine and six respectively.

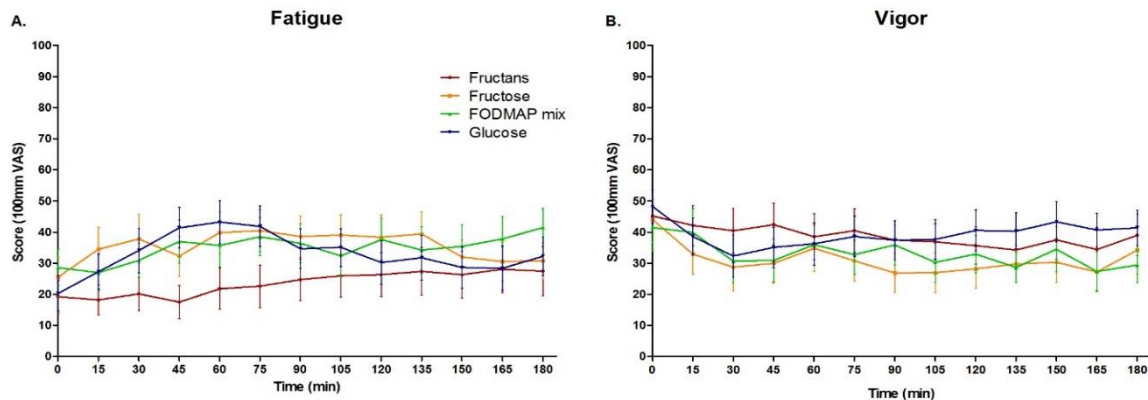


Figure 11: Overview of scores for fatigue and vigor at different time points and for the four different solutions. (A) Fructans are associated with lower scores for fatigue. However, after correcting for the different time points, no significant difference was found between the four solutions. (B) No significant difference in scores for vigor was detected between the four solutions after correction for the different time points.

Patient data and comparison with healthy volunteers

In IBS patients, analysis of the POMS questionnaire did not reveal any significant differences in fatigue (data not shown), anger (data not shown) and depression (Figure 12C) between fructans and glucose after correcting for the different time points. Mean scores over all time points however, were significantly higher for anger (fructans: 6.53 ± 0.46 VAS; glucose: 5.41 ± 0.34 VAS; $p = 0.0037$) and depression (fructans: 13.55 ± 2.00 VAS; glucose: 9.13 ± 0.67 VAS; $p = 0.0334$) following fructan infusion. A significant decrease from baseline was detected for vigor following fructan infusion ($p = 0.0151$) (Figure 12A). Furthermore, there was a trend for lower vigor scores following fructans compared to glucose in the patient population after correcting for the different time points ($p = 0.0553$). As shown in Figure 12B, IBS patients started the experiment with an increased tensed feeling, but this tension seemed to reduce over time (fructans: $p = 0.0585$; glucose: $p = 0.0083$). Mean tension scores over all time points were significantly greater for fructans (fructans: 18.69 ± 2.15 VAS; glucose: 10.95 ± 1.20 VAS; $p < 0.0001$), but did not differ significantly between the two tested solutions after correcting for the different time points.

Mean scores for fatigue, anger, tension and depression were significantly higher in IBS patients compared to healthy controls for both fructans and glucose (p -values < 0.0001). Mean values of vigor were significantly lower in patients compared to healthy volunteers following fructan infusion ($p = 0.0002$) and glucose infusion ($p = 0.0428$).

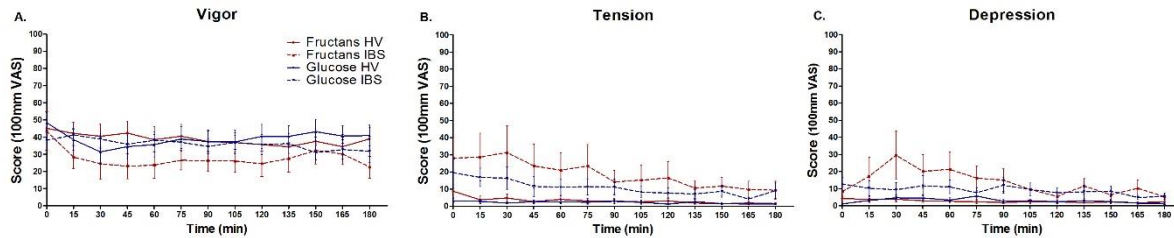


Figure 12: Vigor, tension and depression scores following fructans and glucose infusion in healthy volunteers (HV) and patients (IBS). Vigor was significantly lower in patients compared to healthy controls following fructans and glucose infusion ($p = 0.0002$ and $p = 0.0428$, respectively). Compared to healthy volunteers, patients started the experiment with increased levels of tension ($p < 0.0001$), which decreased over time. Mean depression scores were significantly greater in patients than in healthy controls both for fructans and glucose ($p < 0.0001$).

3.2.5.2 SAM

Healthy volunteers

Average scores for valence in the healthy volunteers stayed above the neutral score of five throughout the experiment for all solutions. Scores tended to increase towards the end of the experiment for the glucose solution. For the three FODMAP solutions, valence scores rather showed a slight drop during the first 30 minutes of the experiment, where after they stayed stable until the end of the experiment. Only fructose and FODMAP mix induced a significant change in valence scores compared to baseline (fructose: $p = 0.0043$, FODMAP mix: $p = 0.0137$). Although the three FODMAP solutions did not induce a significant difference in valence compared to glucose after correction for the different time points, fructose and FODMAP mix showed a trend towards lower mean valence scores (fructose: $p = 0.053$, FODMAP mix: $p = 0.0887$). Differences in mean valence scores were found for fructose compared to the other three solutions when analyzed over all time points (Table 8).

No significant differences were found for arousal in healthy volunteers following the different solutions after correction for the different time points. However, mean arousal scores over all time points were significantly greater for the FODMAP solutions compared to glucose (Table 8). Mean arousal scores varied between 3.5 and 5 (neutral score) throughout the experiment for all solutions. Dominance scores decreased significantly from baseline following the FODMAP mix ($p = 0.0009$), but not for the other solutions. Furthermore, after correction for the different time points, FODMAP mix induced significantly lower scores for dominance compared to the glucose solution ($p = 0.0063$). Mean scores for dominance over all time points differed significantly between all four solutions, except between fructans and fructose (Table 8). Average dominance scores stayed around the neutral score of five or higher for all solutions during the entire experiment.

Table 8: Mean SAM-scores over all time points for the four challenge solutions in healthy volunteers. Significant *p*-values are marked in bold.

	Valence		Arousal		Dominance	
	Mean ± SEM	<i>p</i> -value	Mean ± SEM	<i>p</i> -value	Mean ± SEM	<i>p</i> -value
Fructans vs.	6.28 ± 0.06		4.50 ± 0.08		5.55 ± 0.04	
Fructose	5.95 ± 0.07	0.0023	4.6 ± 0.08	0.4787	5.6 ± 0.05	0.4655
FODMAP mix	6.24 ± 0.06	0.5748	4.57 ± 0.09	0.5506	5.11 ± 0.05	< 0.0001
Glucose	6.30 ± 0.08	0.6494	4.27 ± 0.04	0.027	6.01 ± 0.02	< 0.0001
Fructose vs.						
FODMAP mix		0.0002		0.793		< 0.0001
Glucose		0.0158		0.0024		< 0.0001
FODMAP mix vs.						
Glucose		0.6246		0.0111		< 0.0001

Patient data and comparison with healthy volunteers

Within the patient population, no significant differences were observed in valence and dominance between fructans and glucose after correction for the different time points. However, arousal was significantly lower following glucose infusion compared to fructans ($p = 0.0122$). When comparing mean scores over all time points following fructan or glucose infusion between healthy volunteers and patients, patients scored significantly less on all three moods. Mean values are summarized in Table 9.

Table 9: SAM-scores of healthy volunteers (HV) and patients (IBS). Significant *p*-values are marked in bold.

	Valence		Arousal		Dominance	
	Mean ± SEM	<i>p</i> -value	Mean ± SEM	<i>p</i> -value	Mean ± SEM	<i>p</i> -value
IBS						
Fructans	5.71 ± 0.09	0.2419	3.96 ± 0.12	0.0122	3.51 ± 0.13	0.3971
Glucose	5.74 ± 0.06		3.57 ± 0.12		5.03 ± 0.07	
Fructans						
HV	6.28 ± 0.06	0.0002	4.50 ± 0.08	0.0012	5.55 ± 0.04	< 0.0001
IBS	5.71 ± 0.09		3.96 ± 0.12		3.51 ± 0.13	
Glucose						
HV	6.30 ± 0.08	0.0002	4.27 ± 0.39	< 0.0001	6.01 ± 0.02	< 0.0001
IBS	5.74 ± 0.06		3.57 ± 0.12		5.03 ± 0.07	

3.2.5.3 PANAS

Healthy volunteers

PA and NA were measured at five different time points: (1) before the infusion, (2) after the infusion, (3) one hour after the infusion, (4) two hours after the infusion and (5) three hours after the infusion. In healthy volunteers, no significant differences were found in PA and NA between the four solutions at the different time points. Moreover, none of the solutions induced a significant change in PA or NA over the five different time points (data not shown).

Patient data and comparison with healthy volunteers

PA within patients was not significantly different between fructans and glucose at any time point (Figure 13A). Furthermore, infusion of glucose did not influence PA of the patients, so no significant differences between the different time points were detected. Fructan infusion however, did induce a significant decrease in PA from baseline ($p = 0.0027$). No significant differences in NA were observed in patients between both infusions at the five different time points. Moreover, fructan or glucose infusion did not induce a significant change from baseline in NA levels (Figure 13B).

When PA levels in IBS patients were compared with levels in healthy controls, no significant difference was found per time point for fructans (Figure 13A). However, a trend for higher NA in patients before the start of the infusion and immediately after infusion of fructans was observed (before: $p = 0.0579$, after: $p = 0.0960$) (Figure 13B), indicating that patients contend with more negative feelings than healthy controls at the start of the experiment and immediately after infusion. Furthermore, a significantly higher NA was detected in patients three hours after infusion compared to healthy controls ($p = 0.0393$). Glucose did not induce significant differences in PA, but induced significantly greater NA levels in patients than in healthy controls at all five time points (before: $p = 0.011$; after: $p = 0.0071$; 1h after: $p = 0.0027$; 2h after: $p = 0.006$; 3h after: $p = 0.006$).

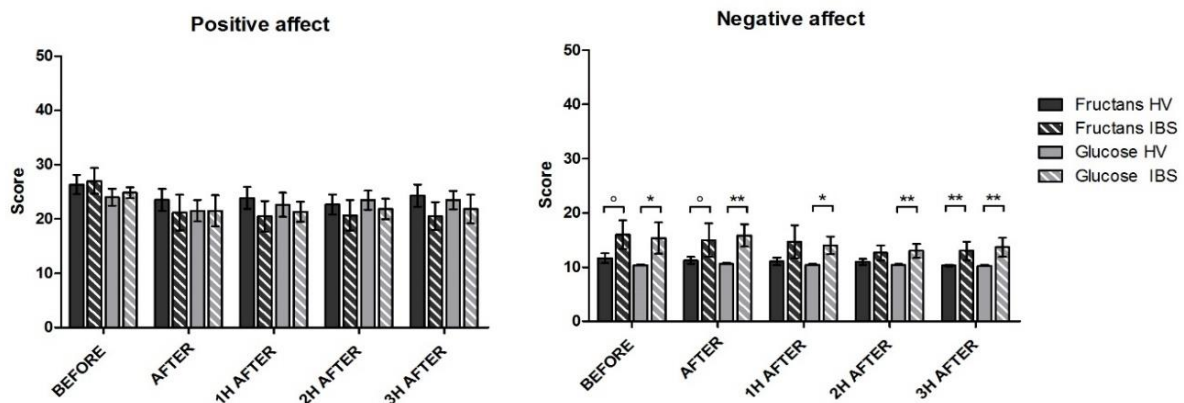


Figure 13: Positive and negative affect (PA and NA) in healthy volunteers (HV) and patients (IBS). Fructans induced a significant decrease in PA in patients. Glucose did not induce a significant change from baseline in PA. NA was significantly higher in patients compared to HV three hours after fructan infusion. Glucose induced elevated levels of NA in patients compared to healthy controls at all five time points. * $p < 0.05$, ** $p < 0.01$, ° $0.1 < p < 0.05$.

3.2.5.4 STAI

Healthy volunteer and patient data

State-trait anxiety was assessed at the same time points as the PANAS questionnaire. In healthy volunteers, state-trait anxiety scores were not significantly different between the four solutions at the five different time points. Moreover, none of the solutions induced a significant change in state-trait anxiety over the time of the experiment. The same results were found in the patient population. Furthermore, no significant differences between healthy volunteers and patients were observed for state-trait anxiety at any time point (data not shown).

4 Discussion and conclusion

FODMAPs are important triggers of functional gut symptoms by inducing luminal distention via a combination of osmotic effects and gas production in the intestines. However, the role of FODMAPs in upper GI physiology and symptom generation has never been described to date. In addition to GI symptoms, FGIDs such as IBS are often associated with extra-intestinal symptoms, indicating the presence of a communication pathway between the gut and the brain. The present study was designed to investigate the gastric response and symptom generation after different FODMAP (fructans, fructose, FODMAP mix) challenges, in comparison to a glucose control, delivered intragastrically in healthy volunteers and IBS patients. In a second aspect of the study, the putative impact of these FODMAP solutions on emotion and mood were assessed.

4.1 Healthy volunteers

The data of the current study reveal that in healthy volunteers, fructans induce a significantly lower GA response compared to the other three solutions. However, no significant difference in tolerance of the infused nutrient volume was found between the four solutions. Additionally, after recovery of the IGP drop, the fructan solution is associated with a significantly higher IGP during the three hours post infusion compared to the other three solutions. Regarding symptom induction, some significant differences were observed when comparing mean scores over all time points. The three FODMAP challenges induced significantly higher scores for flatulence and bloating compared to glucose. Furthermore, an increase in cramps was reported following fructose and FODMAP mix infusion. However, these differences lost their significance when a more complex mixed models analysis (that corrected for the different time points) was applied, and this is at least in part attributable to the current sample size. Differences in psychological symptoms were revealed for fatigue after infusion of fructans and for vigor following fructose and FODMAP mix compared with the glucose solution. However, significance was also reversed when using the mixed models statistical analysis.

4.1.1 Possible mechanisms for acute effect of fructans on gastric physiology

Gastric accommodation is a vagovagal reflex pathway. The vagal afferents, also called vagal mechanoreceptors, are localized in the muscle wall of the stomach and are activated by physiological levels of distention of the stomach. This information is transferred to the CNS where vagal efferents are activated. This will lead in turn to the relaxation of the fundic muscles (119). A first concept that comes to mind in explaining the differences in behavior of fructans in the stomach could be that fructans cause less activation of these mechanoreceptors and therefore are associated with impaired accommodation.

A fact to consider is that there was a considerable difference in concentration and osmolality between the fructan solution and the other three solutions. A previous study of Vist *et al.* reported that the osmolality and carbohydrate content affect gastric emptying. More specifically, a lower carbohydrate content and a lower osmolality are associated with faster gastric emptying (120). Another study however, reported that osmolality between the range of 243 to 374 mOsm/kg had no effect on gastric emptying rate (121). In our study design, the osmolality of the fructan solution (66 mOsm/kg) is considerably lower than the osmolality range tested in the aforementioned study, whereas osmolarities of the other solutions were higher. Consequently, the emptying time is probably shorter for fructans and less fructans are stored in the stomach. This may lead to a reduced activation of tension and stretch mechanoreceptors and therefore less activation of the vagovagal reflex pathway of GA. Testing this hypothesis requires studies with simultaneous measurement of gastric emptying.

A second hypothesis that could explain why fructans behave differently very rapidly after their infusion in the stomach is nutrient sensing. Throughout the GI tract, different nutrient sensing receptors, such as sweet taste receptors, amino acid taste receptors, fatty acid taste receptors and bitter taste receptors can be found (122). However, it seems likely that only receptors that are located into the stomach can help to explain the acute effect of fructans on gastric relaxation. Although activation of tension-sensitive mechanoreceptors is considered the most important factor in inducing GA, chemosensory receptors also play a role. For example, it has previously been demonstrated that intragastric infusion of a bitter compound inhibits GA to infusion of a nutrient drink (123). Bitter compounds are sensed in the stomach by the TAS2R, a G-protein coupled receptor (GPCR). Their effect on GA involves release of Ca^{2+} from intracellular and extracellular stores, leading to contraction of the gastric muscles (123). Sugars on the other hand are known to bind to the G-protein coupled sweet taste TAS1R. Therefore it seems unlikely that fructans and other sugars would exert their actions through the bitter taste receptor. In terms of putative sweet taste receptor involvement, a possible line of thinking might be that although they bind to the same receptor, fructans activate different signaling pathways than the other tested carbohydrates, leading to less pronounced accommodation. It is not unlikely that other signaling pathways may be activated by different ligands of the same receptor. This phenomenon has already been described for artificial sweeteners and natural sugars. These two compounds bind to the same receptor, but stimulate the production of different secondary messengers through the activation of different signaling pathways (124). Another possibility is that there is an unexplored receptor mechanism involved in the actions of fructans in the stomach, of which the mechanism of action resembles that of the bitter taste TAS2R receptor.

Nutrient sensing by taste receptors is suggested to modulate hormone secretion in the gut (125). Subsequently, another possible mechanism involved can be fructan mediated stimulation of the release of hormones that inhibit the accommodation reflex. There are various hormones which affect the stomach, but only two have been shown to lead to gastric contraction: ghrelin and motilin (126). Ghrelin is an acetylated peptide hormone of the motilin family and is produced preprandially and mainly by fundic cells in the stomach (127). It exerts its various functions, including stimulation of appetite, food intake and increasing gastric tone, through the growth hormone secretagogue (GHS) receptor located in the ENS, on the vagus nerve and in the hypothalamus and the pituitary (127, 128). A study of Ang *et al.* has shown that infusion of ghrelin was associated with an impaired accommodation reflex, but not with early satiation (128). This is noteworthy, because in normal conditions, an impaired GA is associated with enhanced satiation (129). Therefore, if fructans exert their effect through ghrelin, the findings of Ang *et al.* help explain the less pronounced GA after infusion of fructans in the absence of a significant difference in food intake between fructans and the other three solutions found in the current study. Given ghrelin levels decrease upon food intake (130), fructans might induce a decreased drop in ghrelin levels compared to the other carbohydrates due to differences in caloric content, osmolarity or carbohydrate content. However, it is unlikely that this mechanism solely underlies the differences found in the current study.

The second hormone stimulating contraction of the gastric smooth muscles is motilin. Similar to ghrelin, motilin is also a peptide hormone. It is produced by the M-cells of the small intestine in the fasted state (131). Plasma levels of motilin peak together with the start of phase III contractions of the MMC (31). A study of Coulie *et al.* previously showed that two pathways are involved in the motor effects of motilin receptor activation on interdigestive GI motility. Activation of smooth muscle motilin receptors induces contractions of gastric muscles. On the other hand, activation of neural motilin receptors on cholinergic neurons is involved in phase III induction (132). The secretion of motilin is inhibited by the presence of food in the duodenum (131). Similarly to what is suggested for ghrelin, a decreased drop in motilin levels following fructan infusion might explain the different behavior of fructans on gastric physiology. Again this different action on motilin secretion might be attributed to osmolarity, concentration, caloric or molecular differences between the FODMAP solutions.

Reversely, another suggestion could be that fructans have a reduced effect on secretion of hormones that relax the gastric muscles, such as glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), secretin and gastrin. GLP-1 is produced by the L cells in the small intestine and colon in response to meal ingestion. CCK is secreted by the I cells and secretin by the S cells in the duodenum and the jejunum. Lastly, gastrin is produced in the stomach antrum and proximal duodenum by G cells (131). These four hormones are known to stimulate gastric relaxation (133-136). Consequently, if ingestion of fructans leads to a reduced production and secretion of these hormones, either because of the concentration and/or osmolarity differences or because of

molecular differences between the FODMAPs, this may lead to impaired GA. However, this theory is less likely for gastrin and CCK, since the secretion of these hormones is mainly stimulated by proteins and fat respectively (22).

Notably, although numerically differences in nadir IGP were observed, with the least pronounced maximal drop in IGP associated with the fructan solution, no significant differences in nadir IGP between the four solutions could be found. This can be explained by the current size of the study population and by the variability in nadir IGP between the different healthy volunteers per tested solution. The observation of significant differences in mean IGP for fructans compared to the other solutions, in the absence of significantly different nadir IGP, can be explained by the fact that statistical analysis was performed in Prism, where no time correction was implemented. This further highlights the need for an increased sample size.

4.1.2 Possible mechanisms for the delayed effect of FODMAPs on gastric physiology

Some of the mechanisms discussed above, for example the alteration in secretion of peptide hormones, might also play a role in the prolonged actions of FODMAPs on gastric physiology and therefore could explain the observed continuous increase in IGP after recovery for all solutions, and the elevated IGP associated with fructans. Another potential mechanism underlying these findings could be the occurrence of duodenogastric reflux, with increased reflux following fructan infusion. A study of Koek *et al.* reported physiological duodenogastric reflux after a meal in healthy volunteers (137). This reflux of bilirubin-containing fluid from the duodenum into the stomach is caused by enhanced retrograde duodenal motor activity and an increase in fluid content after a meal. In the present study, motor activity of the small intestine was not measured, so therefore future work is needed to test this as a potential hypothesis to explain the postprandial increase in pressure.

The elevated IGP associated with fructans throughout the experiment could be induced by a combination of gastroduodenal coordination, where by gastric relaxation is determined by distention of the small intestine, and colonic distention. De Ponti *et al.* showed that duodenal distention induces gastric relaxation via a vagal enterogastric reflex pathway (138). Furthermore, these researchers found that this mechanism is nutrient and region specific. Carbohydrate infusion into the proximal intestine was not shown to affect gastric relaxation, whereas distal intestinal infusion of carbohydrates was able to decrease gastric tone (139). Additionally, similar crosstalk between the colon and the stomach is reported. Distention of the colon induces a decrease in gastric tone through a vagal mediated reflex pathway (138). We can hypothesize that infusion of the fructan solution is associated with a less prominent enterogastric response and therefore increased pressures because of its lower carbohydrate content compared to the other test solutions.

4.1.3 Mechanisms behind symptom induction

Overall, symptom scores in healthy volunteers stayed relatively low throughout the experiment. However, differences are found for flatulence following all three FODMAP solutions compared to glucose. The highest mean scores were reported following fructose infusion, although this did not survive corrections for multiple testing at the current sample size. These observations of increased flatulence are in line with the results of a study of Ong *et al.*, where an increased gas production was reported after consumption of a high FODMAP diet (HFD) compared to a low FODMAP diet (LFD) (15). Furthermore, all FODMAP solutions induced elevated scores for bloating and fructose and the FODMAP mix are associated with significantly increased cramps. These symptoms are induced by the molecular characteristics of FODMAPs. FODMAPs are carbohydrates that are poorly absorbed in the human body. During their passage through the intestines, unabsorbed FODMAPs undergo rapid fermentation by gut bacteria. This process produces H₂ and CH₄ gases. Additionally, FODMAPs are osmotically active. They attract water into the intestinal lumen, increasing the liquidity of luminal contents. Together these two processes cause luminal distention which can trigger several symptoms such as cramps, bloating and flatulence (16). For the other symptoms assessed, scores stayed very low throughout the experiment.

The human body lacks hydrolases for the breakdown of fructans, so they are always completely malabsorbed. Therefore, we expected the highest symptom scores for fructans. Moreover, Shepherd *et al.* reported numerically higher symptom scores for fructans than for fructose in patients with IBS (69). Surprisingly, this is not what we observed in the healthy volunteers. According to the results of the current study, fructose had a higher potency of inducing symptoms than the other FODMAP solutions. A possible explanation for these results could be the occurrence of fructose malabsorption (FM) among the healthy volunteers. FM is not rare in the healthy state, occurring in approximately 40% of the population (69). A way to detect FM is via hydrogen and/or methane (H₂ or CH₄, respectively) breath testing. Bacterial fermentation of the unabsorbed carbohydrate is the only source of H₂ or CH₄ in the human body. For the breath test, fructose is administered orally where after the person is asked to exhale in a machine that calculates the H₂ or CH₄ concentration in the expired air. Depending on the rise in breath H₂ or CH₄ from baseline, the degree of malabsorption of the carbohydrate is able to be correlated (140, 141). Unfortunately, participants of this study were not tested for FM. Consequently, there is a possibility that some of the healthy volunteers poorly absorbed fructose leading to higher symptom scores for some of the evaluated symptoms. When considering symptom scores per healthy participant following fructose challenge, only one participant scored consistently high on the majority of the symptoms. Four participants reported considerably elevated scores for bloating and cramps, however, which participants reported these symptoms differed between both symptoms. In total, seven healthy volunteers were associated with elevated levels of at least one of the assessed symptoms. As a consequence, we can conclude that there is a clear heterogeneity in symptom scores within the

healthy population, suggestive for a high-symptom group as evidence for FM. Nevertheless, breath testing can be used to determine definitely the occurrence of FM in the healthy population.

Another factor that could explain the found results could be the notably lower concentration of fructans (38g/L) compared to the fructose solution (100g/L). These concentrations were chosen based on previous studies (69, 113) and to be representative of serving sizes and quantities naturally found in the diet (142-144). The amount of fructans delivered into the intestines were therefore purposely designed to be lower than the amount of fructose. These differences in properties of the solutions could also contribute to differences in symptom scores between the four solutions.

A symptom that was not assessed by one of the questionnaires, but which was frequently reported by the healthy volunteers following one of the three FODMAP solutions was diarrhea. This symptom is also induced by the increased water content in intestinal lumen through the osmotic effect of FODMAPs. For further studies, scoring on VAS for diarrhea would allow objective measurement, since it was a prominent symptom after ingestion of FODMAPs.

4.1.4 Psychological questionnaires

The POMS questionnaire did not reveal significant differences in fatigue, vigor, anger, tension or depression between the four solutions after correction for different time points. However, there was a trend for reduced fatigue following the fructan infusion compared to the other three solutions, mainly during the initial two hours of the experiment. This is in contrast with the results of Shepherd *et al.*, which demonstrate numerically higher, although not significantly higher scores in fatigue after the fructan infusion compared to glucose and fructose (69). Also Ong *et al.* did not find a significant difference in lethargy between the LFD and the HFD (15). This demonstrates the need for a larger study population and also for more extensive studies on the effect of FODMAPs on fatigue.

Valence decreased significantly from baseline following fructose and FODMAP mix in healthy volunteers. Furthermore, there was a trend for lower scores of valence following fructose compared to glucose. If some of the volunteers were indeed fructose malabsorbers, the underlying mechanism behind the drop in valence could be an abnormal availability of tryptophan (TRP), the precursor to serotonin. Ledochowski *et al.* previously reported an association between fructose malabsorption and lower TRP levels and serotonin availability. They concluded that these low TRP levels may be involved in depressive symptoms, such as loss of pleasure/valence (145). However, concerning our results, a few considerations regarding this hypothesis need to be taken into account. First, if indeed this mechanism was involved, one would expect a similar increase in depression scores after fructose and FODMAP mix ingestion. However, depression scores assessed by the POMS questionnaire remained almost at minimal throughout the experiment for all solutions. Furthermore, no significant change in NA was observed. Secondly, a study of Williams *et al.* reported an initial decrease in plasma TRP levels in healthy volunteers approximately two

hours following acute TRP depletion (ATD), and nadir TRP at approximately six hours post ATD (146). This implies that if fructose challenge has similar acute effects on TRP as ATD, the decrease in TRP is probably not acute enough to explain our findings. Alternatively, if decreased TRP levels are a prolonged effect of FM, this would likewise not explain the significant drop in valence scores, as it would be associated with consistently lower TRP levels. As a consequence of these considerations, we cannot say definitely that TRP depletion plays a role in our findings and that our observations reveal a real difference in valence rather than a coincidental dissimilarity. A larger study population is needed to better estimate the effect of FODMAPs on valence. In addition, breath testing is needed to determine whether fructose malabsorption occurs among the healthy volunteers.

The overall conclusion of the results of the psychological questionnaires in healthy volunteers is that the differences in psychological symptoms between the four solutions remains very limited. This is not surprising, since only a small number of healthy participants were included and they had no previous psychological problems. Furthermore, there is minimal or no disruption of the BGA in the healthy population as there is in the IBS patient group (discussed below).

4.2 IBS patients

Although patient numbers in this pilot study are limited, some interesting results are already observed regarding GA, symptom induction and effect of FODMAPs on emotional state. A first surprising finding of the current study is the reversed effect of fructans on GA in patients compared to healthy volunteers. Patients seem to have a much more pronounced accommodation reflex in response to fructan infusion compared to glucose infusion, where after IGP recovers to similar levels as measured following glucose infusion. Similar to what was seen in healthy volunteers, IGP tended to increase over time following both fructans and glucose. With respect to symptom induction, fructans induced considerably more flatulence and cramps than glucose in the patient population. Mean scores for bloating, nausea, belching, flatulence, cramps and pain were significantly increased in patients compared to healthy controls for both solutions. Furthermore, differences in emotional state and mood between patients and healthy volunteers were revealed, whereas significant differences between fructans and glucose in patients stayed limited.

4.2.1 Possible mechanisms for the acute effect of fructans on gastric physiology in IBS

The impaired GA during fructan infusion observed in healthy volunteers, was not present in patients. In addition, fructans seem to have the complete opposite effect in IBS patients than in the healthy population, inducing a significantly greater accommodation response in the former group. Furthermore, the underlying mechanism behind this exaggerated relaxation reflex in IBS must be specific for fructans, as glucose infusion was not associated with prominently different GA compared to healthy controls. In accordance with this reasoning, we can exclude that the action of

fructans on gastric physiology are mediated by hypersensitive gastric mechanoreceptors in IBS patients. If mechanoreceptor hypersensitivity was involved, GA should also be increased during glucose infusion, since volume infused did not significantly differ between the two solutions. However, this was not observed in the current study. Several studies have reported an association between nausea and gastric relaxation (147-149). Although our results show elevated levels for nausea in IBS patients during fructan infusion compared to glucose, this difference was not significant during the initial 15 minutes of the experiment (when GA occurs). This may be too quick to draw direct causal effects for the GA discrepancies seen between fructan and glucose infusion in the patient population. Also, the osmolarity, concentration or caloric dissimilarities cannot explain the differences in GA. As previously stated, a lower osmolarity and carbohydrate content is associated with faster gastric emptying (120), and therefore a decreased gastric storage. Alternatively, supposing that gastric emptying can be impaired in IBS patients (150), leading to increased gastric storage of the infused solution, differences would thought to be present for glucose also.

Nutrient sensing is a credible factor to be involved in GA discrepancies. If indeed fructans bind to TAS1R but activate different signaling pathways and secondary messengers, a potential explanation could be that this mechanism is impaired or even reversed in IBS by either changes in the activated signaling pathways, or in the secondary messengers. Alterations in the function, expression or conformation of the TAS1R itself are less likely, as this would also have an effect on gastric responses to glucose. Another line of thought cited for the observations regarding GA in healthy volunteers was the presence of a hitherto unidentified receptor mechanism for fructans. If such a receptor is expressed and responsible for impaired accommodation in healthy volunteers, a mutation or a lack of this receptor could explain the observations in patients.

The hormonal hypothesis described for healthy volunteers can also be applied to explain the patient results. As previously stated, ghrelin and motilin both induce contraction of the gastric muscles. Hypersensitive fructan sensing, followed by a rapid and exaggerated drop in ghrelin and motilin secretion, might explain the greater GA found in IBS patients following fructan infusion. In addition, it can be suggested that GLP-1, secretin, CCK and/or gastrin release, however impaired in healthy volunteers, may be hypersensitive for fructans in IBS patients, leading to an altered accommodation reflex following fructan challenge.

Lastly, bacterial overgrowth, commonly reported in IBS, could help explain the greater GA following fructan infusion. When bacterial overgrowth is present, fructans are already fermented in the small intestine (87). As a consequence, increased luminal distension of the small intestine is induced, leading to a greater GA via the enterogastric reflex pathway described above. This hypothesis is applicable in the current study, since all solutions were liquid, and therefore have a fast gastric emptying rate.

4.2.2 Possible mechanisms for late effect of FODMAPs on gastric physiology in IBS

The current study shows that IGP following fructan infusion reaches similar levels to glucose infusion after a considerably more prominent GA. Furthermore, similar to what was observed in healthy volunteers, IGP increases over time following both fructan and glucose infusion. These two observations can again be explained by duodenogastric reflux, as indicated above. A potential explanation for the equalization of IGPs after distinguishable GA between both solutions is that fructans induce an increased reflux of bilirubin-containing fluid to the stomach compared to glucose. Additionally, gastroduodenal and colo-gastric reflex pathways described above can also play a role in the same way as described for healthy volunteers.

4.2.3 Mechanisms behind symptom induction

IBS is characterized by visceral hypersensitivity, the excessive perception of physiological gut stimuli (68). A study of Posserud *et al.* reported that altered rectal perception is associated with GI symptom severity in IBS (151). In addition, visceral hypersensitivity has also been reported in other parts of the GI tract, such as the small intestine or the colon (75, 152). The present study reports higher scores for bloating, nausea, belching, flatulence, cramps and pain in patients compared to healthy volunteers, implying an increased visceral hypersensitivity in the patient population. This increased visceral perception can be facilitated by abnormalities at different levels in the communication between the gut and the brain: at the level of mechanoreceptors, afferent pathways or the CNS. Another mechanism that might be involved in visceral hypersensitivity is the increased release of inflammatory mediators by mast cells, activating mechanoreceptors and nerve endings (153). As a result of this visceral hypersensitivity, patients tend to react more to luminal distention, caused by the osmotic effect of FODMAPs and gas production following FODMAP fermentation described above, leading to higher symptom scores.

Surprisingly, these elevated symptom levels were not only reported following fructan infusion, but also for the control solution, glucose. Notably, in this study, symptom scores in general and especially for belching, flatulence, cramps and pain following glucose infusion were elevated by particularly high scores in one of the patients. The other IBS patients reported only minor symptoms after glucose infusion. This highlights the need for further investigation and the inclusion of more patients into the study to produce more accurate results. Furthermore, an important aspect to take into account in clinical practice is the nocebo effect, the counterpart of the placebo effect. It has been demonstrated that the psychological state of the patient influences the intensity and severity of symptoms that are experienced (154). On recruitment, patients were informed about the tested carbohydrates and their effects in IBS. Although they were blinded to the identity of the solution infused, they were mentally prepared and had a negative expectation of experiencing symptoms. This mind-set might explain the increased symptom scores after glucose infusion. In addition, IBS

patients are much more aware of what they can and cannot eat to avoid symptoms, making them more biased (65). These two factors together enable the occurrence of a placebo effect in these patients, which explains the increased symptom scores in patients following glucose infusion.

4.2.4 Psychological questionnaires

We observed increased levels of fatigue, anger, depression, tension and NA and decreased levels of vigor, arousal, valence and dominance in patients compared to healthy volunteers for the fructan and glucose conditions. This indicates that IBS patients are characterized by a more negative psychological state compared to healthy controls, supporting the placebo effect described above, and also confirming the well-known association between IBS and psychological disorders, such as depression and anxiety (155-157). This highlights the involvement of the CNS in the pathophysiology of IBS. An important factor in this association is 5-HT, a neurotransmitter in the brain. 5-HT plays a role in the regulation of mood and appetite. Dysfunction of the 5-HT system has been implicated in psychological and gut disorders (37). The central differences between patients and healthy volunteers reported in our results may also form a basis for the symptomatic differences found between these two groups.

One of the aims of this study was to investigate the role of FODMAPs on gut-brain signaling. Fructan infusion led to a significant decrease from baseline for vigor and PA in patients. This decrease in vigor may be explained by the reported increase in symptoms. When symptom intensity rises, people feel less comfortable and less powerful. PA was measured by assessing different items: attentive, interested, alert, excited, enthusiastic, inspired, proud, determined, strong, and active. The sum of these scores decreased after fructan infusion. Furthermore, following fructan infusion, increased arousal compared to glucose and elevated NA compared to healthy controls were observed, indicating that fructan ingestion might exert central effects, changing mood and emotion. However, as the sample size of the patient population was limited, further research and additional specific and sensitive measures are needed to confirm these results and to identify the mechanism involved.

4.3 Limitations of the study

The most important limitation of the current study is the small number of IBS patients. As a consequence, statistical analysis could not be accurately performed, and rather showed trends or non-significant results instead of any strong significances. Therefore, patient results must be interpreted with caution and retested when more patients are included to provide more accurate results. Furthermore, it must be noted that patients were recruited via an advert in a local newspaper and as a result, the patients recruited tended to have more of a somewhat 'mild' severity of IBS (158). This could explain why only limited differences in psychological symptoms were reported in this study.

Another limitation could be the variance in osmolarity between the four solutions. In particular, the fructan solution's osmolarity was considerably lower than the osmolarity of the other three solutions. It has already been stated that osmolarity has an effect on gastric emptying time. Consequently, it might also affect the GA reflex. To ensure that osmolarity differences are not a significant confounder for discrepancies found in GA in healthy volunteers, the gastric effect to FODMAP infusion should be retested with similar osmolarity for all solutions, or simultaneous measurement of gastric emptying should be added to study its impact as a co-variable.

4.4 Clinical implications and future research directions

The results of the current study show that FODMAPs are involved in upper GI symptom generation and meal induced aggravation in healthy volunteers and IBS patients. Dietary exclusion or limitation of these carbohydrates continues to be a good strategy to help IBS patients manage their GI symptoms. Future research should be directed towards unraveling the neural, sensory or hormonal mechanisms involved in the differences in gastric relaxation reflex following fructan infusion in healthy volunteers and patients. Furthermore, the effects of other FODMAPs on gastric response remain unknown. Limited patient numbers in this study kept us from accurately identifying the effect of FODMAPs on mood and emotion in IBS patients. In future studies, patient numbers must be expanded in order to evaluate if FODMAP intake has an effect on the BGA. Moreover, upper GI effects and actions on the BGA of FODMAPs in FD patients remain unexplored. Nevertheless, these findings contribute to evidence supporting the low FODMAP approach in the dietary management of specific upper GI symptoms in IBS. In addition, they highlight the potential role of specific foods in the signalling and modulating of emotions. Further data in this study will help our incomplete understanding of the heterogeneity of IBS (and other FGIDs) and may lead to the development of specific therapeutic agents.

4.5 Conclusion

In conclusion, gastric responses to FODMAP infusion can vary between different FODMAP types. We have demonstrated that fructans induce a reduced GA in healthy volunteers compared to the other FODMAPs and an exaggerated GA in patients. The exact mechanisms underlying these gastric response to fructans remain unknown. Fructose was associated with the highest symptom scores in the healthy study population. Osmolarity differences might contribute to some of these reported differences. Lastly, the patients in this study were characterized by a negative psychological state, which might have an influence on symptom perception. However, the number of patients in this study was limited. Therefore, results must be interpreted with caution and further research is needed.

Korte samenvatting

Functionele gastro-intestinale aandoeningen vormen een heterogene groep van maagdarmaandoeningen en komen voor in een groot deel van de populatie. Ze worden gekenmerkt door een variabele combinatie van chronische of terugkerende gastro-intestinale klachten in de afwezigheid van structurele problemen (2). De diagnose van deze aandoeningen is gebaseerd op symptomen die met de aandoening gepaard gaan (45).

Prikkelbare darmsyndroom (PDS) is een van de meest prevalentie functionele gastro-intestinale aandoeningen en komt voor in 10–15% van de bevolking (5, 68). Deze aandoening wordt gekarakteriseerd door abdominale klachten en een verandering in de stoelgang in afwezigheid van een structurele oorzaak die deze symptomen kan verklaren (5). Andere klachten die geassocieerd worden met PDS zijn diarree of constipatie, opgeblazen gevoel, winderigheid, urgente ontlasting en het gevoel van onvolledige ontlasting (6). Er bestaan drie klinische varianten van PDS: diarree dominante PDS, constipatie dominante PDS en het gemiddelde type (70). PDS heeft niet alleen een impact op de kwaliteit van het leven, maar brengt daarnaast ook een belangrijke kost met zich mee, zowel voor de patiënten als voor de samenleving, door een afname in productiviteit en een toename in afwezigheid op het werk, aantal consultaties en onnodige testen (68, 69).

Hoewel onderzoek rond PDS de laatste jaren sterk uitgebreid is, is de pathofysiologie ervan nog steeds niet volledig ontrafeld. Naast abnormale gastro-intestinale motiliteit (72) en viscerale hypersensitiviteit (68) spelen voeding (82) en psychologische factoren (77) een belangrijke rol in de pathogenese van PDS. Fermenteerbare Oligosaccharide, Disaccharide, Monosaccharide en polyolen (FODMAPs), een groep van natuurlijk voorkomende korte-keten koolhydraten, werden recent geïdentificeerd als actoren in symptoomontwikkeling in PDS (16) omwille van hun malabsorptie in het maagdarmsstelsel. Hierdoor zijn ze beschikbaar voor bacteriële fermentatie, met de productie van gasen zoals waterstof- en methaangas als gevolg. Verder hebben FODMAPs ook osmotische effecten, waardoor de waterinhoud in de darmen toeneemt. Deze twee processen leiden samen tot luminale distentie, wat gastro-intestinale symptomen kan veroorzaken (14). Welke effecten FODMAPs hebben het op bovenste deel van het maagdarmsstelsel, en in het specifiek op de maagrespons, is tot op heden echter nog niet bestudeerd.

Psychologische problemen, zoals angst of depressie, komen vaak gelijktijdig voor met PDS (17) en het is vaak de combinatie van beiden die de patiënten naar medische hulp drijft (99). Een belangrijk systeem betrokken in deze link tussen functionele maagdarmaandoeningen en psychologische aandoeningen is de "brain-gut axis" (BGA) (19). De BGA is een bidirectionele connectie tussen de hersenen en het maagdarmsstelsel en bestaat uit neurale, hormonale en immuunpathways (102). Er wordt verondersteld dat dysregulatie van de BGA betrokken is in symptoomontwikkeling in functionele gastro-intestinale aandoeningen (105).

Doel van de studie

In deze masterthesis willen we de rol van FODMAPs in bovenste gastro-intestinale effecten en gastro-intestinale symptoomontwikkeling nagaan in gezonde vrijwilligers en in PDS patiënten. Hiernaast willen we meer inzicht krijgen in de effecten van FODMAPs op de gemoedstoestand en emoties in beide studiepopulaties. Onze hypothese is dat fructanen geassocieerd zijn met hogere symptoomscores dan de andere geteste FODMAPs door algemene malabsorptie, en dat hogere symptoomlevels en grotere emotionele effecten bereikt worden in patiënten.

Materiaal en methoden

Vijftien gezonde vrijwilligers en zes patiënten met PDS met een leeftijd tussen de 18 en 65 jaar namen deel aan de studie. De gezonde vrijwilligers kregen tijdens vier bezoeken, drie dagen tot twee weken gescheiden van elkaar, vier koolhydraatoplossingen gerandomiseerd toegediend: hoog in fructanen (38g/L), hoog in fructose (100g/L), een FODMAP mix (80g/L) en glucose (100g/L) als controle-oplossing (Tabel 3). De patiëntenpopulatie kreeg enkel de fructanenoplossing en glucose-oplossing toegediend onder dezelfde condities. De deelnemers van de studie waren geblindeerd voor de identiteit van de toegediende oplossing.

In nuchtere toestand werd een manometrieprobe en een sonde via de neus tot in de maag opgeschoven zodanig dat een van de 36 sensors ter hoogte van de onderste slokdarmsfincter gepositioneerd was en de probe zo ver mogelijk naar of in het duodenum zat. Na een stabilisatieperiode en wanneer de vrijwilliger in een late fase II of fase III van de hongercyclus was, werd een van de vier/twee oplossingen toegediend aan een snelheid van 60 mL/min. De infusie werd stopgezet bij volledige verzadiging. Manometrische metingen werden uitgevoerd vanaf het begin van de infusie tot drie uur na de infusie (Figuur 5). Vragenlijsten voor het beoordelen van symptoomintensiteit en psychologische toestand werden op vooraf bepaalde momenten ingevuld (Figuur 6). Data werden geanalyseerd met *t*-toetsen, ANOVA en mixed models in Prism en SAS en werden getoond als gemiddelde \pm standaard error.

Resultaten

Intragastrische drukmetingen

In de gezonde populatie werd tijdens de infusie van fructanen een kleinere daling in intragastrische druk waargenomen dan tijdens infusie van de andere oplossingen (fructose: $p = 0.0003$; FODMAP mix: $p = 0.0002$; glucose: $p = 0.0002$) (Figuur 7A). In de patiëntenpopulatie induceerden fructanen een sterker uitgesproken gastrische accommodatie reflex ten opzichte van de glucose-oplossing in patiënten ($p < 0.0001$) en ten opzichte van fructanen in de gezonde vrijwilligers ($p = 0.0003$) (Figuur 7B).

Na herstel van intragastrische drukken werd in beide studiepopulaties voor alle oplossingen een consistente toename in intragastrische druk over de tijd waargenomen (Figuur 8A en 8B). In de gezonde populatie waren fructanen geassocieerd met hogere intragastrische drukken in vergelijking met de andere oplossingen gedurende de volledige meting (Figuur 8A). In patiënten vertoonden fructanen een sterkere toename in intragastrische druk na drukherstel in vergelijking met glucose (Figuur 8B).

Gastro-intestinale symptomen

Wanneer gemiddelde scores over alle tijdstippen vergeleken werden in de gezonde populatie, was fructose geassocieerd met de significant hogere scores voor opgeblazen gevoel dan de andere oplossingen (fructans: $p = 0.0003$; FODMAP mix: $p = 0.014$; glucose: $p < 0.0001$). Fructanen en de FODMAP mix veroorzaakte hogere gemiddelde scores dan glucose ($p = 0.0055$ en $p < 0.0001$, respectievelijk). Echter, enkel fructanen veroorzaakten een significante toename ten opzichte van baseline voor opgeblazen gevoel ($p = 0.0254$) (Figuur 9D). Gemiddelde scores voor winderigheid over alle tijdstippen waren significant hoger voor alle FODMAP-oplossingen in vergelijking met glucose (fructans: $p = 0.0003$; fructose: $p < 0.0001$; FODMAP mix: $p = 0.0005$). Fructose en FODMAP mix induceerde hogere scores voor krampen in vergelijking met fructanen en glucose (p -values < 0.0001). Echter, na correctie voor de verschillende tijdstippen werd enkel nog een trend gevonden voor hogere scores voor winderigheid en krampen na infusie van de FODMAP mix in vergelijking met glucose (winderigheid: $p = 0.0995$; krampen: $p = 0.0800$)

In de patiëntenpopulatie werd geen significant verschil in opgeblazen gevoel, nausea en pijn waargenomen na toediening van fructanen vergeleken met glucose en wanneer gecorrigeerd werd voor de verschillende tijdstippen (Figuur 10). Krampen waren significant toegenomen na infusie van fructanen in vergelijking met glucose ($p = 0.0246$). Voor winderigheid werd een trend gedetecteerd voor hogere scores na blootstelling aan fructanen ($p = 0.0553$). Gemiddelde scores (over alle tijdstippen) voor opgeblazen gevoel, nausea, opboeren van lucht, winderigheid, krampen en pijn waren significant hoger in de patiëntenpopulatie in vergelijking met de gezonde vrijwilligers ($p < 0.0001$).

Psychologische toestand

Gemiddelde scores voor vermoeidheid over alle tijdstippen waren significant lager voor fructanen in vergelijking met de andere oplossingen in gezonde vrijwilligers (fructose: $p < 0.0001$; FODMAP mix: $p < 0.0001$; glucose: $p = 0.001$) (Figuur 11A). Na correctie voor de verschillende tijdstippen werd er echter geen significant verschil meer gevonden. Gezonde vrijwilligers vertoonden significant lagere scores in controlegevoel na de FODMAP mix in vergelijking met de controle-oplossing ($p = 0.0063$) (Tabel 6). Voor alle andere emoties en gemoedstoestanden geëvalueerd in deze studie werden in gezonde vrijwilligers geen significante verschillen gevonden.

De patiëntenpopulatie vertoonde een trend naar lagere scores voor kracht na de infusie van fructanen in vergelijking met glucose ($p = 0.0553$) (Figuur 12A). Omgekeerd werden voor opwinding hogere levels waargenomen na toediening van fructanen in vergelijking met glucose ($p = 0.0122$). Positief affect daalde significant na toediening van fructanen in PDS patiënten ($p = 0.0027$) (Figuur 13A). In vergelijking met gezonde vrijwilligers werden significant hogere levels voor vermoeidheid, boosheid, gespannen gevoel en depressie waargenomen in patiënten, zowel voor fructanen als voor glucose (p -values < 0.0001) (Figuur 12). Gemiddelde scores voor kracht, plezier, opwinding en controlegevoel lagen significant lager in de patiëntenpopulatie vergeleken met de controlegroep ($p < 0.05$). Negatieve gevoelens waren op verschillende tijdstippen hoger in patiënten dan in gezonde vrijwilligers, zowel na voor fructanen als glucose (Figuur 13B).

Discussie

Een eerste belangrijke bevinding van de huidige studie was de verminderde maagaccommodatie in gezonde vrijwilligers na toediening van fructanen in vergelijking met de andere oplossingen en een significant toegenomen accommodatie na toediening van fructanen in patiënten. Verschillende hypothesen kunnen aangehaald worden om het acuut effect van fructanen op de fysiologie van de maag te verklaren. Fructanen zouden kunnen zorgen voor een verminderde activatie van mechanoreceptoren als gevolg van hun lagere osmolariteit en snellere maaglediging (120). Deze hypothese kan echter uitgesloten worden in de patiëntenpopulatie aangezien het hier lijkt te gaan om een fructan-specifiek effect.

Binding van fructanen op de TAS1-receptor kan leiden tot activatie van andere signalisatiepathways en/of second messengers dan de andere koolhydraten in de gezonde populatie. In de patiëntenpopulatie zouden afwijkingen in de signalisatiepathways of second messengers aanwezig kunnen zijn, waardoor het omgekeerde effect waargenomen wordt. Een andere mogelijke hypothese is dat er een fructanenreceptor in de maag bestaat waarvan de identiteit tot op heden nog niet gekend is. Mutaties in deze receptor kunnen in PDS patiënten leiden tot een toegenomen accommodatiereflex na binding van fructanen.

Anderzijds kunnen fructanen geassocieerd zijn met een minder uitgesproken daling in de vrijzetting van ghreline en motiline na voedselinname. Beide peptidehormonen hebben een inhiberend effect op maagrelaxatie (126). Toediening van ghreline vermindert de accommodatie reflex, maar leidt niet tot vroegtijdige verzadiging (128). Dit kan de verminderde accommodatie na toediening van fructanen in de afwezigheid van een significant verschil in voedselinname helpen verklaren. Omgekeerd kan men redeneren dat de fructanenoplossing, door de lagere osmolariteit en koolhydraatinhoud, leidt tot een verminderde vrijzetting van hormonen die de relaxatie van de maagspieren stimuleren, zoals glucagon-like peptide-1 (GLP-1), secretine, gastrine en cholecystokinine (CCK). De toegenomen accommodatie die waargenomen werd in patiënten kan verklaard worden door hypersensitiviteit voor fructanen, wat leidt tot een verhoogde release van

maag-relaxerende peptidehormonen en/of een snelle afname in maag-contraherende peptidehormonen.

De stijging van de intragastische druk tijdens de drie uur na de infusie in beide studiegroepen kan verklaard worden door duodenogastrische reflux van bilirubine-bevattende vloeistof (137). De verhoogde intragastrische druk na toediening van fructanen gedurende het volledig experiment en in vergelijking met de andere oplossingen kan geïnduceerd worden door een combinatie van gastroduodenale coördinatie en colonische distentie. De hypothese die gesteld kan worden is dat fructanen geassocieerd met een afgenomen enterogastrische respons door hun lagere osmolariteit en bijgevolg hogere intragastrische drukken induceren. Hiernaast kunnen bovengenoemde concepten, zoals de gewijzigde hormoonsecretie, ook een rol spelen.

Symptomen zoals opgeblazen gevoel, winderigheid en krampen worden veroorzaakt door de bacteriële fermentatie en osmotische effecten van FODMAPs. De hogere symptoomscores na fructose en FODMAP mix infusie in gezonde vrijwilligers kunnen verklaard worden door fructose malabsorptie die mogelijks aanwezig is in (een deel van) de gezonde vrijwilligers. Echter, aangezien dit niet getest werd in de huidige studie, kan dit niet met volledige zekerheid gesteld worden. Hiernaast kunnen de lagere concentratie en osmolariteit van fructanen de lagere symptoomscores na toediening van fructanen verklaren. Viscerale hypersensitiviteit speelt een rol in de toegenomen symptoomscores in patiënten. Een verklaring voor het feit dat symptoomscores zowel verhoogd zijn voor fructanen als voor glucose kan gevonden worden in het nocebo effect, waar een negatieve psychologische instelling kan leiden tot een toename in intensiteit van symptomen. De aanwezigheid van een negatievere psychologische instelling in patiënten wordt bevestigd door de resultaten van de psychologische vragenlijsten. Hiernaast ondersteunen de psychologische resultaten de rol van de brain-gut axis in PDS.

We kunnen concluderen dat fructanen een afwijkend effect hebben op de maagfysiologie, met een afgenomen accommodatie reflex in gezonde personen en een toegenomen maagrelaxatie in patiënten. Echter, het achterhalen van de neurale, sensorische en/of hormonale mechanismen die aan de basis liggen van deze verschillen vereist verder onderzoek. Verschillen in osmolariteit en koolhydraatinhoud kunnen enkele gevonden verschillen in symptoomniveaus verklaren. Een uitbreiding van de studiepopulatie is noodzakelijk om meer accurate resultaten te bekomen en de effecten van FODMAPs op symptoomontwikkeling en psychologische toestand te kunnen achterhalen.

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Appendix 1: IBS screening questionnaire

VRAGENLIJST DARMKLACHTEN

Datum: .../.../..... Leeftijd: jaar Vrouw Man

1. PIJN OF ONGEMAK IN DE BUIK

1. Heeft U Vaak last van terugkerende pijn of ongemak in de buik?
0 Nee
1 Ja

Indien U ja antwoordde: beantwoord de volgende vragen
Indien U geen enkel hokje aankruiste: ga naar vraag 3.

2. VERLOOP VAN DARMKLACHTEN



- 2.1. Heeft U die terugkerende abdominale pijn of ongemak sedert minstens 6 maanden?
0 Nee
1 Ja



- 2.2. Heeft u de laatste 3 maanden minstens 3 dagen per maand last van pijn of ongemak in de buik?
0 Nee
1 Ja



- 2.3. Merkt U vaak verbetering na het maken van stoelgang?
0 Nee
1 Ja



- 2.3. Merkt U ook een verandering op in het aantal stoelgangen (meer of minder vaak)?
0 Nee
1 Ja



- 2.4. Merkt U ook een verandering in het uitzicht van Uw stoelgang (harder of zachter)?
0 Nee
1 Ja

3. PRIKKELBARE DARM SYNDROOM

Dit is een beschrijving van het prikkelbare darm syndroom (soms spastisch colon genoemd)

Spastisch colon is een stoornis van de darmwerking, gekenmerkt door telkens terugkerende buikpijn en ongemak, samen met veranderingen in de stoelgang, diarree, constipatie of een combinatie van de twee, typisch over maanden of jaren. Andere symptomen omvatten een opgezwollen gevoel in de buikstreek, de gewaarwording van overmatige gassen en een gevoel van onvoldoende ledigen van de stoelgang. De buikpijn is meestal erg zeurend met af en toe een pijnlijke krampaanval en verbetert door naar het toilet te gaan.

Lijdt U zelf aan een prikkelbare darm/spastisch colon zoals hierboven beschreven?

- 0 Nee
1 Ja

4. ANDERE KLACHTEN



4.1. Hebt U gedurende de laatste 3 maanden ooit bloed in de stoelgang opgemerkt?

- 0 Nee
1 Ja



4.2. Hoe vaak hebt U gedurende de laatste 3 maanden ooit pikzwarte stoelgang opgemerkt?

- 0 Nee
1 Ja



4.3. Heeft Uw dokter U verteld dat U bloedarmoede hebt of ijzertekort (bij vrouwen, niet door overvloedige maandstonden)?

- 0 Nee
1 Ja
2 Niet van toepassing (overvloedige maandstonden)



4.4. Hebt U gedurende de laatste 3 maanden Uw temperatuur gemeten en gezien dat dit boven de 38°C was?

- 0 Nee
1 Ja



4.5. Bent U de laatste 3 maanden meer dan 4 kg vermagerd zonder hiervoor dieet te volgen?

- 0 Nee
1 Ja



4.6. Indien U ouder bent dan 50, hebt U onlangs een grote verandering in het patroon van Uw stoelgang opgemerkt (verandering in hoe vaak U stoelgang maakt of hoe hard of zacht de stoelgang is) ?

- 0 Nee
1 Ja
2 Niet van toepassing (jonger dan 50)



4.7. Hebt U een ouder, broer of zus die één van de volgende aandoeningen heeft of gehad heeft?

4.7.A. Kanker van de dikke darm?

- 0 Nee 1 Ja

4.7.B. Ziekte van Crohn of Colitis Ulcerosa?

- 0 Nee 1 Ja

4.7.C. Coeliakie?

- 0 Nee 1 Ja

5. Aanvullende onderzoeken: ENKEL VOOR DE ARTS

Onderging deze patient voor zijn/haar buikklachten (aankruisen indien ja):

- 1 Colonoscopy
2 Schildklier functie test
3 Test voor coeliakie
4 Lactose tolerantie test
5 Gastroscopie
6 Echo abdomen
7 CT abdomen

Appendix 2: Symptom questionnaire

1. Honger

geen honger veel honger

2. Verwachte hoeveelheid te eten

niets zeer veel

3. Verzadiging

Helemaal niet heel erg

I. Opgeblazen gevoel

niets zeer uitgesproken

II. Volheid

niets zeer uitgesproken

III. Misselijkheid

niets zeer uitgesproken

IV. Opboeren van lucht

niets zeer uitgesproken

V. Winderigheid

niets zeer uitgesproken

VI. Krampen

niets zeer uitgesproken

VII. Pijn

niets zeer uitgesproken

Appendix 3: POMS questionnaire

Ik voel me VERMOEID:

HELEMAAL NIET |-----| HEEL ERG

Ik voel me KRACHTIG:

HELEMAAL NIET |-----| HEEL ERG

Ik voel me BOOS:

HELEMAAL NIET |-----| HEEL ERG

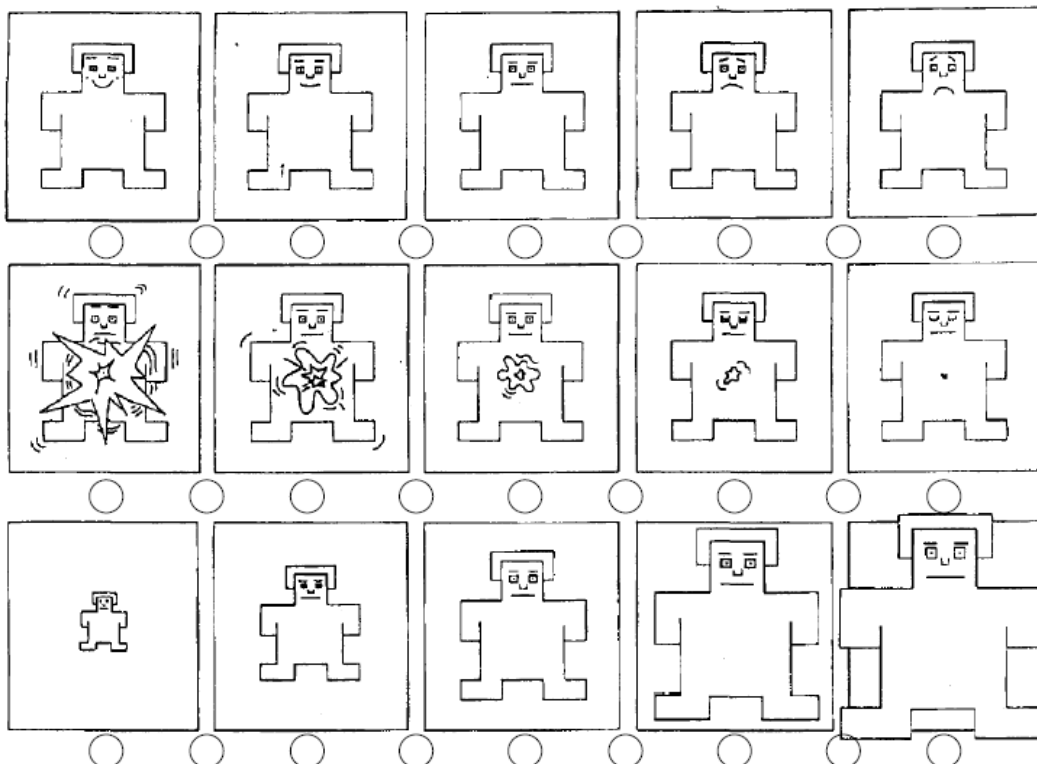
Ik voel me GESPANNEN/ANGSTIG:

HELEMAAL NIET |-----| HEEL ERG

Ik voel me NEERSLACHTIG:

HELEMAAL NIET |-----| HEEL ERG

Appendix 4: SAM questionnaire



Appendix 5: PANAS questionnaire

PANAS

Copyright Watson, Clark & Tellegen, Ndl. Vertaling: F. Peeters (Rijksuniversiteit Limburg / RIAGG Maastricht)

Instructies:

Hieronder ziet u een aantal woorden die verschillende gevoelens en emoties beschrijven. Wilt u ieder woord lezen en aangeven in welke mate u zich **momenteel** zo voelt? U kan het betreffende hokje aankruisen.

	nauwelijks of helemaal niet	een beetje	tamelijk	vrij sterk	in zeer hoge mate
1. Aandachtig	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. Vijandig	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. Geïnteresseerd	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. Prikkelbaar	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. Alert	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. Schuldig	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. Uitgelaten	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. Beschaamd	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. Enthousiast	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. Nerveus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. Geïnspireerd	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12. Rusteloos	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13. Trots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14. Overstuur	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15. Vastberaden	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16. Van streek	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17. Sterk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18. Bang	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
19. Actief	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20. Angstig	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Appendix 6: STAI questionnaire

ZELF-BEOORDELINGS VRAGENLIJST

STAI-versie DY-1, ontwikkeld door H.M. van der Ploeg, P.B. Defares en C.D. Spielberger

Toelichting: Hieronder vindt u een aantal uitspraken, die mensen hebben gebruikt om zichzelf te beschrijven. Lees iedere uitspraak door en zet dan een kringetje om het cijfer rechts van die uitspraak om daarmee aan te geven hoe u zich *nu voelt*, dus *nu op dit moment*. Er zijn geen goede of slechte antwoorden. Denk niet te lang na en geef uw eerste indruk, die is de beste. Het gaat er dus om dat u weergeeft wat u *op dit moment* voelt.

	geheel niet	een beetje	tamelijk veel	zeer veel
1. Ik voel me kalm	1	2	3	4
2. Ik voel me veilig	1	2	3	4
3. Ik ben gespannen	1	2	3	4
4. Ik voel me onrustig	1	2	3	4
5. Ik voel me op mijn gemak	1	2	3	4
6. Ik ben in de war	1	2	3	4
7. Ik pieker over nare dingen die kunnen gebeuren	1	2	3	4
8. Ik voel me voldaan	1	2	3	4
9. Ik ben bang	1	2	3	4
10. Ik voel me aangenaam	1	2	3	4
11. Ik voel me zeker	1	2	3	4
12. Ik voel me nerveus	1	2	3	4
13. Ik ben zenuwachtig	1	2	3	4
14. Ik ben besluiteloos	1	2	3	4
15. Ik ben ontspannen	1	2	3	4
16. Ik voel me tevreden	1	2	3	4
17. Ik maak me zorgen	1	2	3	4
18. Ik voel me gejaagd	1	2	3	4
19. Ik voel me evenwichtig	1	2	3	4
20. Ik voel me prettig	1	2	3	4

