The interplay between diet, gastrointestinal complaints and gut microbiota: more than a gut feeling?

Iris Rijnaarts

Propositions

1. Medical doctors should always include dieticians in the treatment of Irritable Bowel Syndrome patients.
   (this thesis)

2. Both functional bowel disorder characteristics and the gut microbiota are too variable over time to be accurately investigated by means of cross-sectional studies.
   (this thesis)

3. Collaborations between researchers and companies are essential for the implementation of science-based knowledge and applications in daily practice.

4. Communication training for scientists is essential for increasing knowledge transfer and the general public's trust in science.

5. Personal development is more important than scientific development when doing a PhD.

6. Collaborating with professionals from different disciplines requires learning to speak different languages.

7. Foods that can be grown in the Netherlands should not be imported.

8. To reduce disease and death, the government should enforce rules for a healthy lifestyle as they did with the restrictive measures during the Covid-19 pandemic.

Propositions belonging to the thesis, entitled
The interplay between diet, gastrointestinal complaints and gut microbiota: more than a gut feeling?

Iris Rijnaarts
Wageningen, 24 June 2022

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Thesis

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CHAPTER 1

GENERAL INTRODUCTION

Iris Rijnaarts

Nearly 2500 years ago, the importance of gut health was already recognized by the ancient Greek Hippocrates, who stated that *'all diseases begin in the gut'*[1]. Today, the importance of the gut is also reflected in our language, as we can 'have a gut feeling' or 'feel butterflies in the stomach'. Indeed, the link between the gut and feelings has biological foundations, as gut health is linked to mental wellbeing via the gut-brain axis, which is associated with the gut microbiota[2]. A different gut microbiota composition compared to controls has been observed in several diseases or disorders such as autism[3], Parkinson's disease[4, 5], inflammatory bowel disease[6] and functional gastrointestinal disorders (FGID)[7, 8]. FGID are the most common diagnosis in clinical gastroenterology, in which people have gastrointestinal (GI) complaints, but structural or biochemical abnormalities seem absent[9]. Research has estimated that up to 35% of the Western population has a FGID[10], which leads to an increase in health care use and costs of 35-59% compared to people without FGID[11].

For adults, FGID are classified into six categories: esophageal, gastroduodenal, bowel, abdominal pain, biliary and anorectal disorders, of which functional bowel disorders are the most prevalent[10, 12]. The most well-known disorder by the public is Irritable Bowel Syndrome (IBS), which is defined by chronic abdominal pain that is associated with a change in form and frequency of stool, and pain related to bowel movements[9]. The global prevalence of IBS is estimated around 11%, and prevalence rates are 1.5-3 times higher in women than in men[13]. IBS can be further subdivided into subtypes based on the predominant stool pattern (*e.g.* stools present ≥25% of the time), namely constipation predominant IBS (IBS-C), diarrhea predominant IBS (IBS-D), mixed IBS in which stools are alternating between diarrhea and constipation (IBS-M), and the so-called unclassified IBS in which patients do not have an altered stool pattern but do experience abdominal pain and bloating (IBS-U)[9]. Although IBS is not a life-threatening disorder, it greatly diminishes quality of life (QoL) and daily functioning, and often co-occurs with chronic fatigue, fibromyalgia, headache, anxiety and depression[14-20].

Another frequent bowel disorder is constipation, which has a global prevalence ranging between 5-20%, depending on the definition used[21-23]. Constipation is characterized by hard stools and infrequent bowel movements, and can greatly decrease QoL and increase the risk for several diseases and all-cause mortality[24-30]. Both constipation and IBS are diagnosed using the Rome IV diagnostic criteria, which are derived from a consensus by a group of experts[9]. The absence of frequent abdominal pain and bloating in constipation is in theory the main differentiator with IBS-C, illustrated in Figure 1[9]. However, studies have shown that the Rome criteria are unable to distinguish IBS-C and constipation, and patients switch between diagnoses[31, 32]. This problem occurs not solely for IBS-C and constipation, as differentiation between other functional bowel disorders is difficult due to their large symptom overlap[12]. Furthermore, research has shown that only a fraction of people

who experience constipation complaints fit the Rome criteria for constipation or IBS-C, but still experience substantial symptoms[33-35]. These people are a group that is frequently missed in research and health care.

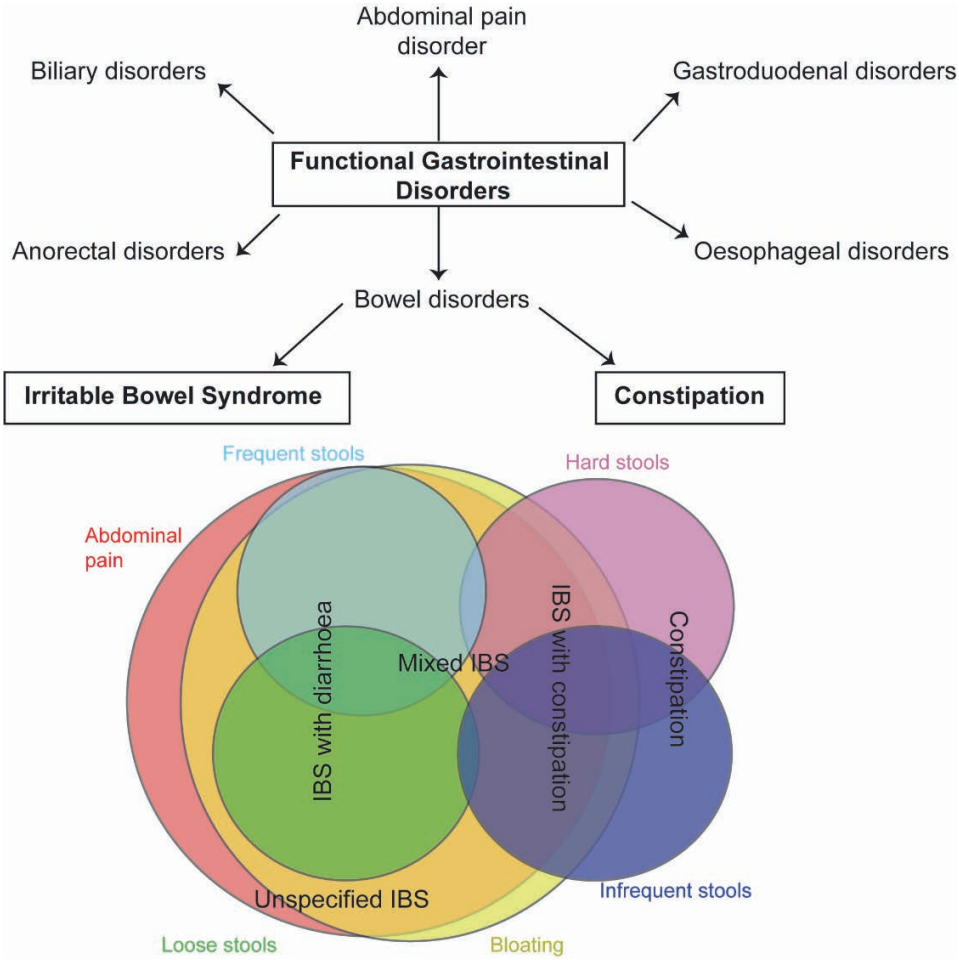

Figure 1. Overview of types and symptoms of Irritable Bowel Syndrome and Constipation
Adapted from[12], with information from[9, 10, 12].

Diet plays an important role in health and prevention and treatment of bowel disorders. For IBS, diet can be a trigger of symptoms, as nearly 90% of patients report to experience GI complaints induced by specific foods[36]. However, these triggers differ largely between patients, and it is unclear what causes a patient to experience symptoms after consumption or not. Some have suggested that psychological state can affect the response to a trigger[37], but this has been contradicted by others[38]. For constipation, a low fiber intake was associated with a

higher prevalence of constipation[39], and an increased fiber and fluid intake can improve constipation complaints[40]. However, it remains a challenge to sustainably increase dietary fiber intake, not only in constipated adults but also for the general population, as median intakes are far below the recommendations in Western societies[41, 42].

Diet is not only linked to GI symptoms, but has also been considered as one of the major modulators of the gut microbiota[43]. There is growing evidence that the gut microbiota in IBS and constipation differs from controls (see section 1.3)[44, 45], but large differences are found between studies and consensus seems elusive[44, 46, 47]. The identification of a microbial signature for IBS as well as other disorders is hampered by the large individual variation in gut microbiota and GI symptoms and the instability of both over time[48-52]. Since most studies are cross-sectional, they are unable to detect these dynamics. Furthermore, psychological status is often not taken into account, but has frequently been associated to the gut microbiota, and anxiety and depression is highly prevalent in IBS[44, 53, 54]. Lastly, cohort-specific characteristics can also differ between studies and limit consensus[55].

A further challenge for studies lies in the selection of participants for trials. For studies with fiber interventions, dietary assessment is part of the screening process of eligible participants. However, most dietary assessment methods are time consuming and more extensive than needed for screening[56-59]. This places an unnecessary burden on both participant and researcher, and practical tools for dietary screening are therefore warranted.

To summarize, in IBS the exact interplay between GI complaints, diet and the gut microbiota remains elusive, and further identification of associations and patterns taking time dynamics of symptoms and the gut microbiota into account are needed. Furthermore, the role of psychological status needs to be considered in these investigations. Methods to sustainably increase dietary fiber intake for both adults with and without constipation, and to improve participant screening for such trials are needed. The work in this thesis will focus on these aspects, and the background will be further introduced in this chapter.

1.1 Digestion, gut microbiota and the relationship with dietary fibers

The GI tract is the tract that extends from the mouth, followed by the esophagus, stomach, small intestine, large intestine, rectum to the anus. The GI tract can still be seen as the “outside” of the body, since the intestinal wall prevents unwanted compounds and bacteria to enter the body[60]. Food enters the GI tract via the mouth, where digestion is already started. A large proportion of foods are absorbed in the

GI tract, with exception of most dietary fibers and some fiber-binding fats, cholesterol and minerals, which are metabolized by the gut microbiota and/or excreted via the feces[60, 61].

1.1.1 Dietary fibers

Dietary fibers are indigestible carbohydrates derived from plants and include polysaccharides, lignin, oligosaccharides and resistant starches[62]. Fibers vary in their solubility, viscosity, bulking capacity and fermentability, and therefore each fiber can have a different effect on GI transit time, the gut microbiota and stool pattern[63] (Figure 2). GI transit time in turn can greatly impact the gut microbiota due to the water and nutrient availability in the colon[64, 65]. Insoluble fibers can bind to water, increasing stool weight and water content, which stimulates gut peristalsis. Therefore, they have a laxative effect and decrease GI transit time[66-68]. Soluble fibers are generally highly fermentable and can form gels that can impact intestinal motility by delaying gastric emptying. Furthermore, they increase satiety and short-chain fatty acid (SCFA) production[69-72]. A last group are the so-called prebiotics, which are substrates (often soluble, non-viscous fibers) that are fermentable by the colonic gut microbiota and modulate the SCFA production, which can have health benefits[73, 74]. A diet high in fibers has been associated with reduction of risk of several diseases and GI complaints[62, 75-78]. Due to the delay in gastric emptying time, fibers reduce the postprandial glucose and insulin peak and can prevent the development of insulin resistance, which is seen as a cause of many chronic diseases[79, 80].

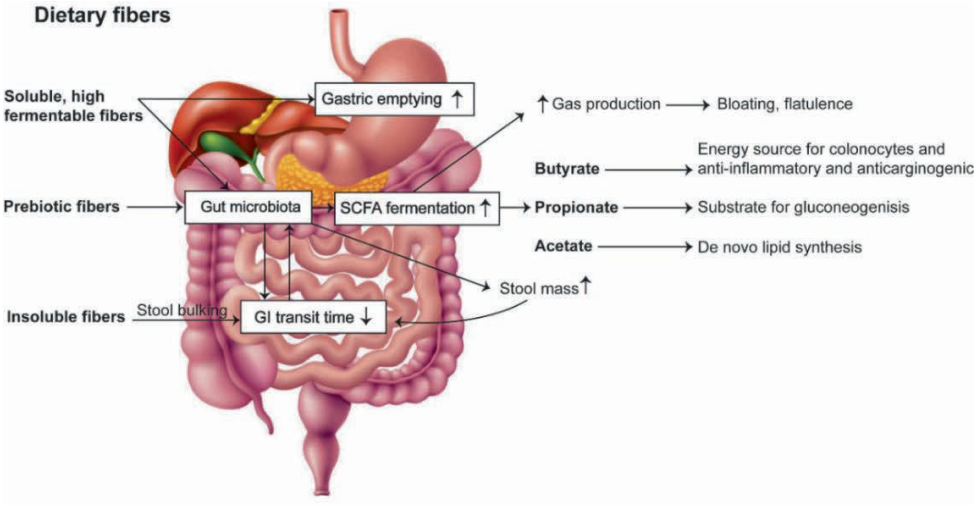

Figure 1. The effects of different dietary fibers on gastro-intestinal transit time and gut microbiota. Adapted from [63], with information from [43, 81-83].

1.1.2 The gut microbiota and SCFA

The gut microbiota comprises trillions of bacteria, viruses, fungi and archaea, of which bacterial species from the phyla *Firmicutes* and *Bacteroidetes* are dominating[84], which are mainly present in the colon. Although a healthy or normal gut microbiota profile still needs to be defined, research showed that a high microbial richness (amount of species) and diversity (combination of richness with evenness, *e.g.* the relative differences in the abundances of different species) are associated with a good health status[85, 86]. As indicated before, diet is one of the most important environmental factors shaping the gut microbiota. This already starts at an early age, where initial feeding practice (*e.g.* breastfeeding or formula feeding) greatly impacts the gut microbiota[87, 88]. Later in life, African and Asian populations which have diets high in plant-based foods and dietary fiber, and lower in energy, fat and animal food sources, have been shown to have different gut microbiota than populations consuming a Western diet high in energy, fat and animal food sources[89-91]. A high-fiber diet has been associated with higher levels of microbial diversity and richness[92], and even a 5-day high-fiber intervention already increased the gut microbiota richness and stability[93]. However, this has been contradicted by others, such as the study of O'Keefe and colleagues (2015), which have shown that the gut microbiota profiles in African Americans who were fed a high-fiber African diet did not change[94].

SCFA are the main metabolites which are notably produced when fibers are fermented by the gut microbiota. Butyrate, propionate and acetate are the most predominant SCFA[43, 82]. Butyrate is the most investigated SCFA and the preferred energy source for colonic epithelium, and also has anti-inflammatory and anti-carcinogenic properties[81, 95]. Acetate and propionate are mostly absorbed and transported to the liver, where propionate can be used as a substrate for gluconeogenesis and acetate can support lipid synthesis[95]. The concentration of SCFA varies in the gut, with the highest levels in the caecum and the lowest levels in the distal colon[96]. Research estimates that 80-95% of the SCFA are absorbed or utilized in the gut[97, 98]. Higher levels of SCFA have been suggested to play a role in the prevention and treatment of metabolic syndrome, bowel disorders and different cancers[99], and thus can have a positive impact on health. Furthermore, when African Americans were fed an African diet, gut microbiota profiles remained quite stable but SCFA levels increased[94], indicating that a high-fiber more plant-based diet can beneficially alter SCFA levels.

1.2 Pathophysiology of IBS and constipation

1.2.1 Pathophysiology of IBS

Several mechanisms have been proposed to underlie the pathophysiology of IBS, such as alterations in the gut-brain axis and gut microbiota, visceral hypersensitivity, increased intestinal permeability, low grade inflammation, psychological status and

genetic polymorphisms[100-105] (Figure 3). Furthermore, in approximately 20% of the IBS patients, IBS develops after an acute bacterial, viral or protozoal gastroenteritis, the so-called post-infectious IBS[106].

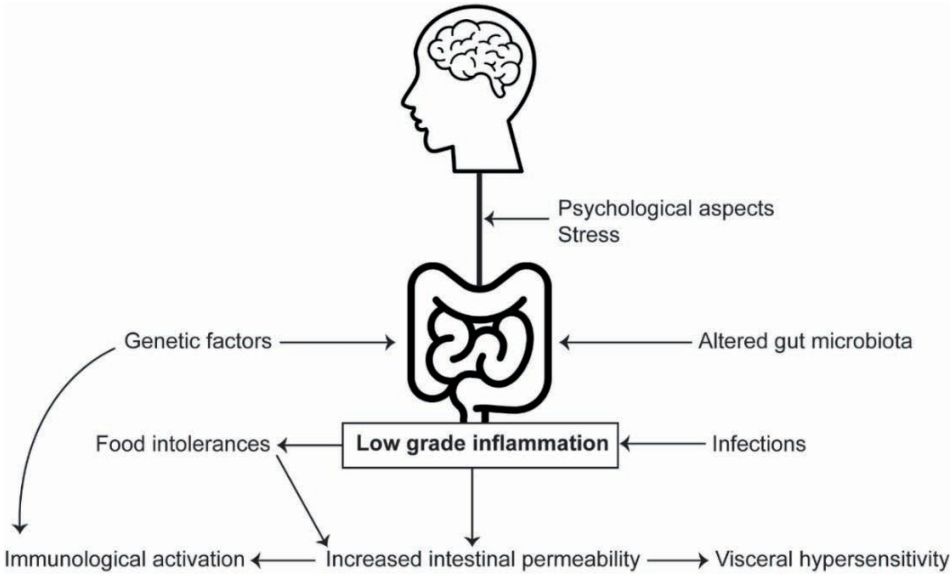

Figure 3. Schematic overview regarding the proposed mechanisms behind the pathophysiology of Irritable Bowel Syndrome. Adapted from[100], with information from[103, 104, 107-109].

Visceral hypersensitivity is one of the most common found alterations, which is reported to be prevalent in 18-60% of the IBS patients[110, 111]. It encompasses a painful response to a intraluminal stimulus. It has been suggested that a low grade inflammation and a change in the intestinal barrier function can play a role, which can be modulated by stress[107]. The role of stress and psychological status is important in the pathophysiology of IBS, as a meta-analysis has shown that childhood abuse or traumas increased the risk for developing IBS[112]. An increased presence of T lymphocytes, mast cells and enterochromaffin cells, which modulate the inflammatory response, are suggested to cause the low grade inflammation in IBS[113].

Furthermore, there is some evidence regarding genetic susceptibility, as IBS often occurs within several family members[114, 115]. It has been suggested that a polymorphism in the serotonin transporter gene might play a role[116, 117], however, a meta-analysis including eight studies did not find an association between presence of this polymorphism and risk of IBS[118]. A higher prevalence of cytokine gene polymorphisms, and specifically Interleukin-10 (IL-10) for general IBS and tumor

necrosis factor (TNF) in Asian IBS populations, have been observed in a different meta-analysis[108], which could be related to the low grade inflammation in the gut.

Lastly, food intolerances have been reported by 50-83% of the IBS patients, but the etiology is poorly understood[119-121]. Patients often report dairy, gluten and fatty/fried foods as causing symptoms[121, 122], however skin prick or IgE antibody testing are often inconclusive[123-125]. Recently, a study by Fritscher-Ravens and colleagues (2019) observed that >50% of the IBS patients who had negative results from skin prick or IgE tests showed alterations in the intestinal mucosal response, resulting in a dysfunction of the GI barrier and presented an atypical food allergy. Patients also reported improvements in symptoms after they excluded these foods from their diet[109]. This provides promising leads for a further understanding of the pathophysiology of IBS and the development of treatments. A next step would be to determine if there are subgroups that can be identified, to further understand which IBS patient will respond to which dietary triggers, in order to further personalize treatment plans.

1.2.2 Pathophysiology of constipation

Constipation can have a primary or secondary origin. Primary constipation results from a dysfunction of the colonic regulation of stool movement. Neuropathy or dysfunction of the colonic muscle can cause a delayed stool transit. Some patients have difficulty with stool evacuation, which can be caused by an impaired coordination of the abdominal and anorectal muscles[126]. Constipation of secondary origin includes medication usage, metabolic disorders such as diabetes mellitus and hypothyroidism, neurological problems such as Parkinson disease, and an unhealthy lifestyle with low intakes of fiber and fluids and low physical activity levels[127]. It is currently unclear if there are differences in the pathophysiology between people who fulfill the Rome criteria for constipation or not. They do not seem to differ in general characteristics such as age, gender, body weight or education level, but people with self-reported constipation seem to have a lower stool frequency and suffer more often from straining[33]. Further work is needed to better define these subgroups and their differences in clinical characteristics.

1.3 Gut microbiota composition and fecal SCFA levels in bowel disorders

The Rome foundation has indicated that there is good evidence that the gut microbiota is altered in functional bowel disorders[12], but a distinct signature remains elusive. This is hampered by the large variation between individuals in diet-gut microbiota associations[48], and the individuality in gut microbiota resilience and possibly the many factors associated with it[128]. In both IBS and constipation, the gut

microbiota has been investigated, often cross-sectionally, of which a summary of the findings are presented below.

1.3.1 Gut microbiota composition and SCFA levels in IBS

Several cross-sectional studies have suggested that microbial alpha diversity (diversity within a sample) and the gut microbiota composition is different between IBS and controls[129-132], but this has been disputed by others[133, 134]. A meta-analysis of Lui and colleagues (2017), assessing differences in microbial taxa which included 13 articles and 12 species or genera, observed lower abundances of *Lactobacillus, Bifidobacterium* and *Faecalibacterium prausnitzii* in IBS compared to controls[135]. *Bifidobacterium* and *Lactobacillus* are often suggested to beneficially affect health due to strengthening the intestinal barrier and modulating the immune response[136].

It has also been suggested that specific gut microbiota profiles might be related to subgroups in the heterogenous IBS population. Several studies have investigated the gut microbiota composition between the IBS subtypes, of which some did observe differences but none were replicated in another study, and others did not find any differences[44]. Jeffrey and colleagues (2011) have not looked into the traditional IBS subtypes, but performed clustering analysis to discover new subgroups that might reveal specific gut microbiota profiles. They discovered three clusters: a normal-like IBS cluster which resembled the gut microbiota of controls, and two distinct IBS clusters, which were mainly defined by an increased *Firmicutes:Bacteroidetes* ratio compared to controls. Interestingly, the depression prevalence was higher in the IBS-normal like cluster, and the two distinct IBS clusters had depression prevalence's similar to the general population. The gut microbiota profiles of the two distinct clusters were furthermore associated with colonic transit time, satiety, bloating and rectal pain threshold[137]. Recently, IBS severity was associated to a specific IBS gut microbiota profile of 90 operational taxonomic units (OTU) in an exploratory cohort, which was then validated in a new cohort[133]. These studies provide new leads in investigating the gut microbiota in the heterogenous IBS population, however, both studies were cross-sectional and the stability of these findings are yet to be investigated.

Another important factor to consider in gut microbiota research in IBS patients is the increased prevalence of anxious and depressive symptoms[16]. A recent meta-analysis of Senada and colleagues (2020) including 16 articles has shown that patients with major depressive disorders have an different gut microbiota profile compared to controls, and that probiotic treatment improved depressive symptoms[138]. However, almost none of the included studies considered the possible effects of diet or medication use as confounder in their investigations, which may have influenced the results. Little research has been conducted about gut microbiota composition in anxious patients, but a meta-analysis has shown that probiotic

treatment reduced anxious symptoms[139]. Psychological status is therefore an important factor to consider when assessing the gut microbiota in IBS patients, which is often not done.

Additionally to differences in the gut microbiota, differences in SCFA levels in feces have been reported. Sun and colleagues (2018) have performed a meta-analysis regarding the SCFA levels in IBS including case-control studies (n=9), randomized controlled trials (n=4) or self-controlled studies (n=2). They found significant higher levels of fecal propionate compared to controls, and differences between the IBS subtypes: IBS-C had lower levels of propionate and butyrate, and IBS-D had higher levels of butyrate compared to controls[140]. In a different trial, differences in fecal SCFA were associated with bloating, abdominal pain and a reduced QoL[141]. Some have therefore suggested that fecal SCFA might be a diagnostic biomarker for IBS[142]. However, differences in fecal SCFA are not only observed in IBS but also other bowel diseases such as adenomatous polyposis and colorectal cancer[143], thus lack discriminatory power. Moreover, it can be debated whether these differences are due to IBS or due to an altered colonic transit time, which has been shown to impact fecal SCFA levels[144]. It is therefore questionable if fecal SCFA are a good biomarker candidate for IBS.

1.3.2 Gut microbiota composition and SCFA levels in constipation

In a trial investigating elderly with constipation, higher abundances of *Bacteroides, Ruminococcus, Lachnospiraceae* and *Prevotella* and lower abundances of *Akkermansia* and *Veillonella* were seen[46]. However, these differences were not observed in adults with constipation. Khalif and colleagues (2005) reported lower abundances of *Bifidobacterium* and *Lactobacillus*[145], while Mancabelli and colleagues (2017) observed lower abundances of *Bacteroides, Roseburia* and *Coprococcus*, and higher abundances of *Faecalibacterium* compared to controls[47]. *Roseburia, Coprococcus* and *Fecalibacterium* are taxa that have been described to be able to produce butyrate, and these are hypothesized to fasten colonic transit due to the motility-stimulating effect of butyrate[146]. Although *Faecalibacterium* is considered a butyrate-producing microbe, it has been associated with the pathophysiology of constipation via the reduction of stool volume[147]. Recently, a study including 48 adults with a slow colonic transit but without constipation showed that alpha diversity was positively associated with descending colon transit time but not stool consistency, indicating that alpha diversity increased when transit time was faster[144].

Little research has been conducted regarding fecal SCFA levels in constipation. A shorter colonic transit time has been associated with higher levels of fecal SCFA[144], possibly due to less time for SCFA absorption in the gut. In a study including 90 patients with constipation, SCFA from ascending colon specimens has been

negatively associated with constipation severity, and levels were lower compared to cancer patients[148]. These results provide some first clues that SCFA levels might be altered in constipation, but more research is needed.

1.3.3 Summary of findings regarding gut microbiota composition and SCFA levels in IBS and constipation

Overall, it remains unclear whether gut microbiota diversity or composition as well as fecal SCFA levels are different in IBS or constipated people compared to controls. Currently most research is based on cross-sectional designs which do not include the large within and between person variability in the gut microbiota. Furthermore, important confounders such the diet, medication or psychological status are also not always included, but could alter a study result. Longitudinal studies taking these confounders into account are needed, to advance in the discovery regarding a microbial signature in IBS and constipation.

1.4 Current dietary treatments of IBS and constipation

1.4.1 Dietary treatments in IBS and its challenges

In 2021, the American College of Gastroenterology provided a clinical guideline on the management of IBS, as curing IBS is not possible but management of symptoms is. Possible effective treatment options for IBS patients include medications such as antispasmodics or laxatives, peppermint oil, psychotherapy, probiotics, soluble fiber supplements or the elimination of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) in the diet [149]. However, it must be noted that the quality of the evidence was often low to very low, with the exception for medications and soluble fibers. Furthermore, most recommendations were conditional, indicating that some patients will benefit from a therapy but others do not [149].

The FODMAP diet is often suggested to be the first in line as treatment for IBS[150]. FODMAPs are groups of carbohydrates in the diet that are hypothesized to increase intestinal osmolarity and gas production, and thus increase GI symptoms and bloating[151, 152]. The diet includes a 6-week elimination period in which all FODMAPs are avoided. If symptoms are reduced, then a gradual reintroduction of FODMAPs takes place to identify which FODMAP can cause symptoms, which are then eliminated from the patients' diet. A recent meta-analysis found that the FODMAP diet reduced IBS severity more than a control diet (habitual intake or traditional IBS diet, *e.g.* distribute smaller meals over the day and avoid foods that can cause extensive gas or bloating), and was associated with an increase in QoL[153]. However, the trials assessing this efficacy have a high risk of bias as study durations are short and often do not include the reintroduction phase of the diet, and effects might be

driven by a possible placebo effect as blinding is not feasible[154]. Furthermore, there are concerns about the long-term safety of the diet due to the risk of nutrient deficiencies and alterations in the gut microbiota since many foods are excluded[155-157]. The diet is therefore advised to be only followed under guidance of a dietician. Nutrient adequacy in the IBS population already deserves some attention, as the majority of the IBS patients report to have adjusted their diet to reduce symptoms, but only 12% did this under supervision of a dietician[36]. The impact of these adjustments on diet quality are unclear, as some observed a lower diet quality and lower fiber intake[158, 159], but others have not[160]. Furthermore, it is currently unclear whether these dietary adjustments differ in subgroups of the IBS population, and therefore each subgroup needs its own dietary strategy. This highlights the need for a better understanding of the diet in the IBS population and patients' current strategies.

Dietary fiber supplements are often prescribed to IBS patients. Two different meta-analyses have shown positive effects of soluble dietary fibers on symptom improvement, while insoluble fibers did not improve symptoms[161, 162]. A randomized controlled trial even observed worsening of symptoms after supplementation of the insoluble fiber bran[163]. Increasing dietary fiber intake via the diet did not show symptom improvement in a different meta-analysis[164]. These differences in effectiveness are likely due to the different physiological effects of dietary fiber in the gut, as soluble, moderately fermentable and vicious fibers such as psyllium produce little gas, while highly fermentable fibers can increase gas production and therefore can cause abdominal pain and bloating[165]. Furthermore, foods contain mixed amounts of different types of fiber, which might hamper the positive effects on complaints.

1.4.2 Dietary treatments in constipation and its challenges

Treatments can depend on the cause of constipation, but general approaches include laxatives and increasing fluid and fiber intake, either via supplements or via the diet[126]. Meta-analyses have shown that fiber supplements were effective in increasing stool frequency and improving stool pattern[166, 167], but that fiber supplements can also increase flatulence and bloating[168]. The effects of dietary fiber from the diet in adults with constipation has been sparsely investigated. Anti and colleagues (1998) have shown that a fiber intake >25 g/day increased stool frequency, which was more pronounced in patients who drank >2 L/day[40]. Furthermore, increasing fiber intake >28g/day improved constipation symptoms in women with pelvic floor disorders and constipation, and gradually increasing the intake improved tolerability[169]. Although constipation guidelines do not discriminate between an increase in fiber via the diet or supplements, it can be argued that increasing fibers via the diet has a substantial advantage over supplements. Regardless of having constipation, consumption of high-fiber food sources such as

fruits, vegetables, whole grains, legumes and nuts are part of the dietary guidelines[170]. Consumption of these high-fiber food sources are considered positive for health as they are associated with a reduction of the risk of for example coronary heart disease[171-173] and obesity[174-176]. Furthermore, a high-fiber intake is associated with a reduction of risk for diseases such as diabetes mellitus, cardiovascular disease and colorectal cancer[62, 75-78].

Recommendations for dietary fiber vary across the globe, but the majority of the general population are not meeting them (Figure 4). In the Netherlands, a fiber intake of 14 g/1000 kcal is recommended by the Dutch Health council, which equals to 30 g/day for females or 40 g/day for males[177], regardless of having constipation or not. Currently there are no guidelines specified for type of fiber but it is recommended to consume a variation of different fiber types, as they have different functions in health[178]. Median intakes in the Netherlands are around 18 and 23 g/day for females and males, respectively[179]. Many intervention studies have been done in different populations to improve diet quality including fiber intake[180-182], but also with high-fiber diets such as the Mediterranean diet[183-186]. However, long-term adherence to a completely different diet such as the Mediterranean diet has been shown difficult[187, 188]. Other interventions have focused on specific high-fiber food sources, such as fruit and vegetables[189-191] or whole grain[68, 192-194]. However, to reach the dietary fiber recommendations, an increase in multiple high-fiber food sources is often needed.

Personalized dietary advice (PDA) has been suggested as a new method to improve long-term adherence to healthy diets, and have the ability to reach larger populations due to its possible digital applications. Personalized nutrition entails adapting dietary advice to each individual, as it has been shown that each individual can respond differently to the same food[202], possibly due to genetics, lifestyle, gut microbiota and environment[203, 204]. But personalized nutrition also encompasses tailored messages, which is more effective for behavior change as it meets the behavior, needs and beliefs of an individual[205, 206]. There are large differences in type of interventions and personalization, but several PDA trials have been shown effective in improving dietary intake, metabolic markers and wellbeing (Table 1)[202, 207-216]. Only Karagiozoglou-Lampoudi and colleagues (2012) has developed a PDA targeting dietary fiber intake, but this included face-to-face guidance for children with refractory functional constipation[212]. A digital high-fiber PDA for adults with or without constipation has not been developed yet.

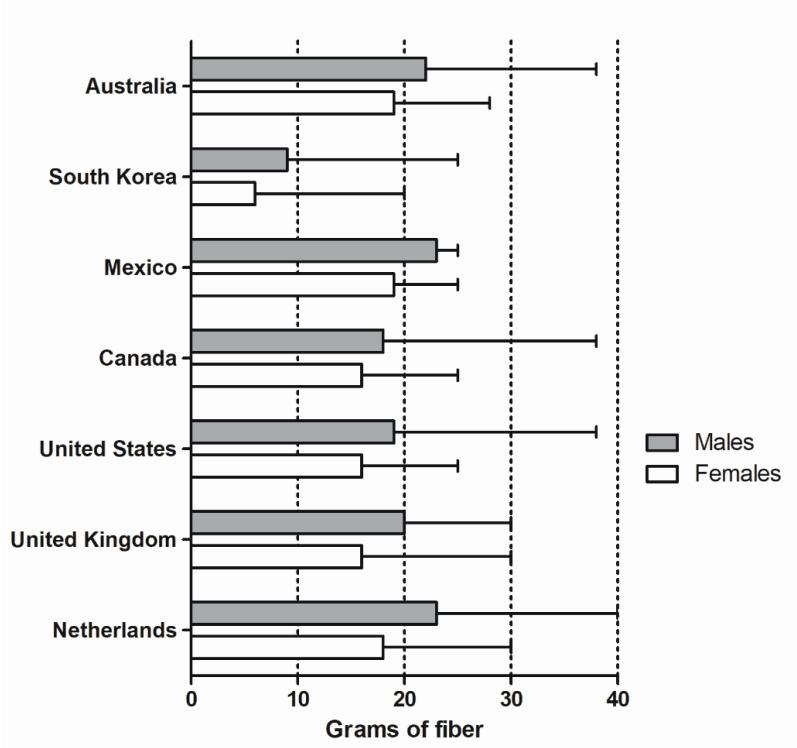

Figure 4. Fiber recommendations and intake for different countries, stratified for gender. Bars represents median or average fiber intake while the error bar represents the national recommendation. Information from[177, 179, 195-201]

Lastly, the screening of participants for these trials has been shown challenging. An inclusion criterion is often a low fiber intake, but dietary assessment methods are often more extensive than needed for screening[59]. Furthermore, completion time for dietary assessment methods such as the Food Frequency Questionnaire or a single 24hr recall is estimated between 20-60 minutes[56, 58], which can be a burden for both participant and researcher. No validated biomarker to date for dietary fiber intake has been found yet. Plasma Alkylresorcinol has been suggested as a biomarker for whole grain or rye intake[217-219], but has shown poor correlations with total fiber intake and other grain sources[220], and cannot be used for this purpose in fiber trials. Short dietary screeners have also been developed, of which one of the most frequently used is the PrimeScreen[221]. Although the PrimeScreen assesses the intake of vegetables, fruits and whole grain foods, it misses other high-fiber categories such as nuts, seeds and legumes, and is therefore not optimal to screen for fiber intake. This shows the need for a tool that improves the practical aspects of fiber intake screening for trials.

1.4.3 Summary of findings regarding dietary treatments in IBS and constipation

In summary, current treatments are not optimal for all IBS patients. The FODMAP diet seems to be effective, but there are concerns about the long-term safety and efficacy of the diet. IBS patients often adjust their diet to reduce symptoms, but there is a low percentage of patients who does this under supervision of a dietician. Soluble fibers can be beneficial for IBS patients, while there is no evidence of a clinical benefit of increasing fiber from the diet or insoluble fiber supplementation.

Fiber supplements are effective in increasing stool frequency in adults with constipation, but few studies have investigated the effects of a high-fiber diet on constipation complaints. However, it has several advantages for overall health and diet quality to improve intake of high-fiber food sources. It remains difficult to increase fiber intake towards the recommendations, not only in adults with constipation but also the general population. PDA has been suggested as a new method to improve long-term adherence, but a high-fiber PDA for adults has not been developed yet. Lastly, trials are hampered by unpractical dietary fiber screening which places an unnecessary burden on both participant and researcher. New tools to facilitate screening of fiber intake are needed.

Table 1. Overview of the effects of personalized dietary advice (PDA) interventions on diet, metabolic markers and wellbeing

First author and year	Study population	Intervention	Main conclusions (compared to control)
Dietary intake or quality			
Bianchi 2020 [207]	n=80 pregnant women	9-week intervention. Digital PDA to improve diet quality and three face-to-face meetings with a dietician. Control: general booklet with dietary advice	↑ Diet quality
Celis-Morales 2017 [208]	n=1269 adults from 7 European countries, having a disease was excluded	Food4Me trial. 6-month digital PDA with 3 levels of personalization or control (non-personalized dietary advice) L1: personalized based on dietary intake L2: personalized based on dietary intake and phenotype L3: personalized based on dietary intake, phenotype and genotype	↓ Red meat, salt and saturated fat intake. ↔ Dietary fiber intake. ↑ Folate intake and diet quality. No differences between different levels of PDA.
Demark-Wahnefried 2007 [209, 210]	n=519 locoregional breast or prostate cancer patients	FRESH START trial. 10-month intervention tailored or non-tailored emails with information regarding fruit and vegetables, total and saturated fat intake and/or increasing exercise.	↑ Meet ≥2 behavior goals, exercise, fruit and vegetable intake. ↓ Total fat and saturated fat consumption and BMI. Two year follow-up: ↑ diet quality, ↓ total fat and saturated fat consumption
Garner 2017 [211]	n=23 adults with inflammatory arthritis	6-month intervention: twice an individualized nutrition and exercise counselling session. Control: standard care.	↑ Vitamin C, iron, fiber, vitamin A and folate intake. Both groups improved in physical activity. No significances due to small sample size.
Karagiozoglou-Lampoudi (2012) [212]	n=86 children with refractory functional constipation	1-month intervention: written instructions + one face-to-face meeting with dietician who provided a personalized meal plan and high-fiber recipes. Control: written instructions.	↔ Consumption of protein and fat. ↑ Energy, carbohydrate, fiber and water intake

Metabolic markers			
Di Renzo 2020 [213]	n=52 women at risk for cardiovascular disease	6-month intervention. Personalized Mediterranean diet and generic physical activity advice. No control group.	↓ Body weight, BMI, waist circumference, fat mass, lean mass, HDL and LDL cholesterol and TG/cHDL ratio
Valentini 2015 [214]	n=62 healthy older adults aged 65-85 years	RISTOMED trial. 8-week digital PDA including 2 capsules per day of VSL#3 probiotics. Control received PDA but no probiotics.	Control: ↓ inflammatory markers, plasma cholesterol and glucose. Intervention (compared to control): ↑ folate, vitamin B12 and fecal *Bifidobacterium.*
Zeevi 2015 [202]	Cohorts designed to represent non-diabetic adult general population.	PDA was developed in a large cohort (n=800) based on response to standardized meals, continuous glucose monitoring, microbiota, anthropometrics, lifestyle and medical history. This was validated in a separate cohort (n=100), and a cross-over trial was performed (n=26). Intervention: “good” diet based on PDA, control: “bad” diet opposite of their PDA.	Cross-over trial: ↑ Improvement in glucose metabolism, fecal *Roseburia inulinivorans, Eubacterium eligens* and *Bacteroides vulgates.* ↓ Fluctuations of blood glucose levels and fecal *Bifidobacterium adolescentis*
Quality of life and wellbeing			
Doets 2019 [215]	n=59 older sedentary adults ≥60 years	9-week intervention with digital personalized advice regarding protein, energy, saturated fat, omega-3 fatty acids, salt, vitamin D and liquids, and aerobic and resistance exercise. Control: generic advice (leaflet)	↔ Self-perceived health, resilience and motivation, mental health and energy. ↓ Body fat percentage and hip circumference. ↑ Physical functioning (both groups)
Willems 2016 [216]	n=462 cancer survivors	6-month intervention. Online tailored advice regarding return to work, fatigue, anxiety, depression, social relationship, physical activity, diet and smoking cessation, cancer residual symptoms. Control: waitlist	↓ Depression, fatigue ↑ Emotional and social functioning

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PDA, personalized dietary advice; TG, triglycerides. ↓ = decrease, ↑ = increase, ↔ = no change.

Outline and aims

Much remains unknown about the interplay between diet, gut microbiota and psychological status in IBS and how this is related to GI complaints as well as distorted defecation such as constipation. Furthermore, dietary fiber supplements seem effective for treatment in adults suffering from constipation, but the effects of a high-fiber diet on complaints are less investigated, and sustainably increasing fiber intake remains a challenge. Tools are lacking to improve dietary fiber intake and to screen for a low fiber intake for eligibility in these trials. These tools could not only benefit a constipated population but also the general population, as a high-fiber diet is positive for most. The work in this thesis (Figure 5) aims to 1) investigate associations in IBS patients between groups of different GI complaints (subtype and severity) and the diet, gut microbiota and SCFA, taking psychological status into account (part I); and 2) to create science for impact, by investigating the effects of a novel practical personalized dietary advice web-tool on dietary fiber intake and GI complaints, as well as developing a practical tool for fiber intake screening (part II). The overall aim of this thesis is to improve gut health (GI complaints and QoL) via the diet.

To identify which dietary triggers are associated with IBS symptoms, an inventory by using a large online questionnaire is described, which assesses associations between IBS subtype and symptom severity with dietary triggers and psychological status **(Chapter 2)**. Given the chronic nature of IBS, **Chapter 3** investigated temporal dynamics in IBS severity, and gut microbiota and SCFA levels and their associations with diet and psychological status. In **Chapter 4**, the development and validation of a short fiber screening questionnaire is described, as questionnaires assessing diets are often too complex and time-consuming for recruitment of subjects based on fiber intake. This tool was subsequently used to recruit study participants with a relative low fiber intake for the PDA intervention studies described in **Chapters 5 and 6**. The development and validation of a high-fiber PDA web-tool to improve dietary fiber intake is described in **Chapter 5.** The efficacy of this tool was validated in adults without GI complaints. In **Chapter 6,** this high-fiber PDA web-tool was further developed and tested in a population with constipation complaints, aiming to assess the efficacy of the web-tool in increasing dietary fiber intake and subsequently reducing constipation complaints. Finally, in **Chapter 7** the results of this thesis are discussed, and suggestions for future research are given.

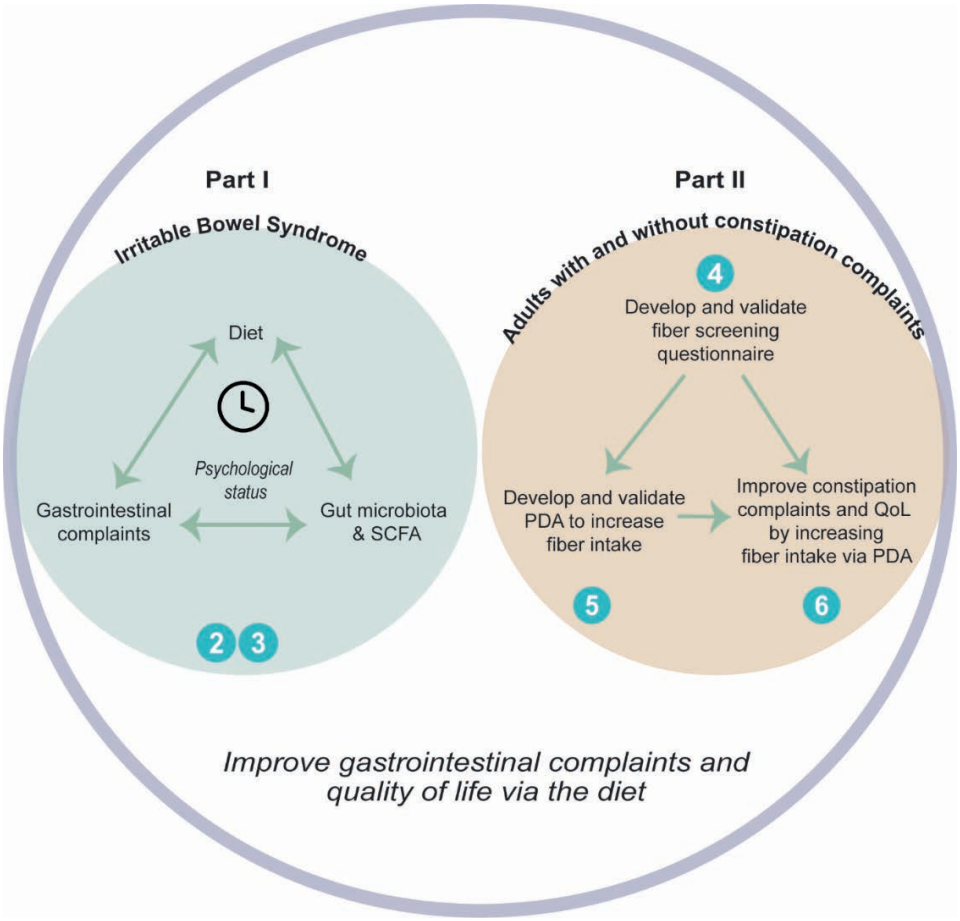

Figure 5. Schematic overview of thesis topics. Numbers indicate thesis chapters in which each topic is discussed. Abbreviations: PDA, personalized dietary advice; QoL, quality of life; SCFA, short-chain fatty acids.

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CHAPTER 2

SUBTYPES AND SEVERITY OF IRRITABLE BOWEL SYNDROME PATIENTS ARE NOT RELATED TO PATIENTS' SELF-REPORTED DIETARY TRIGGERS: RESULTS FROM AN ONLINE SURVEY IN DUTCH ADULTS

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Abstract

Background: Diet plays an important role in symptom management of Irritable Bowel Syndrome (IBS). However, current diet therapies are not optimal nor successful for everyone.
Objective: To investigate whether subgroups based on IBS subtypes or severity identify different self-reported dietary triggers, and whether these are associated with severity and psychological factors.
Design: Online cross-sectional survey
Participants: 1601 IBS patients who fulfilled the Rome IV criteria or had an IBS diagnosis
Main outcomes: self-reported response to 44 pre-selected dietary triggers, IBS quality of life, anxiety and depression. Subgroups were based on subtypes or severity.
Statistical analysis: Response to dietary triggers was analyzed using multiple correspondence analysis (MCA). Moreover, a foodscore was calculated to quantify the number and severity of responses to dietary triggers.
Results: Response to greasy foods, onions, cabbage, spicy and fried foods were mentioned most often (ranging between 55-65%). Response to dietary triggers differed between subtypes and severity groups, but absolute differences were small. MCA analysis did not reveal clustering between dietary triggers, and ellipses for the subtypes overlapped. Some clustering was seen when ellipses were drawn for severity, which indicates that severity explained a fraction of the variation in response to dietary triggers, and subtypes did not. The foodscore was not significantly different between subtypes, but was significantly higher with higher levels of severity (mild=20.9±17, moderate=29.2±19, severe=37.9±20, $p<.001$), having depressive (no=31.4±20, yes=37.4±20, $p<.001$) or anxious symptoms (no=30.7±20, yes=35.2±20, $p<.001$), and lower quality of life (lower QoL=38.5±19, higher QoL=26.5±19, $p<.001$).
Conclusion: patients with different IBS subtypes or IBS severity do not identify different self-reported dietary triggers. Patients with more severe IBS and who experience anxiety or depression tend to respond severe to more dietary triggers. IBS severity seems a better classifier than Rome IV criteria regarding diet. Dietary treatment needs to be individualized under guidance of a dietician.

Keywords: Functional Bowel disorder; Diet; Treatment: Psychologic factors; Subgroups

Introduction

Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder, which is characterized by abdominal pain and abnormal defecation patterns, and global prevalence is estimated between 10-20%[1-6]. The pathophysiology is unknown, but is suggested to include altered intestinal permeability, gastrointestinal motility, gut microbiota composition, low grade inflammation and visceral hypersensitivity[7-10]. IBS is diagnosed using the Rome IV criteria, and can be divided into subtypes: constipation predominant IBS (IBS-C), diarrhea predominant IBS (IBS-D), IBS with a mix of constipation and diarrhea (IBS-M) or IBS with no specific stool pattern, so-called unclassified IBS (IBS-U)[11]. Moreover, based on a validated questionnaire that assesses complaints and its impact on daily life, patients can be also classified as having mild, moderate or severe IBS[12].

Although IBS does not harm the intestines nor is a life-threatening disorder, it strongly affects quality of life (QoL) and impairs daily functioning[2]. Moreover, IBS patients frequently present comorbidities, such as depression, anxiety or chronic fatigue[13-16]. Guidelines for treatment of IBS include medication, psychological interventions or dietary adjustments[17]. Diet is a known trigger of symptoms: nearly 90% of IBS patients in a survey which included 135 IBS patients reported to have gastro-intestinal complaints induced by specific foods[18]. Foods reported to cause symptoms were spicy and fatty foods, vegetables and cereal-based foods[18]. The majority of IBS patients reported to have adjusted their diet to reduce symptoms, but only 12% did this under supervision of a dietician[18]. The most frequently advised diet focusses on exclusion of foods high in Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAP). Although effective for some IBS patients, the complexity of the FODMAP diet limits its use and compliance[19-21]. Moreover, excluding foods from different food groups may lead to nutritional deficiencies.

Currently, it is unclear why certain patients benefit from diet therapies, where others do not. Possibly, the large heterogeneity of the population and the multifaceted pathophysiology of IBS affect the response. Indeed, Simren and colleagues showed that anxious IBS patients responded to more foods with severe complaints than non-anxious patients. No difference in response to foods was found between the IBS subtypes[22]. However, Böhn and colleagues did not find any difference between anxious and non-anxious IBS patients in regards to food allergens[23]. It is questionable whether these studies were large enough to capture all facets of self-perceived food intolerance of the heterogenous IBS population and assess differences between subgroups such as the IBS subtypes.

Thus, more insight is needed to understand the interplay between dietary triggers, IBS characteristics and depression or anxiety. Therefore, we investigated whether

subgroups based on IBS subtypes and IBS severity identify different dietary triggers. Additionally, we investigated whether the number of dietary triggers to which a patient responds and severity of complaints linked to dietary triggers, is associated with IBS-QoL, depression or anxiety.

Methods

We performed a nationwide cross-sectional online survey in the Netherlands from January until May 2018. Participants were recruited via several platforms, including a national newspaper, the Dutch IBS patient association, social media and recruitment websites of Wageningen University & Research. Since recruitment was online and open, no response rate could be calculated. All information collected was self-reported. Figure 1 shows the flowchart of the included participants (complete questionnaire, consent, >18 years, and IBS diagnosis or fulfillment of the ROME IV criteria). If participants had filled in the questionnaire twice, only the most recent one was used (n=4); this was checked using e-mail address of the participant and city of residence.

The survey was performed using the platform Limesurvey version 2.50 (Limesurvey GmbH. / LimeSurvey: An Open Source survey tool /LimeSurvey GmbH, Hamburg, Germany. URL http://www.limesurvey.org) and was developed and monitored by the research team. The questionnaire was pre-tested by several colleagues and IBS patients from the Dutch IBS patient association, who provided feedback on clarity and completion time, which was estimated around 45-60 minutes. Participants had to complete a CAPTCHA code for loading and saving the survey. Among the participants, 25 vouchers of €10 and 10 vouchers of €25 for (web) shops were raffled as incentive, using Excel formula's for generating random number. If this number matched the participant survey ID, the participant was contacted for the incentive. Survey data was downloaded from Limesurvey sever into Excel and SPSS files, which was protected by the most common secure socket layer method (encryption), and was in fulfillment of the European Privacy Law. Participants consented to sharing their data with the researchers before filling in the survey. The medical ethical committee of Wageningen decided that no formal ethical approval was needed, due to the low burden and risk of the study. This study was registered at Clinicaltrials.gov, under number NCT03824821.

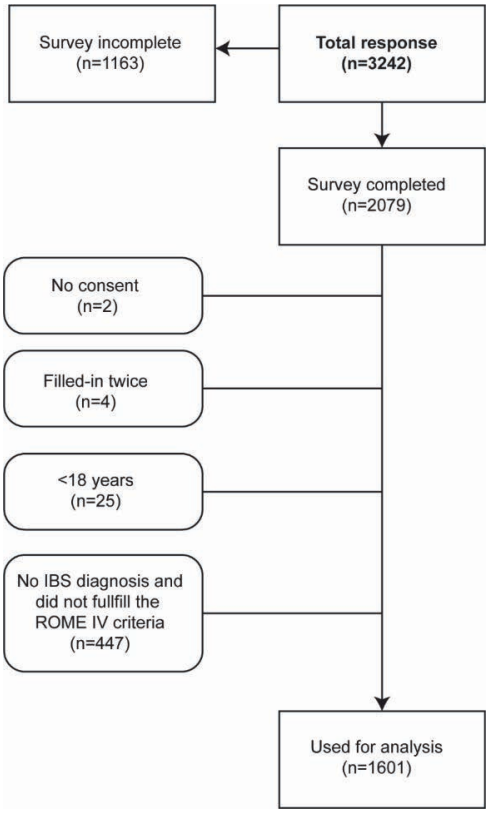

Figure 1. Flowchart of included participants from a cross-sectional online survey in Dutch IBS patients. Duplicate responses were checked by duplicate e-mail address in combination with city of residence. When duplicate responses were found, only the most recently filled in response was included. Incomplete responses were often within the first few questions; probably due to total completion time (estimated between 30 and 60 minutes). Abbreviations: IBS; Irritable Bowel Syndrome.

IBS characteristics

An overview of the validity and reliability of questionnaires assessed in the survey can be found in Table 1. Patients were classified into subtypes IBS-C, IBS-D, IBS-M or IBS-U, based on their most frequent self-reported stool types[24], by ranking their stool types over the last four weeks from most frequent to least frequent using the Bristol stool chart[1]. The three most frequently reported stool types were used to decide to which subgroup patients belonged. The validated 14-item Birmingham questionnaire was used to validate IBS subtype grouping[25]. Symptom severity was assessed using the validated IBS-Symptom Severity Score (IBS-SSS)[12, 26]. Based on this score, IBS patients were classified for their severity into mild (≤175), moderate (175-300) or severe (≥300) IBS[12].

Table 1. Validity of the questionnaires used in a cross-sectional online survey in 1601 Dutch IBS patients

First author (year)	Questionnaire	Method	Validity measures	Additional research
Roalfe, A.K. (2008)[25]	Birmingham Questionnaire: 14-items on a 6-point Likert scale. Gives a score for pain, diarrhea, and constipation of the last 4 weeks.	Re-test 1 week later, based on Rome II questionnaire. Compared to IBS-QoL	**Pain:** Cronbach α=0.74, validity r = -0.4 to -0.6, reproducibility ICC=0.75**. Constipation:** Cronbach α=0.79, validity r = -0.1 to -0.3, reproducibility ICC=0.78. **Diarrhea:** Cronbach α=0.90, validity r = -0.3 to -0.5, reproducibility ICC=0.81. **Overall:** Cronbach α=0.75, validity r = -0.5 to -0.7, reproducibility ICC=0.78	
Blake, M. R. (2016) [24]	Bristol stool chart: has 7 types of different stool with pictures.	Comparison with stool water, classification by experts and comparison between IBS-D and healthy and duplicate stools.	Correlation with stool water r=0.49. Differences between healthy and IBS-D patients was found (p<.0001). Overall, 977/1204 (81%) of the stools were correctly classified: substantial accuracy = 0.78). sustainable reliability was 76%, but lower reliability for type 2 (63%) and type 3 (62%).	
Zigmond, A.S. (1983)[29]	Hospital Anxiety and Depression Score (HADS); 11-items on a 5-point Likert scale. Ranges from 0-21, a score ≥8 indicates having anxious or depressive symptoms	Compared to psychological interviews	**Anxiety:** internal consistency between each item and total score= 0.41 to 0.76, correlation with interview r=0.74, 5% false positive, 1% false negative. **Depression:** internal consistency between each item and total score= 0.30 to 0.60. correlation with interview r= 0.70. 1% false positive, 1% false negative	Literature review by Bjeland et al (2002) compared 19 studies that investigated validity of the HADS. They conclude that the HADS performs well as a screening questionnaire for separate dimensions of anxiety and depression[30].

Patrick, D.L. (1998) [27]	IBS-Quality of Life (IBS-QoL), 34-items on a 5-point Likert scale. the score ranges from 0-100; 100 indicating good QoL	Re-test 1 week later, compared with SF-36, PWGB, SCL90-R	**Overall:** Cronbach α=0.95, internal reliability=0.95, reproducibility ICC=0.86. **Subscales**: Cronbach α=0.74-0.92, reproducibility ICC=0.65-0.89	Andrea, D.A. et al (2008) reproducing the original article, but with a special focus on IBS-D patients. Was compared with HRQOL. The questionnaire demonstrated very good construct validity.[28]
Francis, C.Y. (1997) [12]	IBS-Symptom Severity Score (IBS-SSS). Includes 5 items regarding pain (intensity and number of days), abdominal distention, satisfaction of bowel habit and interference of daily life of the last 10 days on a 10-point scale. Gives a score between 0-500, and groups of severity can be made: mild (≤175), moderate (175-300) and severe (≥300) IBS.	Three different groups of IBS patients, comparison to clinical rating by gastroenterologists, re-test 1 day later	Good reproducibility (Δ6 range: -107;75 on a score from 0-500). Able to pick up improvements after treatment	Literature review by Mujagic et al (2015) conclude that IBS-SSS includes the largest number of questions related to pain, and “appears to be the best retrospective instrument that can be used for the assessment of broader GI-symptom severity in IBS, including abdominal pain” [26]

Abbreviations: HADS, Hospital Anxiety and Depression Score; HRQOL, Health Related QoL; IBS, Irritable Bowel Syndrome, IBS-D; diarrhea-predominant IBS; IBS-QoL, IBS Quality of Life Questionnaire; IBS-SSS, IBS Symptom Severity Score; ICC, Intra-class correlation coefficient; PWGB, Psychological General Well-Being Scale; QoL, Quality of Life; SF-36, Medical Outcome Study Short Form 36; SL90-R, Symptom Checklist.

Psychological assessment

Patients completed the validated 34-item Irritable Bowel Syndrome Quality of Life (IBS-QoL) questionnaire, to compute a score for overall IBS-QoL[27, 28]. Participants also completed the validated screening Hospital Anxiety and Depression score (HADS)[29]. A score of ≥8 was indicative for having anxious or depressive symptoms[30].

Dietary triggers

Foods known for initiating IBS symptoms ("dietary triggers") were identified based on previous research[18, 22], and were split up into 8 food categories and 36 food products, as shown in figure 2A and 2B. Participants scored all 44 dietary triggers on a 3-point Likert scale (0=no complaints, 1=little complaints, 2=severe complaints, I don't know, I don't use this). From this data, similar to Simren and colleagues[22], we calculated an overall foodscore by summing the 44 items and multiplying by the Likert scale score. Since some patients respond severely to few dietary triggers or have some complaints to many dietary triggers, the foodscore enabled us to quantify the response for each patient and summarize this in one score. Moreover, we used the foodscore to test for associations between dietary triggers and IBS-QoL, IBS-SSS, anxiety and depression. To prevent underestimation of the score, answer options "I don't know" or "I don't use this" were handled as missing instead of zero when computing the foodscore. By standardizing the foodscore to a scale of 0-100, by taking into account their personal maximum (=44 minus number of missings multiplied by 2), the sum scores were corrected to prevent that patients with higher scores on fewer items received the same score as patients with lower scores for more items. The formula for the foodscore is as follows:

$$\frac{(\#products\ mild\ complaints \times 1) + (\#products\ severe\ complaints \times 2)}{personal\ maximum: (44 - \#\ missings) \times 2} \times 100$$

For example, if a participant answered "I don't know" to 10 out of 44 food products, their maximal possible foodscore was 68 points (34 items, maximum score of 2 points per food). Therefore, their summed score was divided by 68 and multiplied by 100. A score of 100 indicates that a participant responds to all products severely, and 0 indicates that a participant experienced no complaints to any of the triggers.

Statistical analysis

Data was presented as mean ± standard deviation for continuous data, or median (interquartile range) when data was skewed. For categorical data, counts and percentages were given. To test for differences between groups, Analysis of Variance (ANOVA) and Bonferroni post-hoc testing and correction[31], Kruskall-Wallis testing when not normally distributed or a chi-square test for categorical data was used. Data was stratified for IBS subtypes and severity groups. Moreover, foodscore

results were stratified for age (median split), gender, anxious or depressive symptoms (based on HADS cut-offs) and IBS-QoL scores (median split), to assess possible differences.

Foodscore data was analyzed using multiple linear regression, to assess associations in separate models between foodscore (independent variable) and IBS-QoL, IBS-SSS, anxiety and depression (dependent variables). Regression analysis was corrected for age, gender and Body Mass Index (BMI) in model 1, and in model 2 anxiety and depression were added. Moreover, crude dietary trigger data was analyzed using Multiple Correspondence Analysis (MCA), to assess if there were certain patterns within the dietary trigger responses. MCA can be seen as a qualitative version of principal component analysis, and allows us to analyze patterns of several categorical variables per subject[32]. Answer options "I don't know" or "I don't use this" were included in the MCA analysis, to obtain a complete overview. Ellipses for IBS subtype and IBS severity groups were drawn based on a 95% confidence interval. Statistical analyses were performed using SPSS version 23 (IBM Corp, Chicago, USA) and R version 3.5 (R Core Team 2013, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/, Boston, USA), and a p-value <0.05 was considered significant.

Results

Participant characteristics

This study included 1601 participants, with a median age of 47 (29 – 60) years and 291 (18%) were male. Patient characteristics, stratified for IBS subtype or IBS severity are shown in Table 2. IBS subtype classification was in accordance with the Birmingham diarrhea and constipation score, which was high or low accordingly with the subtype and significantly different between IBS subtypes (p<.001). Age, gender, body weight, BMI, IBS-SSS and IBS-QoL differed significantly between the IBS subtypes. Among the IBS subtypes, a comparable percentage of patients with anxious or depressive symptoms were seen. In contrast, between the three IBS severity groups, IBS-QoL, anxiety and depression scores were significantly different (p<.001). Of the total population, only 584 (36%) was currently using medication, predominantly by severe IBS patients. Antibiotics was the least used medication (n=50, 3%), fiber supplementation was the most used (n=469, 29%). Significant differences between IBS subtypes were found for medications related to subtype complaints, *i.e.* IBS-D patients significantly used more antidiarrheal medications (p<.001) and IBS-C patients used significantly more laxatives (p<.001).

Of the 1601 participants, 1143 (71%) participants indicated to have changed their diet due to abdominal complaints, of which 480 (30%) participants reported to have

Table 2. Participant characteristics, stratified by IBS subtype or IBS severity

	IBS subtypes					IBS severity groups			
	IBS-C (n=545)	**IBS-D (n=557)**	**IBS-M (n=420)**	**IBS-U (n=79)**	**P-value**	**Mild IBS (n=174)**	**Moderate IBS (n=661)**	**Severe IBS (n=766)**	**P-value**
Age (years)	47 (28 – 59)[a,b]	48 (31 – 61)[a]	47 (29-60)[a,b]	40 (24 – 57)[b]	**.040**	53 (32 – 64)[a]	48 (29 – 62)[a]	44 (28 – 58)[b]	**.000**
Gender, male	82 (15)	123 (22)	66 (16)	20 (25)	**.003**	48 (28)	135 (20)	108 (14)	**.000**
BMI (kg/m^2)	23.2±3.9[a]	24.5±4.4[b]	24.1±4.1[b]	22.7±3.4[a]	**.000**	23.5±3.4[a]	23.7±3.8[a]	24.2±4.5[a]	**.049**
Current smokers	35 (6)	46 (8)	36 (9)	6 (8)	.583	8 (5)	35 (5)	80 (10)	**.000**
Educational level					.067				**.002**
High school or vocational secondary education	126 (23)	143 (26)	135 (32)	16 (20)		40 (23)	154 (23)	228 (30)	
Higher or academic education	419 (77)	414 (74)	283 (68)	63 (80)		134 (77)	507 (77)	538 (70)	
IBS-SSS	275±85[a]	288±81[a,b]	293±88[b]	300±79[a,b]	**.004**				
IBS-SSS groups					**.013**	N/A	N/A	N/A	
Mild	71 (13)	49 (9)	51 (12)	3 (4)					
Moderate	234 (43)	242 (43)	152 (36)	33 (42)					
Severe	240 (44)	266 (48)	217 (52)	43 (54)					
IBS subtypes	N/A	N/A	N/A	N/A					
IBS-C						71 (41)	234 (35)	240 (31)	**.013**
IBS-D						49 (28)	242 (37)	266 (35)	
IBS-M						51 (29)	152 (23)	217 (28)	
IBS-U						3 (2)	33 (5)	43 (6)	
Birmingham score									
Constipation	51.2±25[a]	21.2±19[b]	43.3±24[c]	32.6±18[d]	**.000**	29.8±24[a]	36.2±25[b]	41.0±27[c]	**.000**
Diarrhea	13.5±11[a]	33.0±18[b]	26.3±15[c]	17.6±14[a]	**.000**	16.6±13[a]	20.7±15[b]	28.2±19[c]	**.000**
Pain	45.8±19[a]	48.7±19[a,b]	49.2±19[b]	47.8±17[a,b]	**.021**	24.7±12[a]	41±14[b]	58.8±16[c]	**.000**
IBS-QoL	75.5±18[a]	70.7±20[b]	71.1±20[b]	73.9±20[a,b]	**.000**	87.9±9[a]	79.1±14[b]	63.5±20[c]	**.000**

Anxiety score	6 (4 – 10)	6 (4 – 10)	6 (4 – 9)	6 (4 – 10)	.636	4 (3 – 7)[a]	6 (4 – 9)[b]	7 (5 – 11)[c]	**.000**
Having anxious symptoms	214 (39)	228 (41)	159 (38)	33 (42)	.770	37 (21)	228 (34)	369 (48)	**.000**
Depression score	3 (1 – 6)	3 (1 – 6)	3 (1 – 7)	3 (1 – 6)	.198	1 (0 – 3)[a]	3 (1 – 5)[b]	4 (2 – 8)[c]	**.000**
Having depressive symptoms	88 (16)	102 (18)	84 (20)	8 (10)	.127	8 (5)	79 (12)	195 (25)	**.000**

Data are presented as mean±SD or median (interquartile range) when skewed. Categorical data is presented as n (%). Different superscripts indicate significance between the subgroups. P-values indicate differences between the different IBS subtype or severity groups, and are tested using an analysis of Variance (ANOVA) and Bonferroni post-hoc testing, Kruskal Wallis when skewed or chi-square testing for categorical data.
Abbreviations: BMI: body mass index, IBS-SSS: Irritable Bowel Syndrome Symptom severity score, IBS-QoL: Irritable Bowel Syndrome quality of life, HADS: hospital anxiety and depression scale

Self-reported data is obtained using validated questionnaires such as the IBS-SSS[12], IBS-QoL (range 0-100; 100 indicates good QoL)[27], Birmingham questionnaire[25], HADS (range from 0-21, score ≥8 indicates substantial anxious or depressive symptoms)[29,30], and the Bristol stool chart which was used to compute the IBS subtypes[24], based on the three most frequent habitual stool types.

done this under supervision of a dietician. Of this subgroup, 59% reported improvements in complaints after guidance by a dietician. Either currently or in the past, 460 (29%) participants reported to have followed the FODMAP diet, which was not significantly different between the IBS subtypes (p=.938), but again was between the severity groups, with a significantly higher percentage of severe IBS patients following the FODMAP diet (mild 20%, moderate 23%, severe 36%, p<.001). After following the FODMAP diet, 238 (52%) participants reported improvements in complaints.

Self-reported dietary trigger differences between IBS subtypes and severity groups

Figure 2A and 2B provide an overview of the prevalence of experiences with dietary triggers for the whole IBS population. The prevalence of "I don't know" ranged between 13-34% and the prevalence of "I don't use this" ranged between 0.5-46%. Response to yeast, spicy foods, potatoes, peppers, tomato, fish, citrus, alcohol and coffee was significantly different between the IBS subtypes (p<.05), but absolute differences were small (Supplementary Table 1). When stratified for IBS severity, all dietary triggers except fish (p=.085) had significantly different prevalence's of having no, mild or severe complaints between mild, moderate and severe IBS (Supplementary Table 2). In general, severe IBS patients more often reported a severe response to a dietary trigger, and mild IBS patients more often reported no complaints. Importantly, both mild, moderate and severe IBS identified the same five foods as most triggering, with a higher number of people in the severe group.

Associations between foodscore and IBS characteristics

Mean foodscore was 32.5±20, and did not differ significantly between the IBS subtypes (p=.073). In contrast to IBS subtypes, the foodscore did differ significantly between IBS severity groups, with a higher foodscore for those with more severe IBS (p<.001). As shown in Table 5, stratification revealed that the foodscore was also significantly different between gender, experiencing anxious or depressive symptoms versus not, and relatively low versus high IBS-QoL, but not for age groups.

IBS-SSS, IBS-QoL, anxiety and depression were significantly associated with the foodscore, even after adjustment of age, gender and BMI (Table 5). In other words, when a participant identified more food products as inducing severe symptoms, this was associated with a higher IBS severity, anxiety and depression score and a lower IBS-QoL. When depression and anxiety were added to the model, this did not change the results for IBS-SSS and IBS-QoL. IBS-QoL was also strongly associated with IBS-SSS (β= -0.118 [-0.128; -0.109], p<.001), this remained when depression and anxiety were added to the model (β= -0.089 [-0.098; -0.080], p<.001).

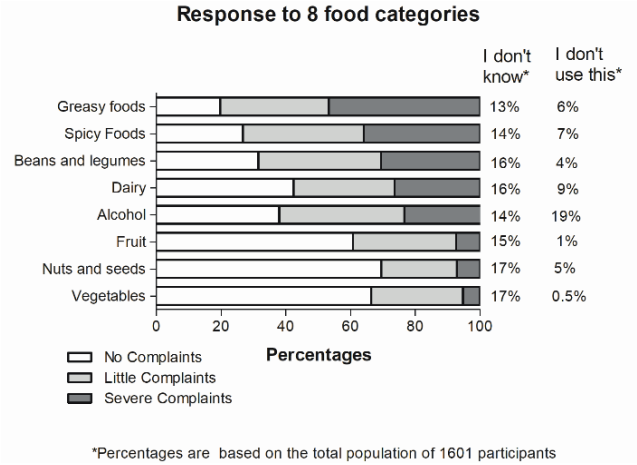

Figure 2A. Self-reported response to dietary triggers of 8 food categories from a cross-sectional online survey in 1601 Dutch IBS patients

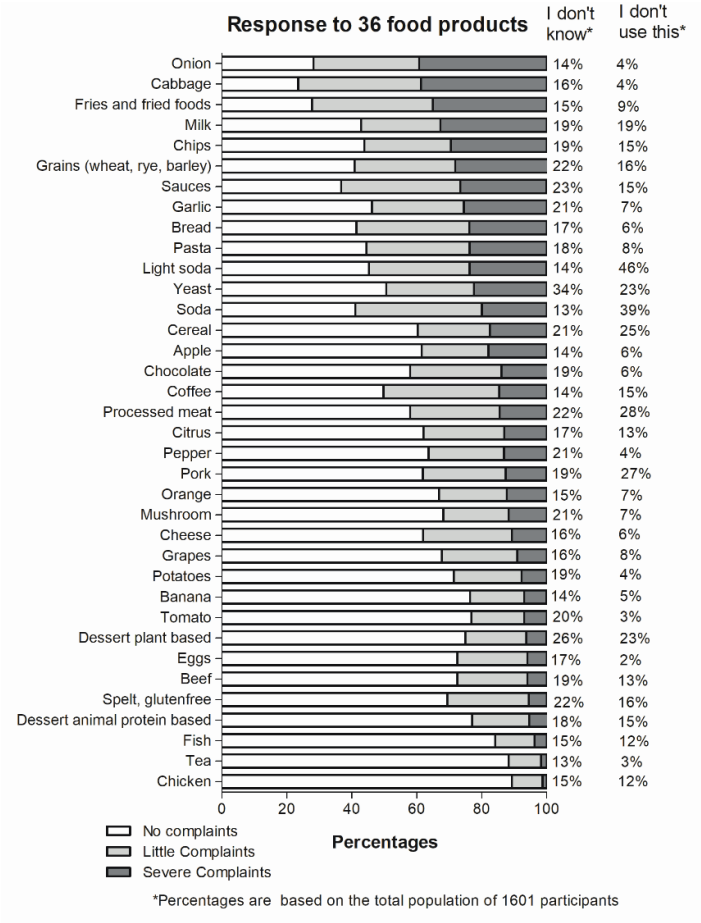

Figure 2B. Self-reported response to dietary triggers of 36 food products from a cross-sectional online survey in 1601 Dutch IBS patients. Patients indicated their response on a 3-point Likert scale “no complaints”, “little complaints” or “severe complaints”. Food categories and products are predefined using literature based on previously reported dietary triggers. Percentages given are excluded participants who indicated “I don’t know” or “I don’t use this”. IBS; Irritable Bowel Syndrome

Multiple correspondence analysis for crude dietary trigger data

The MCA score plot (Figure 3A and B) provides a two-dimensional explanation of variance between the responses to 44 dietary triggers, which showed a large variation between participants. Figure 3A showed no clustering of the IBS subtypes, indicating that the variation in response to dietary triggers is not explained by the IBS subtypes. Figure 3B again showed high variation between subjects but some clustering for patients with mild, moderate and severe IBS. This indicates that IBS severity explained more variation in response to dietary triggers than the IBS subtypes, however much variation remains unexplained.

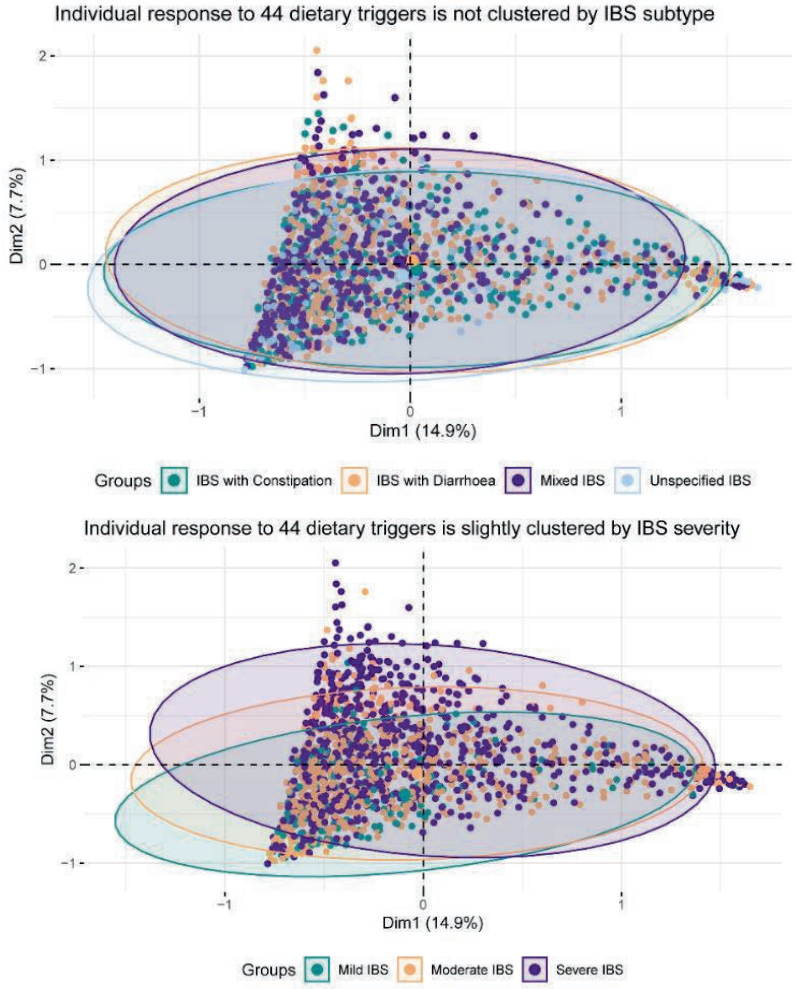

Figure 3A and 3B. Individual response to 44 dietary triggers, clustered by IBS subtypes or IBS severity, based on results from a cross-sectional online survey in 1601 Dutch IBS patients. Answer options “I don’t know” or “I don’t use this” are included in the analysis. Ellipses are drawn based on a 95% confidence interval.

Table 5. Foodscore stratified for IBS characteristics, and multiple linear regression analysis

Foodscore stratified			p-value
IBS subtypes	IBS-C (n=518)	30.9±19	.073
	IBS-D (n=531)	33.8±20	
	IBS-M (n=403)	33.1±20	
	IBS-U (n=75)	30.0±22	
IBS-SSS*	Mild (n=166)	20.9±17[a]	**.000**
	Moderate (n=629)	29.2±19[b]	
	Severe (n=732)	37.9±20[c]	
Age†	<47 years (n=774)	33.3±19	.095
	≥47 years (n=753)	31.6±21	
Gender	Male (n=263)	28.3±21	**.000**
	Female (n=1264)	33.4±20	
Having depressive symptoms‡ n (%)	No (n=1258)	31.4±20	**.000**
	Yes (n=269)	37.4±20	
Having anxious symptoms‡ n (%)	No (n=921)	30.7±20	**.000**
	Yes (n=606)	35.2±20	
IBS-QoL†	<77.9 (n=760)	38.5±19	**.000**
	>77.9 (n=767)	26.5±19	
Multiple linear regression		ß (95% CI)	p-value
IBS-QoL	Crude	-0.33 (-0.38; -0.28)	**.000**
	Model 1	-0.32 (-0.36; -0.27)	**.000**
	Model 2	-0.23 (-0.27; -0.19)	**.000**
IBS-SSS	Crude	1.39 (1.19; 1.59)	**.000**
	Model 1	1.34 (1.14; 1.54)	**.000**
	Model 2	1.16 (0.97; 1.36)	**.000**
Anxiety	Crude	0.03 (0.02; 0.04)	**.000**
	Model 1	0.03 (0.02; 0.04)	**.000**
Depression	Crude	0.03 (0.02; 0.04)	**.000**
	Model 1	0.03 (0.02; 0.04)	**.000**

Foodscore data: presented as mean±SD. Foodscore data is missing of 74 participants. Different superscripts indicate significance between the subgroups. P-values indicate differences between groups, and were tested using chi-square for categorical data, and for continuous data analysis of variance (ANOVA) and Bonferroni post-hoc for ≥3 groups or independent sample t-test for ≤2 groups was used. Abbreviations: IBS-C: IBS constipation predominant, IBS-D: IBS diarrhea predominant, IBS-M: IBS mixed, IBS-U: unspecified, IBS-SSS: Irritable Bowel Syndrome Symptom severity score, IBS-QoL: Irritable Bowel Syndrome quality of life, HADS: hospital anxiety and depression scale. †Subgroups were defined based on a median split. Multiple linear regression: data are reported as ß with 95% confidence intervals (CI), including the p-value of the ß. The foodscore was the independent variable, and IBS-QoL, IBS-SSS, anxiety and depression were dependent variables. Model 1: age, gender and Body Mass Index (BMI) were added. Model 2: like model 1 + anxiety and depression.

Discussion

This study found no clinically relevant differences in self-reported dietary trigger response between IBS subtypes and IBS severity subgroups. Symptom severity seems more suitable for classifying the response to dietary triggers, since IBS-SSS score was positively associated with the foodscore. This was also shown in MCA analysis, where symptom severity explained the variation in crude dietary trigger data better when compared to IBS subtypes, by showing some clustering highlighted by the ellipses. However, no difference in the five most triggering foods was seen between IBS severity groups. This indicates that there is no need for a specific dietary treatment based on IBS subtype or IBS severity, but that dietary treatment needs to be individualized under supervision of a dietician. The foodscore was statistically significantly different between men and women and those with or without signs of anxiety or depression, but differences were small, therefore clinical relevance is questionable. A larger difference in foodscore was seen between patients with a relatively low or high IBS-QoL, indicating that response to dietary triggers and IBS-QoL is associated.

Our study confirms previous findings that self-perceived food intolerance is not different between subtypes[16], but this time in a much larger population. A unique aspect of our study was the nationwide inventory of IBS complaints regarding nutrition, making our power high and our results more representative of the heterogenous IBS population. Although 1163 (36%) dropped out, this is much lower than previously reported in online surveys[33]. Our Dutch population is similar as in a previous European prevalence study, but with a slightly higher female predominance (82% compared to 63%)[2]. Our age and gender population characteristics also resemble previous self-reported food intolerance data [22, 23], indicating our study population is representative of the IBS population, and our results are therefore applicable also to a non-Dutch IBS population. About one third of our population discussed their diet with a dietician, which is higher than the 12% found in an Irish study[18]. We did not find data on dietician guidance in IBS in other countries, indicating the need for further research. The severity prevalence of our population is different than estimated by the Rome Foundation, as only 11% in our population was classified as mild IBS as opposed to the 45% that is estimated to be mild by the Rome Foundation[34]. However, previous studies have shown that severe IBS prevalence may range from 3-69%, depending on the population, and is likely to be underestimated[35]. Possibly, severe patients are more likely to participate in research than mild patients. Although our mild IBS prevalence is low, the number of patients and total sample size are sufficient enough to detect relevant differences between severity groups. In our study, we found that severe IBS patients respond to more dietary triggers more severely. This seems plausible, regardless of the dietary trigger. Causality remains the question, whether the more severe response is a

result of more severe IBS, or the other way around. Due to our cross-sectional observational data, we cannot assess this.

Several known associations, such as between IBS-QoL and IBS-SSS were confirmed in our study, suggesting our questionnaire was well constructed. When interpreting our results, we should not look only for significance; due to our large sample size many of our results reached statistical significance, but not all might be of clinical relevance. One limitation of our study is that we predefined our 44 dietary triggers based on previous research, therefore narrowing the search on beforehand. The amount of a dietary trigger consumed was not taken into account. The percentage of "I don't know" already ranged between 13-34% per food; probably a large percentage of IBS patients are unknown to their personal "threshold" of response to a dietary trigger, making it impossible to take this into account in a self-reported survey. This unknown threshold factor again highlights the importance of dietetic counselling, which can be a method to investigate personal thresholds of dietary triggers. Moreover, data on dietary triggers is self-reported. Although it is known that the placebo effect of diet in IBS is high, self-reported dietary trigger response data remains valuable due to the high impact on daily life of self-perceived complaints after food consumption. Moreover, the sensation of complaints remains similar, regardless whether there are mechanistic reactions or not.

The products that were identified as most important dietary triggers are in line with previous research[18, 22, 23]. Our percentages of "I don't know" ranged between 13-34% and "I don't use this" ranged between 0.5-46% for the different dietary triggers, which is quite high. However, for the 8 main food categories, only 0.6-15.5% of all participants reported to exclude products due to their abdominal complaints. This indicates that the high percentages of "I don't use this" are not explained by the changes participants made in the diet due to symptoms, but that participants do not use these products for other reasons.

Similar to Simren and colleagues, we combined dietary trigger data and computed a continuous foodscore[22], in order to scale how severe a patient responds to a number of products. Our foodscore was different on two important points: first, Simren and colleagues did not provide the option to answer don't use or don't know, which therefore may represent an over- or underestimation. Second, we standardized our foodscore to a scale of 0-100, which makes comparison between participants and future studies easier. However, the exclusion of "I don't know" or "I don't use this" answers may also be a disadvantage of our foodscore calculation. In theory, it is possible that a participant responds to one product severely, but reports "I don't know" to all other products, resulting in a foodscore of 100. However, only 13% of the patients indicated "I don't know" or "I don't use this" for ≥30 of the 44 dietary triggers. When we repeated our analysis without these participants, this did

not change our results (data not shown). In our study, we could not assess validity and reproducibility of the foodscore. However, assessing whether an IBS patient truly responds to a trigger is difficult to test, and no gold standard currently has been developed. Moreover, we did not assess reproducibility, as IBS complaints are variable[36], and therefore reproducibility may not be feasible or representative in this population.

Currently, most treatment plans are based on predominant stool type, but evidence for this is limited. Dietary fiber supplements are mainly advised for IBS-C patients[37], but most studies do not classify the IBS subtypes or only select IBS-C patients in their recruitment[38]. In our study, we did not find any difference in reported response to grains, bread, pasta, cereals, fruit or vegetables between IBS subtypes. For greasy foods, advice is targeted towards IBS-D and bloating patients[39], however, Calderella and colleagues have shown that both IBS-C and IBS-D experience gastro-intestinal symptoms after intraduodenal lipid infusion, but the type of complaints were different. IBS-C patients reported mainly cramping, while IBS-D patients mostly experienced an urgency to defecate[40]. We did not find clinically relevant differences between response to dietary triggers between the subtypes, aiding the hypothesis that diet therapy should not be based solely the Rome IV classifications. Possibly, current classifications are not suitable for identifying which patient will respond to diet therapy. More mechanistic evidence is needed to understand differences in responses between patients. Current dietary treatment plans should be individualized, and the low prevalence of IBS patients visiting a dietician should be increased, as IBS patients are also known to have a lower diet quality[41].

We confirmed the importance of management of mental health when treating IBS patients, as we showed a high scores of anxiety and depression, and an association with severity and IBS-QoL[42, 43]. Although the foodscore was significantly associated with anxiety and depression, beta's and R^2_{adj} were small, which makes its clinical relevance questionable. However, a recent study has shown that IBS symptom severity is strongly correlated with GI-specific anxiety and QoL, but not with general psychological features[44]. Possibly, general anxiety or depression are not associated with dietary trigger response, but GI-specific anxiety is. Nevertheless, (GI-specific) psychological factors are an important aspect to consider when treating IBS patients, as unrelieved pain and functional impairment are risk factors for developing anxiety and depression[45, 46].

In conclusion, our study showed that patients from different IBS subtypes and IBS severity groups do not identify different self-reported dietary triggers. However, IBS severity is associated with the number and severity to which patients respond to a dietary trigger. Moreover, anxiety and depression are important in management of

IBS symptoms, but there may not be a clinically relevant association with the response to dietary triggers. Our data does not support the need of a specific dietary advice for patients with different IBS subtype or IBS severity groups. Dietary treatments plans should be individualized under guidance of a dietician, and the prevalence of IBS patients visiting a dietician needs to be increased. Moreover, IBS severity seems to be a better classifier than the Rome IV criteria for IBS patients in regards to diet. Future studies should investigate new classifications that can identify responders for diet therapy.

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Supplementary Table 1. Self-reported Dietary triggers stratified for IBS subtype, based on results from a cross-sectional online survey in 1601 Dutch IBS patients

Dietary triggers	Complaints	IBS-C (n=545)	IBS-D (n=557)	IBS-M (n=420)	IBS-U (n=79)	p-value
Grains (whole wheat, rye, barley)	None	168 (31)	161 (29)	116 (28)	26 (33)	.411
	Little	109 (20)	133 (24)	96 (23)	20 (25)	
	Severe	100 (18)	107 (19)	101 (24)	18 (23)	
Grains (spelt, gluten free)	None	237 (43)	228 (41)	182 (43)	41 (52)	.074
	Little	80 (15)	101 (18)	61 (15)	6 (8)	
	Severe	12 (2)	22 (4)	17 (4)	4 (5)	
Bread	None	176 (32)	169 (30)	130 (31)	31 (39)	.085
	Little	140 (26)	160 (29)	112 (27)	12 (15)	
	Severe	89 (16)	94 (17)	89 (21)	19 (24)	
Pasta	None	176 (32)	188 (34)	135 (32)	29 (37)	.626
	Little	129 (24)	124 (22)	106 (25)	18 (23)	
	Severe	80 (15)	100 (18)	86 (21)	15 (19)	
Cereal	None	185 (34)	167 (30)	139 (33)	26 (33)	.337
	Little	69 (13)	69 (12)	42 (10)	11 (14)	
	Severe	40 (7)	55 (10)	46 (11)	9 (11)	
Yeast	None	113 (21)	115 (21)	90 (21)	25 (32)	**.027**
	Little	74 (14)	53 (10)	49 (12)	7 (9)	
	Severe	40 (7)	67 (12)	38 (9)	6 (8)	
Spicy foods	None	138 (25)	97 (17)	82 (20)	20 (25)	**.007**
	Little	140 (26)	181 (32)	125 (30)	23 (29)	
	Severe	134 (25)	162 (29)	135 (32)	22 (28)	
Vegetables	None	292 (54)	299 (54)	227 (54)	45 (57)	.717
	Little	120 (22)	138 (25)	107 (26)	21 (27)	
	Severe	24 (4)	22 (4)	22 (5)	1 (1)	
Cabbage	None	105 (19)	94 (17)	80 (19)	23 (29)	.249
	Little	162 (30)	178 (32)	127 (30)	19 (24)	
	Severe	158 (29)	169 (30)	144 (34)	26 (33)	
Onion	None	140 (26)	118 (21)	88 (21)	20 (25)	.153
	Little	137 (25)	155 (28)	114 (27)	18 (23)	
	Severe	154 (28)	177 (32)	156 (37)	24 (30)	
Garlic	None	194 (36)	172 (31)	144 (34)	23 (29)	.372
	Little	95 (17)	124 (22)	90 (21)	18 (23)	
	Severe	92 (17)	104 (19)	83 (20)	16 (20)	
Potatoes	None	317 (58)	293 (53)	223 (53)	50 (63)	**.011**
	Little	67 (12)	104 (19)	75 (18)	13 (17)	
	Severe	23 (4)	35 (6)	34 (8)	3 (4)	
Peppers	None	287 (53)	250 (45)	186 (44)	46 (58)	**.003**
	Little	85 (16)	108 (19)	77 (18)	10 (13)	
	Severe	38 (7)	63 (11)	53 (13)	5 (6)	
Tomato	None	323 (59)	307 (55)	258 (61)	58 (73)	**.016**
	Little	63 (12)	86 (15)	49 (12)	3 (4)	
	Severe	23 (4)	35 (6)	24 (6)	3 (4)	
Mushroom	None	266 (49)	266 (48)	211 (50)	39 (49)	.576
	Little	70 (13)	89 (16)	58 (14)	13 (17)	
	Severe	37 (7)	47 (8)	43 (10)	8 (10)	

Beans and legumes	None	128 (24)	136 (24)	111 (26)	26 (33)	.455
	Little	163 (30)	179 (32)	123 (29)	18 (23)	
	Severe	123 (23)	132 (24)	114 (27)	20 (25)	
Greasy foods	None	71 (13)	92 (17)	71 (17)	21 (27)	.084
	Little	158 (29)	152 (27)	107 (26)	17 (22)	
	Severe	194 (36)	215 (39)	167 (40)	28 (35)	
Sauces	None	122 (24)	120 (22)	100 (24)	23 (29)	.569
	Little	111 (20)	141 (25)	94 (22)	19 (24)	
	Severe	88 (16)	91 (16)	76 (18)	10 (13)	
Chocolate	None	235 (43)	228 (41)	183 (43)	46 (58)	.236
	Little	111 (20)	116 (21)	98 (23)	10 (13)	
	Severe	54 (10)	63 (11)	43 (10)	7 (9)	
Fries and fried foods	None	108 (20)	117 (21)	87 (21)	23 (29)	.211
	Little	162 (30)	152 (27)	116 (28)	19 (24)	
	Severe	125 (23)	159 (29)	122 (29)	17 (22)	
Chips	None	180 (33)	175 (31)	143 (34)	30 (40)	.580
	Little	111 (20)	133 (24)	87 (21)	15 (19)	
	Severe	56 (10)	66 (12)	57 (14)	8 (10)	
Dessert of animal protein	None	170 (31)	144 (26)	127 (30)	31 (39)	.053
	Little	102 (19)	101 (18)	78 (19)	6 (76)	
	Severe	97 (18)	120 (22)	85 (20)	15 (19)	
Plant based dessert	None	209 (38)	227 (41)	159 (38)	36 (46)	.989
	Little	46 (8)	54 (10)	38 (9)	6 (8)	
	Severe	15 (3)	15 (3)	11 (3)	3 (4)	
Beef	None	265 (49)	269 (48)	240 (57)	42 (53)	.264
	Little	66 (12)	81 (15)	50 (12)	8 (10)	
	Severe	15 (3)	25 (4)	23 (5)	5 (6)	
Eggs	None	318 (58)	311 (56)	261 (62)	45 (57)	.242
	Little	88 (16)	108 (19)	68 (16)	15 (19)	
	Severe	25 (5)	31 (6)	20 (5)	0 (0)	
Processed meat	None	148 (27)	143 (26)	145 (35)	26 (33)	.371
	Little	64 (12)	86 (15)	61 (15)	9 (11)	
	Severe	34 (6)	44 (8)	30 (7)	8 (10)	
Pork	None	173 (32)	178 (32)	156 (37)	28 (35)	.950
	Little	65 (12)	78 (14)	66 (16)	12 (15)	
	Severe	35 (6)	37 (7)	29 (7)	8 (10)	
Chicken	None	349 (64)	345 (62)	300 (71)	53 (67)	.172
	Little	29 (5)	50 (9)	28 (7)	4 (5)	
	Severe	3 (1)	7 (1)	5 (1)	0 (0)	
Fish	None	343 (63)	330 (59)	266 (63)	47 (60)	**.020**
	Little	36 (7)	64 (12)	34 (8)	8 (10)	
	Severe	11 (2)	15 (3)	18 (4)	0 (0)	
Dairy	None	193 (35)	157 (28)	129 (31)	28 (35)	.106
	Little	124 (23)	130 (23)	104 (25)	15 (19)	
	Severe	91 (17)	122 (22)	89 (21)	13 (17)	
Cheese	None	274 (50)	247 (44)	220 (52)	37 (47)	.217
	Little	104 (19)	138 (25)	87 (21)	15 (19)	
	Severe	41 (8)	51 (9)	36 (9)	6 (8)	
Milk	None	172 (32)	138 (25)	112 (27)	28 (35)	.058
	Little	81 (15)	90 (16)	78 (19)	9 (11)	
	Severe	106 (19)	133 (24)	90 (21)	15 (19)	

Fruit	None	288 (53)	271 (49)	210 (50)	47 (60)	.242
	Little	141 (26)	153 (28)	121 (29)	15 (19)	
	Severe	26 (6)	37 (7)	32 (8)	4 (5)	
Orange	None	288 (53)	281 (50)	211 (50)	49 (62)	.060
	Little	57 (16)	92 (17)	70 (17)	10 (13)	
	Severe	46 (8)	54 (10)	51 (12)	1 (1)	
Apple	None	269 (49)	260 (47)	210 (50)	46 (58)	.193
	Little	82 (15)	100 (18)	74 (18)	6 (8)	
	Severe	82 (15)	79 (14)	60 (14)	8 (10)	
Banana	None	342 (63)	343 (62)	255 (61)	54 (68)	.370
	Little	71 (13)	77 (14)	62 (15)	7 (9)	
	Severe	34 (6)	24 (4)	30 (7)	3 (4)	
Grapes	None	295 (54)	273 (49)	212 (51)	40 (51)	.161
	Little	89 (16)	110 (20)	71 (17)	11 (14)	
	Severe	31 (6)	33 (6)	38 (9)	7 (9)	
Citrus	None	256 (47)	223 (40)	183 (44)	41 (52)	**.013**
	Little	95 (17)	104 (19)	73 (17)	10 (13)	
	Severe	36 (7)	63 (11)	46 (11)	3 (4)	
Alcohol	None	152 (28)	120 (22)	109 (26)	24 (30)	**.033**
	Little	148 (27)	138 (25)	110 (26)	18 (23)	
	Severe	70 (13)	107 (19)	61 (15)	12 (15)	
Coffee	None	203 (37)	170 (31)	164 (39)	26 (33)	**.040**
	Little	143 (26)	152 (27)	90 (21)	19 (24)	
	Severe	45 (8)	66 (12)	48 (11)	7 (9)	
Tea	None	413 (76)	401 (72)	309 (74)	62 (79)	.436
	Little	37 (7)	53 (10)	39 (9)	5 (6)	
	Severe	6 (1)	7 (1)	9 (21)	1 (1)	
Soda	None	103 (19)	109 (20)	85 (20)	15 (19)	.969
	Little	100 (18)	102 (18)	82 (20)	12 (15)	
	Severe	51 (9)	52 (9)	44 (11)	4 (5)	
Soda light	None	93 (17)	99 (18)	81 (19)	13 (17)	.982
	Little	64 (12)	67 (12)	55 (13)	10 (13)	
	Severe	54 (10)	50 (9)	41 (10)	5 (6)	
Nuts and seeds	None	296 (54)	313 (56)	218 (52)	42 (53)	.468
	Little	98 (18)	96 (17)	87 (21)	11 (14)	
	Severe	24 (4)	31 (6)	5 (6)	5 (6)	

Data is showed as n (%). Participants who indicated “I don’t know” or “I do not use this product” are not shown. Data was tested using chi-square.
IBS-C: IBS with predominantly constipation, IBS-D: IBS with predominantly diarrhea, IBS-M: IBS alternating between diarrhea and constipation, IBS-U: IBS unspecified

Supplementary Table 2. Self-reported dietary triggers stratified for IBS severity, based on results from a cross-sectional online survey in 1601 Dutch IBS patients

Dietary triggers	Complaints	Mild IBS (n=174)	Moderate IBS (n=661)	Severe IBS (n=766)	p-value
Grains (whole wheat, rye, barley)	None	81 (47)	224 (34)	166 (22)	**.000**
	Little	31 (18)	150 (23)	177 (23)	
	Severe	16 (9)	108 (16)	202 (26)	
Grains (spelt, gluten free)	None	89 (51)	299 (45)	300 (39)	**.000**
	Little	11 (6)	89 (13)	148 (19)	
	Severe	3 (2)	15 (2)	37 (5)	
Bread	None	84 (48)	239 (36)	183 (24)	**.000**
	Little	38 (22)	173 (26)	213 (28)	
	Severe	18 (10)	89 (13)	184 (24)	
Pasta	None	86 (49)	248 (37)	194 (25)	**.000**
	Little	30 (17)	149 (22)	198 (26)	
	Severe	15 (9)	85 (13)	181 (24)	
Cereal	None	82 (47)	246 (37)	189 (25)	**.000**
	Little	13 (7)	66 (10)	112 (15)	
	Severe	11 (6)	45 (7)	94 (12)	
Yeast	None	52 (30)	166 (25)	125 (16)	**.000**
	Little	20 (11)	77 (12)	86 (11)	
	Severe	10 (6)	49 (7)	93 (12)	
Spicy foods	None	60 (34)	149 (22)	128 (17)	**.000**
	Little	53 (30)	209 (32)	207 (27)	
	Severe	19 (11)	171 (26)	263 (34)	
Vegetables	None	128 (74)	384 (58)	351 (46)	**.000**
	Little	20 (11)	138 (21)	228 (30)	
	Severe	2 (1)	17 (3)	50 (6)	
Cabbage	None	57 (33)	137 (21)	108 (14)	**.000**
	Little	53 (30)	218 (33)	215 (28)	
	Severe	32 (18)	178 (27)	387 (37)	
Onion	None	68 (39)	174 (26)	124 (16)	**.000**
	Little	42 (24)	185 (28)	197 (26)	
	Severe	36 (21)	185 (28)	290 (38)	
Garlic	None	85 (49)	250 (38)	198 (26)	**.000**
	Little	27 (15)	127 (19)	173 (23)	
	Severe	20 (11)	103 (16)	172 (23)	
Potatoes	None	118 (68)	397 (60)	368 (48)	**.000**
	Little	18 (10)	94 (14)	147 (19)	
	Severe	6 (3)	24 (4)	65 (8)	
Peppers	None	107 (61)	340 (51)	322 (42)	**.000**
	Little	21 (12)	105 (16)	154 (20)	
	Severe	12 (7)	61 (9)	86 (11)	
Tomato	None	123 (71)	424 (64)	399 (52)	**.000**
	Little	10 (6)	67 (10)	124 (16)	
	Severe	7 (4)	29 (4)	49 (6)	
Mushroom	None	108 (62)	353 (53)	321 (42)	**.000**
	Little	17 (10)	77 (12)	136 (18)	
	Severe	7 (4)	47 (7)	81 (11)	

Beans and legumes	None	63 (36)	182 (27)	156 (20)	**.000**
	Little	57 (33)	202 (31)	224 (29)	
	Severe	29 (17)	145 (22)	215 (28)	
Greasy foods	None	38 (22)	106 (16)	111 (14)	**.000**
	Little	56 (32)	196 (30)	182 (24)	
	Severe	42 (24)	239 (36)	323 (42)	
Sauces	None	63 (36)	170 (26)	132 (17)	**.000**
	Little	37 (21)	136 (21)	192 (25)	
	Severe	13 (7)	93 (14)	159 (21)	
Chocolate	None	100 (57)	320 (48)	272 (35)	**.000**
	Little	25 (14)	128 (19)	182 (24)	
	Severe	9 (5)	56 (8)	102 (13)	
Fries and fried foods	None	59 (34)	137 (21)	139 (18)	**.000**
	Little	49 (28)	198 (30)	202 (26)	
	Severe	19 (11)	157 (24)	247 (32)	
Chips	None	83 (48)	226 (34)	219 (29)	**.000**
	Little	23 (13)	148 (22)	175 (23)	
	Severe	11 (6)	54 (8)	122 (16)	
Dessert of animal protein	None	84 (48)	222 (34)	166 (22)	**.000**
	Little	27 (15)	110 (17)	150 (20)	
	Severe	21 (12)	110 (17)	186 (24)	
Plant based dessert	None	83 (48)	282 (43)	266 (35)	**.000**
	Little	9 (5)	37 (6)	98 (13)	
	Severe	2 (1)	11 (2)	31 (4)	
Beef	None	113 (65)	365 (55)	338 (44)	**.000**
	Little	11 (6)	69 (10)	125 (16)	
	Severe	2 (1)	20 (3)	46 (6)	
Eggs	None	126 (72)	406 (61)	403 (53)	**.000**
	Little	10 (11)	104 (16)	155 (20)	
	Severe	5 (3)	28 (4)	43 (6)	
Processed meat	None	76 (44)	226 (34)	160 (21)	**.000**
	Little	9 (5)	91 (14)	120 (16)	
	Severe	6 (3)	33 (5)	77 (10)	
Pork	None	84 (48)	251 (38)	200 (6)	**.000**
	Little	10 (6)	82 (12)	129 (17)	
	Severe	5 (3)	38 (6)	66 (9)	
Chicken	None	129 (74)	447 (68)	471 (61)	**.000**
	Little	5 (3)	32 (5)	74 (10)	
	Severe	0 (0)	7 (1)	8 (1)	
Fish	None	118 (68)	437 (66)	431 (56)	.085
	Little	10 (6)	53 (8)	79 (10)	
	Severe	6 (3)	18 (3)	20 (3)	
Dairy	None	86 (49)	242 (37)	179 (23)	**.000**
	Little	35 (20)	153 (23)	185 (24)	
	Severe	23 (13)	105 (16)	187 (24)	
Cheese	None	114 (65)	354 (54)	310 (40)	**.000**
	Little	24 (14)	127 (19)	193 (25)	
	Severe	8 (5)	48 (7)	78 (10)	
Milk	None	74 (42)	211 (32)	165 (21)	**.000**
	Little	21 (12)	112 (17)	125 (16)	
	Severe	24 (14)	126 (19)	194 (25)	

Fruit	None	106 (61)	383 (58)	327 (43)	**.000**
	Little	30 (17)	159 (24)	241 (31)	
	Severe	7 (4)	22 (3)	70 (9)	
Orange	None	110 (63)	368 (56)	351 (46)	**.000**
	Little	16 (9)	107 (16)	136 (18)	
	Severe	6 (3)	44 (7)	102 (13)	
Apple	None	99 (57)	360 (54)	326 (43)	**.000**
	Little	19 (11)	103 (16)	140 (18)	
	Severe	14 (8)	71 (11)	144 (19)	
Banana	None	125 (72)	437 (66)	432 (56)	**.000**
	Little	7 (4)	82 (12)	128 (17)	
	Severe	8 (5)	24 (4)	59 (8)	
Grapes	None	105 (60)	368 (56)	347 (45)	**.000**
	Little	20 (11)	112 (17)	149 (19)	
	Severe	3 (2)	29 (4)	77 (10)	
Citrus	None	90 (52)	336 (51)	277 (36)	**.000**
	Little	19 (11)	103 (16)	160 (21)	
	Severe	6 (3)	48 (7)	94 (12)	
Alcohol	None	70 (46)	181 (27)	144 (19)	**.000**
	Little	41 (24)	197 (30)	176 (23)	
	Severe	5 (3)	104 (16)	141 (19)	
Coffee	None	89 (51)	239 (36)	235 (31)	**.000**
	Little	36 (21)	186 (28)	182 (24)	
	Severe	6 (3)	64 (10)	96 (12)	
Tea	None	145 (83)	512 (77)	528 (69)	**.000**
	Little	6 (3)	42 (6)	86 (11)	
	Severe	0 (0)	4 (1)	19 (2)	
Soda	None	54 (31)	136 (21)	122 (16)	**.000**
	Little	21 (12)	118 (18)	157 (20)	
	Severe	5 (3)	50 (8)	96 (12)	
Soda light	None	48 (28)	126 (19)	112 (15)	**.000**
	Little	13 (7)	78 (12)	105 (14)	
	Severe	8 (5)	49 (7)	93 (12)	
Nuts and seeds	None	115 (66)	377 (57)	377 (49)	**.006**
	Little	19 (11)	118 (18)	155 (20)	
	Severe	8 (5)	37 (6)	44 (6)	

Data is showed as n (%). Participants who indicated “I don’t know” or “I do not use this product” are not shown. Data was tested using chi-square.

CHAPTER 3

FECAL MICROBIOTA SIGNATURES ARE NOT CONSISTENTLY RELATED TO SYMPTOM SEVERITY IN IRRITABLE BOWEL SYNDROME

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Abstract

Background: Irritable Bowel Syndrome (IBS) is the most prevalent functional bowel disorder, but its pathophysiology is still unknown. Although a gut microbial signature associated with IBS severity has been suggested, its association with IBS severity still remains largely unknown.
Aims: This study aims to assess longitudinal dynamics of gut microbiota and short chain fatty acids (SCFAs) in different IBS severity groups, and study the association with stool pattern, diet, depression, anxiety and quality of life (QoL).
Methods: A longitudinal study was performed, including n=91 IBS patients and n=28 matched controls. All participants collected fecal samples and completed validated questionnaires regarding IBS severity, stool pattern, depression, anxiety and IBS-QoL at two timepoints with four weeks in-between. Diet was assessed at the first timepoint. Gut microbiota composition was determined by sequencing the V4 region of the 16S ribosomal RNA gene, whereas SCFAs were analyzed by High-Performance Liquid Chromatography.
Results: Over time, 36% of IBS patients changed in severity group, and 53% changed in predominant stool pattern. The largest proportion of microbiota variation was explained by the individual (R^2=70.07%). Microbiota alpha diversity and composition, and SCFAs did not consistently differ between IBS severity groups, nor between IBS and controls. Relative abundances of *Bifidobacterium, Terrisporobacter* and *Turicibacter* consistently differed between IBS and controls, but not between IBS severity groups. Large dynamics over time were observed in the association of microbiota composition with questionnaire data, such as symptom severity.
Conclusions: Gut microbiota and SCFA signatures were not consistently associated with IBS severity over time, indicating the importance of repeated sampling in IBS research.

Keywords: Irritable Bowel Syndrome; Gut Microbiota composition; Short-chain Fatty acids; Severity

Introduction

Irritable bowel syndrome (IBS) is the most commonly diagnosed functional gastrointestinal disorder, with a global prevalence around 11%[1]. IBS reduces quality of life (QoL) and increases health care costs[2]. Several factors have been associated with IBS, such as alterations in the gut-brain axis[3], visceral hypersensitivity[4], increased intestinal permeability[5] and altered gut microbiota composition[6-8]. Different studies have been performed to identify gut microbial signatures in IBS patients, but a general consensus in IBS-related profiles is lacking[9]. This inconsistency could be due to the large individual variation in gut microbiota composition and cohort-specific characteristics[10], as well as cross-sectional study designs. Moreover, there is large variation in symptom severity and stool pattern within and between individuals with IBS[11], and instability in gut microbiota over time[12, 13]. Furthermore, studies often do not include other covariates like diet and psychological state, which can be different in IBS and are associated with gut microbiota[9, 14, 15].

Recently, Tap et al (2017) were the first to explore IBS symptom severity related to the gut microbiota, and they cross-sectionally identified a gut microbial signature of 90 operational taxonomic units (OTUs) associated with IBS severity[16], which provides a new research direction to investigate microbial signatures with taking IBS severity into consideration. Although a recent study by Mars and colleagues longitudinally identified species-level taxa associated to severity of symptoms in diarrhea-predominant IBS[17], the consistency of gut microbial signatures associated with IBS symptom severity over time still remains unknown. Moreover, alterations in fecal short-chain fatty acids (SCFAs), including acetate, propionate and butyrate, have also been observed between IBS and controls[18]. These alterations were associated with bloating, abdominal pain and QoL in IBS[19]. However, the association of SCFA with IBS severity and its consistency over time are unknown.

Therefore, we investigated the dynamics of gut microbiota and SCFA levels in different IBS severity groups compared to controls, and the association with stool pattern, diet, depression, anxiety and QoL over a period of four weeks. These dynamics and associations were also investigated between IBS and controls. We hypothesized that over time, IBS severity would demonstrate a regression to the mean, while gut microbiota signatures associated with severity would remain stable.

Methods

This was an observational longitudinal study with two timepoints (T1 and T2) with four weeks in-between, and included IBS patients and controls who were matched for age, gender and Body Mass Index (BMI) at T1 (no significance at group level). All participants signed an informed consent. The study was approved by the medical

ethical committee of Wageningen and was registered at Clinicaltrials.gov (NCT03720314, https://clinicaltrials.gov/ct2/show/NCT03720314).

Study participants

Participants were recruited using the Wageningen University subject database, and recruitment calls on websites and social media. Participants were aged 18-65 years, lived near Wageningen and had a BMI between 18.5-30.0 kg/m^2. IBS patients had to fulfill the Rome IV criteria or had to be diagnosed with IBS by a physician. Exclusion criteria were presence of any other gastrointestinal or systemic diseases, antibiotics use <3 months before study start, pregnancy or breastfeeding. We aimed to include 100 IBS patients and 30 matched controls at T1, to be able to detect a difference of 3.6±4.9% in similarity of microbiota over time[12]. After T1, the 30 IBS patients with the least symptoms and 30 with the most severe symptoms were selected for T2, to assess the regression to the mean hypothesis and gut microbiota dynamics. Controls completed both timepoints.

Gut microbiota profiling

Participants collected a fecal sample at both timepoints. After collection, the fecal material was immediately stored in the participants' home freezer. Fecal samples were transported on dry ice by research staff to the laboratory on average within 1.1±1.2 days, where it was stored immediately at -80ºC until further analysis.

Gut microbiota composition was determined by sequencing the V4 region of the 16S ribosomal RNA (rRNA) gene (Illumina Hiseq2500, 150bp paired end). As previously described, 0.25g feces (wet weight) was used for DNA isolation with the Repeated Bead Beating method[21]. Subsequently, DNA was purified using the Maxwell® 16 Total RNA system (Promega, Madison, WI, USA) with the 16 Tissue LEV Total RNA purification Kit Cartridge (XAS1220). Amplification was performed in duplicate with uniquely barcoded primers[22] 515F (5'-GTGYCAGCMGCCGCGGTAA-3')[23] and 806R (5'-GGACTACNVGGGTWTCTAAT-3')[24]. Reaction conditions and library preparation were performed as described previously[22]. Afterwards, the libraries were purified with the CleanPCR kit (CleanNA, The Netherlands), and sent to Eurofins Genomics Germany GmbH (Konstanz, Germany) for sequencing. NG-Tax 2.0 was used to process the raw sequencing data for Amplicon Sequencing Variant (ASV) picking with default settings and for taxonomic assignments using the SILVA database (version 128)[25, 26]. Sequencing data was submitted to the European Nucleotide Archive with accession number PRJEB44533.

SCFA profiling

SCFAs were measured as described previously with minor modifications[27]. In total, 0.4 grams of feces (wet weight) were mixed thoroughly with 1.6 mL ultrapure water to extract SCFAs (acetate, propionate, and butyrate). Subsequently, the mixture was centrifuged (21130 × g, 10 minutes) to get the supernatant. Subsequently, 0.4mL supernatant was added to 0.6mL 10mM DMSO as the internal standard in 0.1N H_2SO_4 solution, and analyzed by High-Performance Liquid Chromatography (HPLC, LC-2030C, Shimazu, Kyoto, Japan) with a Shodex SH1821 column (Showa Denko K.K., Tokyo, Japan).

Questionnaires

Both IBS and controls completed all questionnaires at T1 and T2 for comparison. IBS severity was assessed using the validated IBS Symptom Severity Score (IBS-SSS), which was used to classify severity (no symptoms ≤75; mild=76-175; moderate=176-300, and severe >300 IBS)[28]. Both of the continuous severity score and severity grouping were used in analysis. QoL was assessed with the 34-item IBS-QoL, which gave a score for total IBS-QoL, and subscales dysphoria, interference with activity, body Image, health worry, food avoidance, social reaction, sexual life and relationship[29]. Participants completed the Hospital Anxiety and Depression Score (HADS)[30]. A score ≥8 indicated substantial depressive or anxious symptoms[31]. Furthermore, the predominant stool pattern of the previous week was assessed by letting participants rank their stools of the week before sampling from most to least frequent, using the seven types of the validated Bristol Stool chart[32]. Participants also indicated the Bristol stool scale of the gut sample. Habitual dietary intake of the previous month was assessed at T1 using a semi-quantitative 83-item Food Frequency Questionnaire[33, 34]. Dietary intake was calculated using the Dutch Food Composition table[35]. Furthermore, IBS patients were asked if they were currently following the Fermentable Oligo, Disaccharides, Monosaccharides, And Polyols (FODMAP) diet. Participants were instructed to keep their diet similar during the study period.

Statistical analyses

Microbiota data was analyzed in R version 4.0.0[36] and questionnaire data in SPSS version 25 (Armonk, NY, USA: IBM Corp.). Continuous data are presented as mean ± standard deviation, or median and interquartile range when skewed. Categorical data are presented as counts and percentages. Differences in the questionnaire data between IBS and controls were tested with an independent sample T-test or Mann-Whitney U test when not normally distributed. Differences in questionnaire data between IBS subgroups and controls were tested with a one-way Analysis of Variance (ANOVA), or a Wilcoxon test when not normally distributed, with Bonferroni corrected post-hoc testing. An unpaired Wilcoxon test was used to test differences

in SCFAs between IBS subgroups and controls, or between IBS and controls. Differences for categorical data were assessed using chi-square tests. Associations of acetate, propionate and butyrate (dependent variable) with questionnaire data (independent variables) were determined using linear mixed models.

Alpha diversity (within sample diversity) and beta diversity (between sample diversity) were calculated at ASV level using Phyloseq[37]. Alpha diversity metrics, ASV richness and Shannon diversity were calculated. To visualize beta diversity, Principle Coordinate analysis (PCoA) based on unweighted (considering presence/absence of ASVs) and weighted (considering ASVs and their relative abundance) Unifrac[38] distances was performed. A unpaired Wilcoxon test was used to compare genus-level taxa between IBS or different severity groups with controls at both timepoints. The p-values for multiple pairwise tests were corrected for multiple testing using Benjamini-Hochberg false-discovery rate (FDR). The Vegan package[39] was used to assess the association of microbiota composition with questionnaire and dietary variables, using Permutational Multivariate Analysis of Variance (PERMANOVA). A (corrected) p-value ≤0.05 was considered statistically significant, and 0.05≤ (corrected) p-value <0.1 was considered a trend.

Results

A total of n=91 IBS and n=30 controls participated, with n=55 IBS and n=28 controls included for longitudinal analyses (Figure 1). Baseline characteristics differed between IBS and controls for IBS-SSS (p<.001), IBS-QoL (p<.001), anxiety (p=.001) and depression (p=.004, Table 1). When data was stratified for IBS severity and compared with controls, IBS-QoL, anxiety and depression remained different (p's<.05). Distribution of the predominant stool patterns was not different between IBS severity groups. There was no significant difference in dietary intake of energy, fat, carbohydrates, polysaccharides, dietary fiber, alcohol or water between IBS and controls (Supplementary Table 1). However, IBS patients had a lower intake of protein, maltose and lactose. Moreover, 22%

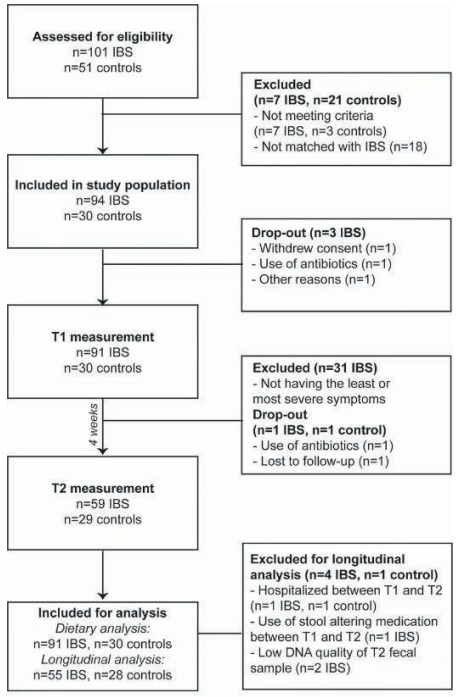

Figure 1. Flowchart of participant recruitment

of IBS patients followed a FODMAP for whom lactose intake was significantly lower to those not following a FODMAP diet (p=.007).

Table 1. Baseline characteristics of the study population

	IBS (n=91)	Control (n=30)	P-value
Age, years	41.7±14.4	39.4±16.9	.500
Gender, n (%) male	19 (21)	4 (13)	.361
BMI, kg/m^2	22.9±2.9	23.2±3.1	.566
IBS-SSS	150 (110 – 230)	55 (27 – 90)	**.000**
Severity groups, n (%)			**.000**
No symptoms (≤75)	7 (8)	19 (63)	
Mild (76-175)	48 (53)	11 (37)	
Moderate (176-300)	24 (26)	0 (0)	
Severe (>300)	12 (13)	0 (0)	
Predominant stool pattern, n (%)			.183
Constipation	26 (29)	14 (47)	
Diarrhoea	32 (35)	6 (20)	
Mixed	19 (21)	4 (13)	
Unspecified	14 (15)	6 (20)	
IBS-QoL	72.2±16.8	98.6±3.6	**.000**
Anxiety			
Score	6.0 (3.0 – 10.0)	4.0 (2.8 – 6.0)	**.001**
Substantial anxious symptoms, n (%)†	35 (38)	3 (10)	**.004**
Depression			
Score	2.0 (1.0 – 5.0)	1.0 (0.0 – 2.2)	**.004**
Substantial depressive symptoms, n (%)†	9 (10)	2.0 1 (3)	.258

Data are presented as mean±standard deviation or median (interquartile range) when skewed. Abbreviations: BMI, Body Mass Index; IBS-SSS, IBS Symptom Severity Score; IBS-QoL, IBS Quality of Life. †Based on the Hospital Anxiety and Depression Score (HADS) cut-off >8.

Instability of IBS symptom severity over time

Instability of the IBS severity score was observed over time (Figure 2A). The severity score decreased by ≥100 in 9 IBS patients and increased by ≥ 100 in 5 IBS patients (Figure 2B). Furthermore, over time, 20 (36%) of the IBS patients changed in severity groups (Supplementary Figure 1A). For other IBS symptoms, a large variation over time was also observed: 29 (53%) of the IBS patients changed in their predominant stool pattern (Supplementary Figure 1B).Total IBS-QoL (Δ=2.4, p=.028), and sub-scores dysphoria (Δ=3.1, p=.032), body image (Δ=3.9, p=.004) and impact on relationship (Δ=4.1, p=.010) increased, while other IBS-QoL sub-scores, anxiety and depression remained stable over time.

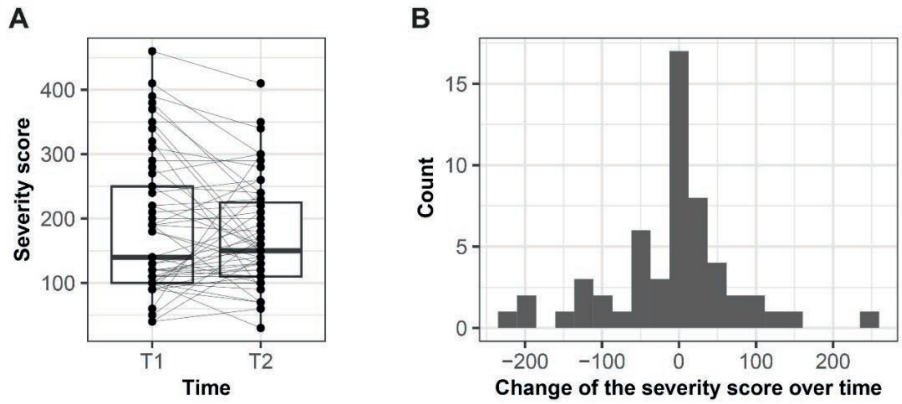

Figure 2. Difference in severity score over time

No difference in microbiota alpha and beta diversity between IBS severity groups and controls

ASV richness (Supplementary Figure 2) and Shannon diversity were not different between IBS and controls (Figure 3A), nor between IBS severity groups and controls (Figure 3B) at neither timepoints. This indicates the number and distribution of microbial ASVs is similar between these groups. PERMANOVA based on unweighted and weighted Unifrac distance (Figure 3F) revealed no significant difference between IBS severity groups and controls. Between IBS and controls, the unweighted Unifrac based observation was similar, while based on weighted Unifrac, a trend at T1 (p=.073) and T2 (p=.064) was observed (Figure 3C). This indicates that the relative abundance of microbial taxa plays a role in the differences of microbiota composition between IBS and controls, rather than the presence or absence of the microbial taxa. Longitudinally, the change in gut microbiota composition in IBS and controls was not different (Figure 3D, p=0.27), indicating that the temporal stability of the microbiota of IBS patients was similar to that of controls. Moreover, microbiota variation over time was not associated with a change in severity score (Figure 3E).

Differences in genus level taxa between IBS and controls were not associated with IBS symptom severity

Relative abundances were different for ten genus level taxa at T1 and eight at T2 between IBS and controls (Figure 4A, Supplementary Table 2). However, of those only *Bifidobacterium*, *Terrisporobacter* and *Turicibacter* were consistently different over time. The relative abundance of *Bifidobacterium* was lower in IBS compared to controls (p_{T1}=.0003; p_{T2}=.0003). In contrast, the relative abundances of

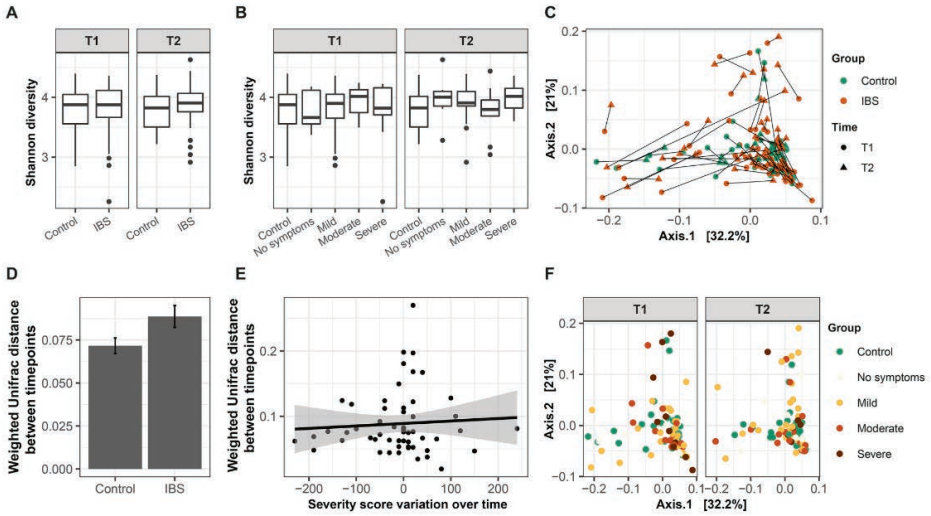

Figure 3. Analysis of gut microbiota alpha and beta diversity for IBS patients and controls and severity groups over time. Shannon diversity displayed as interquartile with boxplot, stratified for IBS and controls (*A*), symptom severity groups and controls (*B*). PCoA of gut microbiota composition based on weighted Unifrac distances, stratified for IBS and controls. Samples taken at different timepoints are connected by solid lines per subject (*C*). Comparison of gut microbiota composition stability based on weighted Unifrac distances over time between IBS patients and controls Values are presented as mean±standard error (*D*). Linear model indicated no association between the changed severity score and weighted Unifrac distance of gut microbiota composition over time (*E*). PCoA of gut microbiota composition based on weighted Unifrac distances, stratified for symptom severity groups and controls (*F*).

Terrisporobacter (p_{T1}=.010; p_{T2}=.004) and *Turicibacter* (p_{T1}=.042; p_{T2}=.0013) were consistently higher in IBS patients over time. However, these taxa were not associated with IBS severity (Figure 4B, Supplementary Table 3). The relative abundances of five genus level taxa at T1 and seven at T2 were significantly different within the severity groups or between severity groups and controls. Remarkably, one uncultured taxon within the *Tenericutes* phylum (p=.021) and *Prevotellaceae_UCG-001* (p=.021) were significantly higher in severe IBS patients at T2 compared to controls. However, none of these taxa were consistently different between IBS severity groups. In addition, the change of the genus-level taxa over time was not correlated with the change of the severity score over time (Supplementary Table 4). Next to IBS severity, we also assessed associations between gut microbiota composition and predominant stool patterns in IBS over time. Some genus-level taxa were associated with predominant stool patterns, such as *Alistipes* with constipation. However, in line with the IBS severity observations these associations were only observed at a single timepoint, and not consistent over time (Supplementary Table 5).

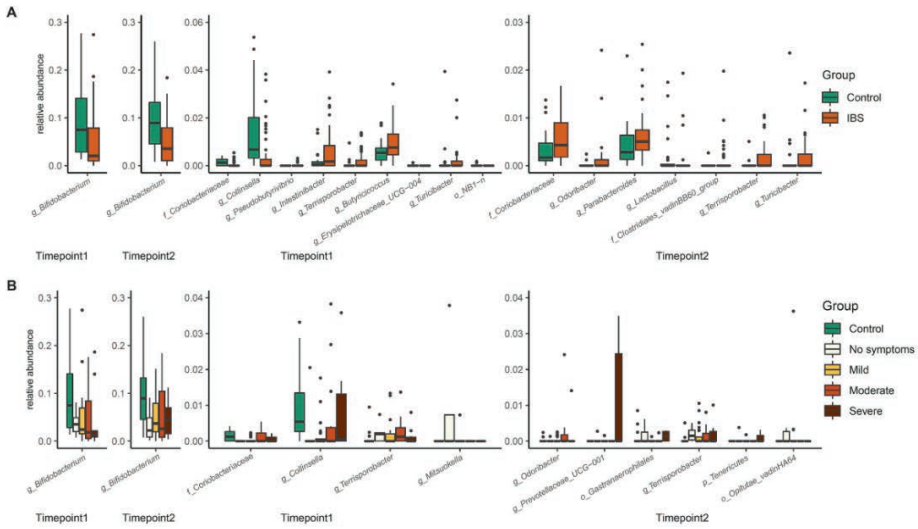

Figure 4. Genus level taxa that significantly differed in relative abundance between IBS patients and controls (*A*), or between severity groups and controls (*B*). Data are presented as interquartile range with boxplot.

Dynamics of the association between questionnaire data and microbiota composition over time

The largest proportion of microbiota variation was explained by the individual (R^2=70.07%), when data of both timepoints was included. Age was significantly and consistently associated with gut microbiota composition at T1 (R^2=2.75%) and T2 (R^2=3.63%, Figure 5). IBS explained a stable proportion of gut microbiota variation over time (R^2_{T1}=2.41%, R^2_{T2}=2.30%). At T1, Bristol stool scale and symptom severity score were significantly associated to gut microbiota composition, and explained, respectively, the first and second largest proportion of gut microbiota variation out of all participant characteristics ($R^2_{\text{Bristol stool}}$=3.69%, R^2_{Severity}=3.17%). However, at T2 the proportion of variation explained by Bristol stool scale and symptom severity score decreased, and was not significant anymore. This indicates large dynamics of explained gut microbiota variation by participant characteristics over time. We did not observe an association between dietary intake and gut microbiota variation. Moreover, no correlations were found between macronutrient, lactose or maltose intake and relative abundance of *Bifidobacterium, Terrisporobacter* and *Turicibacter*.

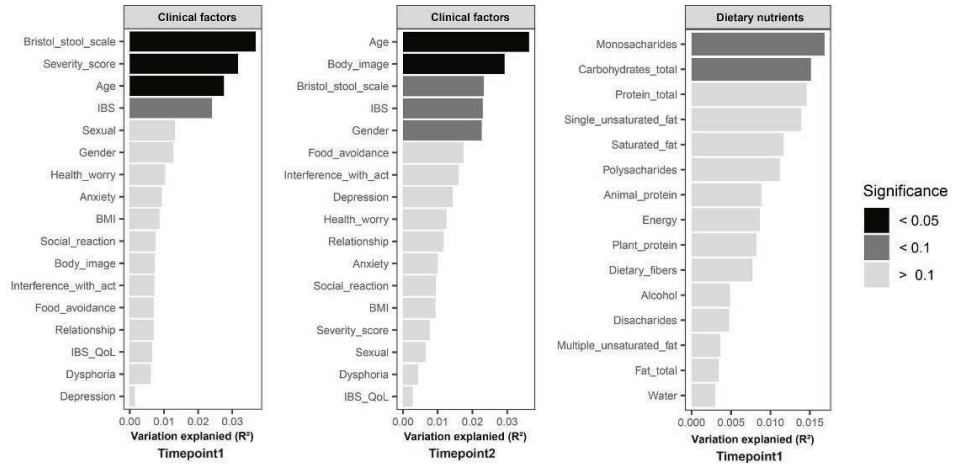

Figure 5. Large dynamics in explained variation of microbiota composition based on weighted Unifrac distances by clinical factors and dietary nutrients.

No difference in SCFAs between IBS, controls and severity groups

Large within and between person variation in SCFA levels was observed at both timepoints (Figure 6), but no differences were found between IBS severity groups or IBS and controls. When subjects were stratified based on the predominant stool pattern, acetate was consistently higher in diarrhea-predominant IBS compared to patients with constipation (p_{T1} =.000; p_{T2}=.008). Linear mixed model analysis revealed that acetate, propionate and butyrate were not associated with psychological factors over time (Supplementary Table 6). No correlations were observed between diet and SCFAs, except for lactose intake and butyrate (r=-.182, p=.046, Supplementary Table 7).

Discussion

We performed a longitudinal study to examine the dynamics of gut microbiota and SCFA levels between IBS severity and controls over time. Moreover, we assessed the association between gut microbiota and SCFAs with stool pattern, IBS-QoL, anxiety, depression and diet. We did not find any difference in gut microbial alpha diversity, composition or SCFAs between the control and IBS severity groups. Multiple taxa were significantly different in relative abundance between IBS and controls were found at different timepoints, but consistent differences were only observed for *Bifidobacterium, Terrisporobacter* and *Turicibacter*. Moreover, consistently lower acetate levels were found in only constipation-predominant IBS patients. Longitudinal analysis showed a large within and between subject variation in IBS severity, predominant stool pattern, and their association with microbiota composition.

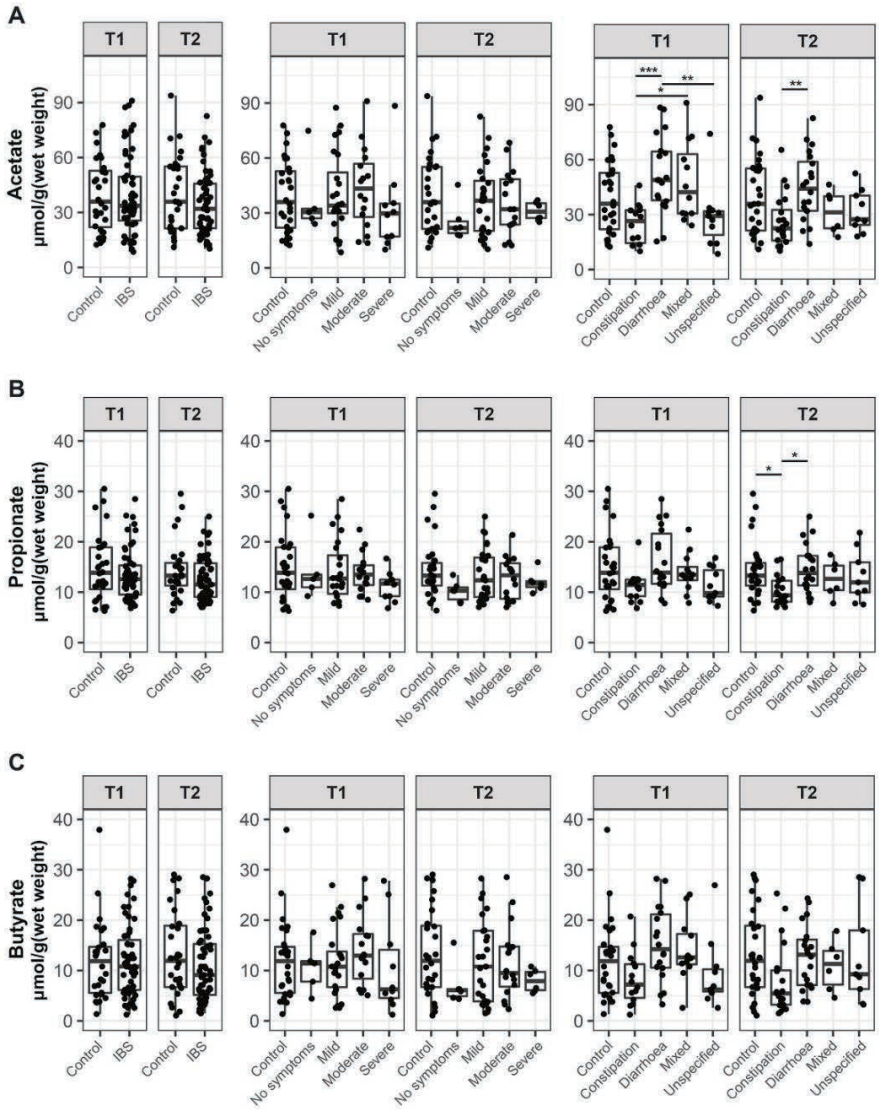

Figure 6. No differences in fecal acetate (*A*), propionate (*B*), and butyrate (*C*) between controls and IBS, and severity groups over time. Lower acetate levels in constipation-predominant IBS patients was seen. Values were presented as interquartile with boxplot. Significance between groups was test by Wilcoxon. * p<0.05; ** p<0.01.

Our results showed no significant difference of gut microbial alpha diversity and composition in IBS, indicating that the number, distribution and phylogenetic identity of microbial ASVs were similar between IBS and controls. This is in line with some studies[16, 40], but not all[41-44]. These conflicting results could be due to different methods used for the analysis, variations in discriminative power of different 16S

rRNA gene regions, variations in inclusion and exclusion criteria, as well as heterogeneity of IBS cohorts[9]. Although the individuality explained the largest proportion of gut microbiota variation when looking at genus level taxa, we observed several consistent gut microbial signatures associated with IBS over time. Lower relative abundance of *Bifidobacterium* in IBS patients has been reported in previous studies with a cross-sectional study design[9], which we found to be consistent over time. Remarkably, we found both *Bifidobacterium* and lactose consumption significantly lower in IBS patients compared to controls. Lactose has been suggested as one of the carbon sources of *Bifidobacterium*[45, 46]. However, the relative abundance of *Bifidobacterium* and lactose were not correlated in our study. Interestingly, *Bifidobacterium* supplements have been reported to improve IBS symptoms[47, 48], however, relief of symptoms was not always associated with an increase in relative abundance of *Bifidobacterium*[49]. This supports our observation that *Bifidobacterium* was not associated with symptom severity, but with IBS itself. Remarkably, we observed consistently higher relative abundance of *Terrisporobacter* and *Turicibacter* in IBS. Both taxa are thought to regulate the biosynthesis and release of serotonin and may play a role in IBS pathophysiology[50-53]. As these taxa have not been associated with IBS before, this finding may provide potential targets for future research.

Due to the accessibility without invasive procedures, fecal samples were the most commonly used to explore the role of gut microbiota in IBS[8]. It is evident that fecal samples only represent the end of the colon and previous studies have shown that comparing samples from small intestine and colon, provide relevant insights into the gut microbiota at other locations in the intestine of IBS patients[37, 51]. Hence, our study cannot exclude that potential key microbes at other locations in relation to IBS are overlooked. Nevertheless, longitudinal studies require repeated sampling without disturbing the intestine, taking samples from other locations nearly impossible without invasive procedures.

In our study, we found some taxa to be associated with IBS severity at one of the two timepoints. However, differences were not consistent over time. IBS symptom severity itself changed drastically within four weeks, and the explained variance of gut microbiota composition by severity also indicated large dynamics over time. Moreover, we did not find any correlation between the change of the severity score over time with the change of the genus-level taxa in relative abundance over time, which indicates that IBS symptom severity seems not the reason causing changes in the relative abundance of gut microbial taxa. A cross-sectional study by Tap et al (2017)[14] and a longitudinal study by Mars et al (2020)[15] have suggested that gut microbial signatures are associated with IBS symptom severity. However, the dynamics of gut microbial signatures over time has not been determined in these two studies. Our study shows that the gut microbial signature associated with

symptom severity is not stable longitudinally. Therefore, caution is needed in identifying signatures based on cross-sectional comparisons, which may change over time.

Increasing evidence indicates that IBS symptoms and gut microbiota composition are associated with carbohydrate intake[54, 55], and especially the FODMAPs[56, 57]. In our study, we did not find an association between gut microbiota composition and carbohydrate intake. Furthermore, after comparing the gut microbiota composition between IBS patients following the FODMAP diet or not, no difference was found. This might be due to the large dietary differences within the FODMAP diet and between studies. Further studies assessing the effects of carbohydrates on IBS symptoms and the gut microbiota are needed.

The main microbial metabolites, SCFAs, have been suggested as a biomarker of IBS[18, 58]. However, approximately 80% of SCFAs produced in the gut are absorbed, and therefore not found in fecal samples[59], which may limit the effectiveness of fecal SCFAs as a biomarker. In our study, we did not find differences in SCFA levels between IBS and controls, while we confirmed that acetate was consistently lower in constipation-predominant IBS compared to diarrhea-predominant IBS[18]. This might be explained by the shorter transit time in diarrhea, leaving less time for absorption of SCFA in the gut, as shown in people with slow colonic transit[60].

Our study is strengthened by the longitudinal design, which enabled us to assess dynamics of gut microbiota and SCFAs associated with IBS severity. Moreover, we assessed diet and psychological factors, which are altered in IBS, thus giving a more complete overview of the IBS patient. However, due to our observational study design we cannot determine causality. In addition, given the large variability over a short period of time in symptom severity scores, maintaining consistently equal sized groups of severity was challenging.

In conclusion, consistent gut microbiota and SCFA signature associated with IBS severity was not found. Interestingly, the relative abundances of the genera *Bifidobacterium*, *Terrisporobacter*, and *Turicibacter* were consistently different between IBS and controls over time, giving directions for future explorations. The importance of inclusion of multiple timepoints was demonstrated by the large within and between person variation of observed IBS severity, stool pattern and their association with gut microbiota composition over time. Hence, conclusion of single-timepoint studies in the past should be reconsidered, and future studies are highly recommended to take time-dynamics into account.

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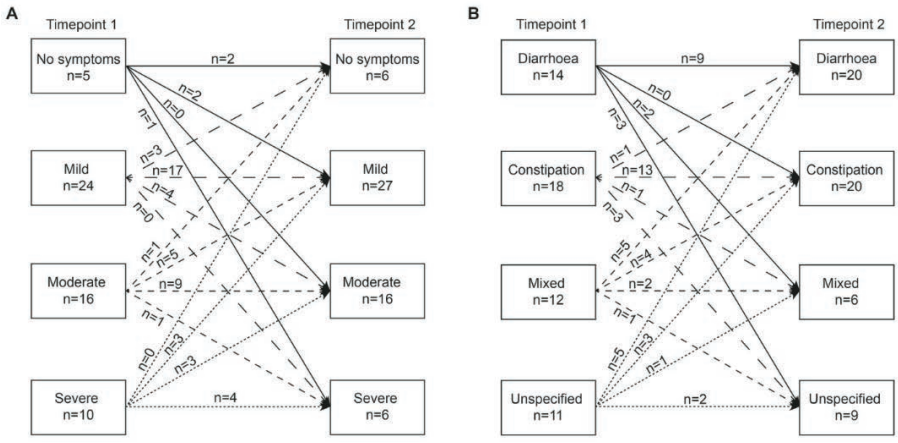
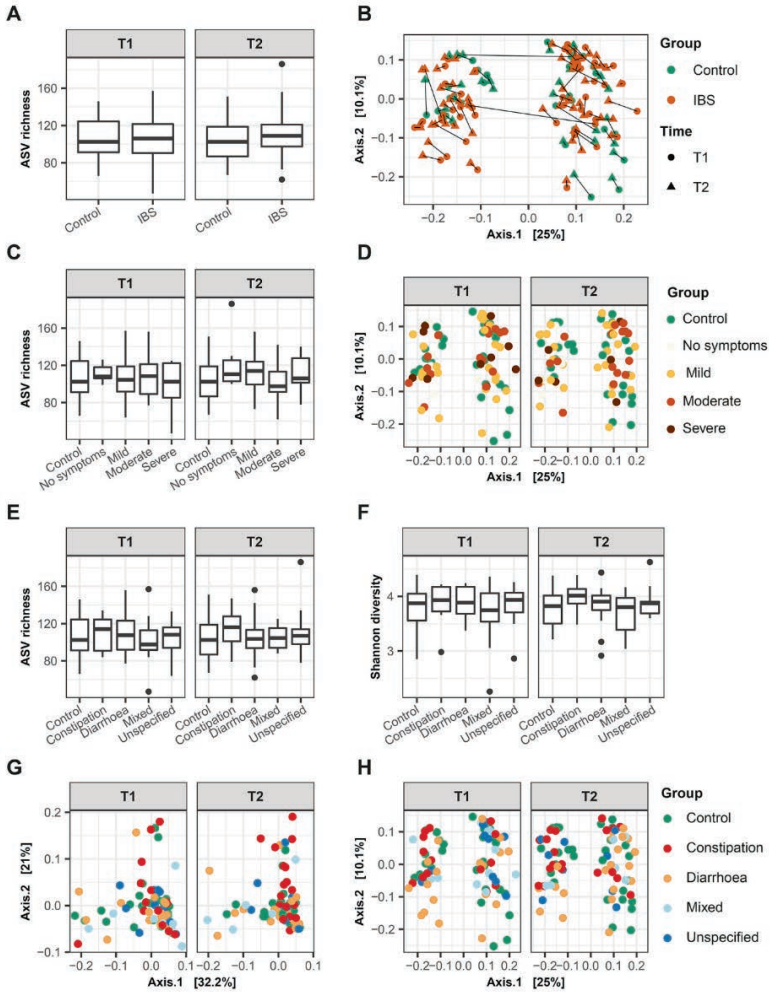
Supplementary Table 1. Dietary intake of the study population at T1

	IBS (n=91)	Control (n=30)	p-value
Energy (kcal)	1942 ± 587	2165 ± 673	*.083*
Protein (g)	70.1 ± 21.4	80.2 ± 25.8	**.036**
Total fat (g)	82.2 ± 29.2	90.6 ± 34.8	.197
Saturated fat (g)	27.2 ± 11.8	31.2 ± 13.0	.118
Total Carbohydrates (g)	205 ± 65	226 ± 64	.137
Glucose (g)	9.8 ± 4.3	8.8 ± 4.2	.295
Fructose (g)	13.6 ± 5.9	12.8 ± 5.4	.565
Lactose (g)	4.6 (2.0 – 10.1)	11.1 (7.0 – 19.0)	**.000**
Maltose (g)	2.0 (1.2 – 2.8)	2.6 (2.0 – 3.6)	**.042**
Saccharides (g)	25.2 ± 9.2	27.4 ± 12.0	.314
Polysaccharides (g)	120 ± 49	135 ± 46	.154
Dietary fiber (g)	24.8 ± 8.3	26.6 ± 7.5	.309
Alcohol (g)	3.2 (0.5 – 9.8)	2.8 (0.0 – 11.8)	.123
Water (g)	2603 ± 696	2517 ± 651	.550
Following the FODMAP diet, n (%)	20 (22)	Not applicable	

Data is presented as mean ± standard deviation or median (interquartile range) when skewed. Differences between groups are tested with an independent sample t-test or Mann-Whitney U test when skewed. The definition of each nutrient is according to the Dutch Food Composition table[35]. In line with the Dutch Food Composition table, dietary fiber is not included in the calculation of total carbohydrates, but treated as a separate category. Abbreviation: IBS; Irritable Bowel Syndrome



Supplementary Figure 1: Dynamics of IBS symptom severity *(A)* and predominant stool patterns *(B)* over time.



Supplementary Figure 2. Comparison in gut microbiota alpha and beta diversity between IBS patients and controls, severity groups, subgroups based on predominant stool pattern. Significance between groups was test by Wilcoxon for alpha diversity, and PEMMANOVA for beta diversity. Comparison of ASV richness between IBS and control *(A)*. PCoA of microbiota composition based on unweighted Unifrac distances, stratified for IBS and controls (*B*). Comparison of ASV richness between severity groups and control *(C)*. PCoA of microbiota composition based on unweighted Unifrac distances, stratified for symptom severity groups and controls (*D*). Comparison of ASV richness between subgroups based on predominant stool pattern *(E)*. Comparison of Shannon diversity between subgroups based on predominant stool pattern *(F)*. PCoA of microbiota composition based on weighted Unifrac distance, stratified for subgroups based on predominant stool pattern (*G*). PCoA of microbiota composition based on unweighted Unifrac distances, stratified for subgroups based on predominant stool pattern (*H*).

Supplementary Table 2. Significant differences in relative abundance differences in genus level taxa between IBS and controls

First timepoint	Control	IBS	p-value
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.0955 ± 0.074	0.0452 ± 0.056	**0.000**
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	0.0014 ± 0.001	0.0005 ± 0.001	**0.001**
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella	0.0147 ± 0.016	0.0041 ± 0.009	**0.000**
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes	0.0205 ± 0.014	0.0160 ± 0.017	*0.051*
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Pseudobutyrivibrio	0.0000 ± 0.000	0.0003 ± 0.001	**0.036**
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Intestinibacter	0.0019 ± 0.004	0.0060 ± 0.009	**0.043**
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Terrisporobacter	0.0005 ± 0.002	0.0020 ± 0.003	**0.010**
k_Bacteria;p_Firmicutes;c_Erysipelotrichia;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Erysipelotrichaceae_UCG-004	0.0001 ± 0.000	0.0000 ± 0.000	**0.048**
k__Bacteria;p__Tenericutes;c__Mollicutes;o__NB1-n;f__;g__	0.0001 ± 0.000	0.0000 ± 0.000	**0.048**
Second timepoint	**Control**	**IBS**	**p-value**
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.1021 ± 0.070	0.0498 ± 0.045	**0.000**
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__uncultured	0.0034 ± 0.004	0.0066 ± 0.011	**0.041**
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Odoribacter	0.0001 ± 0.001	0.0012 ± 0.004	**0.028**
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	0.0041 ± 0.005	0.0072 ± 0.007	**0.010**
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	0.0018 ± 0.004	0.0007 ± 0.003	**0.031**
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales_vadinBB60_group;g__	0.0001 ± 0.001	0.0011 ± 0.003	**0.046**
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus_1	0.0063 ± 0.003	0.0052 ± 0.003	*0.062*
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus_2	0.0172 ± 0.019	0.0105 ± 0.018	*0.071*

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_FCS020_group	0.0025 ± 0.002	0.0017 ± 0.001	*0.064*
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_ventriosum_group	0.0057 ± 0.014	0.0019 ± 0.002	*0.051*
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Terrisporobacter	0.0002 ± 0.001	0.0015 ± 0.003	**0.004**
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Subdoligranulum	0.0426 ± 0.023	0.0345 ± 0.025	*0.065*
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Turicibacter	0.0013 ± 0.005	0.0024 ± 0.005	**0.013**
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__Anaeroplasma	0.0000 ± 0.000	0.0009 ± 0.005	*0.073*

Data is presented as mean ± standard deviation. Differences were tested with unpaired Wilcoxon test. Italic p-values are trends while bold p-values are significantly different between IBS and controls.

Supplementary Table 3. Significant differences in relative abundance differences in genus level taxa between controls and IBS severity groups

First timepoint	Control	No symptoms	Mild	Moderate	Severe
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.0955 ± 0.075[a]	0.0386 ± 0.028[b]	0.0482 ± 0.062[b]	0.0449 ± 0.052[b]	0.0418 ± 0.065[b]
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	0.0014 ± 0.001[a]	0.0000 ± 0.000[b]	0.0003 ± 0.001[a,b]	0.0010 ± 0.002[a,b]	0.0006 ± 0.001[a,b]
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella	0.0147 ± 0.016[a]	0.0041 ± 0.009[b]	0.0017 ± 0.004[b]	0.0054 ± 0.011[a,b]	0.0076 ± 0.012[a,b]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Terrisporobacter	0.0005 ± 0.002[a]	0.0023 ± 0.003[a,b]	0.0018 ± 0.004[a,b]	0.0027 ± 0.004[b]	0.0012 ± 0.003[a,b]
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Mitsuokella	0.0000 ± 0.000[a]	0.0090 ± 0.016[b]	0.0003 ± 0.002[a]	0.0000 ± 0.000[a]	0.0000 ± 0.000[a,b]
Second timepoint	**Control**	**No symptoms**	**Mild**	**Moderate**	**Severe**
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.1021 ± 0.070[a]	0.0332 ± 0.034[a,b]	0.0512 ± 0.041[b]	0.0540 ± 0.059[a,b]	0.0483 ± 0.041[a,b]
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Odoribacter	0.0001 ± 0.001[a]	0.0002 ± 0.001[a,b]	0.0004 ± 0.001[a,b]	0.0023 ± 0.006[b]	0.0023 ± 0.006[a,b]
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotellaceae_UCG-001	0.0000 ± 0.000[a]	0.0005 ± 0.001[a,b]	0.0001 ± 0.000[a,b]	0.0000 ± 0.000[a,b]	0.0112 ± 0.017[b]
k__Bacteria;p__Cyanobacteria;c__Melainabacteria;o__Gastranaerophilales;f__uncultured_bacterium;g__<empty>	0.0006 ± 0.002[a,b]	0.0016 ± 0.003[a,b]	0.0000 ± 0.000[a]	0.0001 ± 0.001[a,b]	0.0014 ± 0.002[b]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Terrisporobacter	0.0002 ± 0.001[a]	0.0019 ± 0.002[b]	0.0013 ± 0.003[a,b]	0.0015 ± 0.002[a,b]	0.0023 ± 0.004[a,b]
k__Bacteria;p__Tenericutes;c__;o__;f__;g__	0.0000 ± 0.000[a]	0.0006 ± 0.002[a,b]	0.0001 ± 0.00[a,b]	0.0000 ± 0.000[a,b]	0.0009 ± 0.001[b]
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutae_vadinHA64;f__uncultured_bacterium;g__<empty>	0.0000 ± 0.000[a]	0.0012 ± 0.002[b]	0.0016 ± 0.007[a,b]	0.0000 ± 0.000[a,b]	0.0000 ± 0.000[a,b]

Data is presented as mean ± standard deviation. Differences were tested with unpaired Wilcoxon test. Different superscript indicates significant differences between subgroups.

Supplementary Table 4. Correlation of the change in relative abundance of genus level taxa with the change of IBS severity score over time

Taxa at genus level	r	p-value
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobrevibacter	-0.062	0.967
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanosphaera	-0.037	0.976
k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__Thermoplasmatales;f__Thermoplasmatales_Incertae_Sedis;g__uncultured	-0.172	0.943
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces	0.091	0.967
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Arcanobacterium	0.172	0.943
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Varibaculum	-0.129	0.967
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.071	0.967
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Rothia	-0.129	0.967
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	-0.096	0.967
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella	0.091	0.967
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Coriobacteriaceae_UCG-002	-0.154	0.943
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Coriobacteriaceae_UCG-003	-0.127	0.967
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Eggerthella	-0.292	0.929
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Enterorhabdus	0.196	0.943
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Gordonibacter	-0.296	0.929
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Olsenella	-0.143	0.967
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Senegalimassilia	-0.036	0.977
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Slackia	0.290	0.929
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__uncultured	0.160	0.943
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__;g__	0.103	0.967

k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__	-0.172	0.943
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides	-0.025	1.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidales_S24-7_group;g__	0.072	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidales_S24-7_group;g__uncultured_bacterium	0.006	1.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Barnesiella	0.058	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Butyricimonas	-0.015	1.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Coprobacter	-0.012	1.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Odoribacter	-0.050	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	-0.065	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas	-0.108	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__uncultured	-0.161	0.943
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__	-0.005	1.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Alloprevotella	-0.021	1.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Paraprevotella	-0.034	0.977
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella	-0.141	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella_2	-0.103	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella_7	0.075	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella_9	-0.060	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotellaceae_NK3B31_group	-0.048	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotellaceae_UCG-001	0.157	0.943
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__uncultured	-0.055	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes	0.060	0.967
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Rikenellaceae_RC9_gut_group	-0.194	0.943
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__uncultured;g__	0.223	0.943

k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__uncultured;g__uncultured_bacterium	-0.207	0.943
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__uncultured	-0.219	0.943
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__;f__;g__	-0.169	0.943
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__uncultured_eukaryote;f__<empty>;g__<empty>	-0.060	0.967
k__Bacteria;p__Cyanobacteria;c__Melainabacteria;o__Gastranaerophilales;f__;g__	-0.110	0.967
k__Bacteria;p__Cyanobacteria;c__Melainabacteria;o__Gastranaerophilales;f__gut_metagenome;g__<empty>	-0.172	0.943
k__Bacteria;p__Cyanobacteria;c__Melainabacteria;o__Gastranaerophilales;f__uncultured_bacterium;g__<empty>	-0.188	0.943
k__Bacteria;p__Cyanobacteria;c__Melainabacteria;o__Gastranaerophilales;f__uncultured_organism;g__<empty>	-0.103	0.967
k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__Elusimicrobiales;f__Elusimicrobiaceae;g__Elusimicrobium	-0.151	0.943
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus	0.232	0.943
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus	0.154	0.943
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	-0.015	1.000
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Pediococcus	0.000	1.000
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Leuconostoc	0.056	0.967
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus	-0.076	0.967
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	-0.307	0.929
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__	-0.120	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;g__Christensenellaceae_R-7_group	-0.041	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;g__uncultured	0.052	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae_1;g__Clostridium_sensu_stricto_1	0.062	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales_vadinBB60_group;g__	0.082	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales_vadinBB60_group;g__gut_metagenome	0.207	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales_vadinBB60_group;g__uncultured_bacterium	0.107	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales_vadinBB60_group;g__uncultured_organism	0.072	0.967

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Defluviitaleaceae;g__Defluviitaleaceae_UCG-011	0.174	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae;g__Eubacterium	-0.077	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Family_XI;g__Parvimonas	-0.108	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Family_XIII;g__Family_XIII_AD3011_group	0.252	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Family_XIII;g__Family_XIII_UCG-001	0.263	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Family_XIII;g__Mogibacterium	0.159	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__	0.048	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Acetitomaculum	0.247	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerosporobacter	-0.056	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes	-0.047	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia	0.052	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Butyrivibrio	0.034	0.977
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus_1	-0.045	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus_2	0.063	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus_3	0.113	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea	-0.068	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Eisenbergiella	0.039	0.969
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Fusicatenibacter	-0.045	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Howardella	0.154	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Hungatella	-0.134	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnoclostridium	-0.096	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospira	-0.075	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_FCS020_group	-0.166	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_ND3007_group	0.023	1.000

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group	-0.048	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_NK4B4_group	-0.127	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-001	-0.164	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-003	-0.108	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-004	0.101	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-008	0.149	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Marvinbryantia	0.175	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Pseudobutyrivibrio	0.057	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Roseburia	0.018	1.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Sellimonas	0.000	1.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Shuttleworthia	0.056	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Tyzzerella	-0.194	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Tyzzerella_3	-0.083	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Tyzzerella_4	0.085	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Bacteroides]_pectinophilus_group	0.014	1.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_eligens_group	0.187	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_hallii_group	0.074	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_ruminantium_group	-0.053	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_ventriosum_group	-0.100	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_xylanophilum_group	-0.036	0.977
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]_gauvreauii_group	-0.060	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]_gnavus_group	-0.194	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]_torques_group	-0.118	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__uncultured	0.145	0.967

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Peptococcus	-0.045	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__uncultured	0.043	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	0.188	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Intestinibacter	0.202	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Terrisporobacter	0.245	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__	0.007	1.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Anaerotruncus	-0.067	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Butyricicoccus	0.088	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Faecalibacterium	-0.121	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Flavonifractor	-0.188	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillibacter	0.027	1.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira	-0.090	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminiclostridium_1	-0.121	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminiclostridium_5	-0.067	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminiclostridium_6	-0.053	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminiclostridium_9	-0.306	0.929
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_NK4A214_group	0.071	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-001	0.129	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-002	0.077	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-003	-0.101	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-004	0.079	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-005	0.208	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-008	0.112	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-009	-0.017	1.000

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-010	-0.162	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-013	-0.100	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-014	-0.096	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus_1	0.205	0.943
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus_2	-0.017	1.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Subdoligranulum	-0.301	0.929
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__[Eubacterium]_coprostanoligenes_group	0.095	0.967
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__uncultured	0.095	0.967
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Catenibacterium	0.045	0.967
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Catenisphaera	0.089	0.967
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Erysipelatoclostridium	-0.306	0.929
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Erysipelotrichaceae_UCG-003	0.053	0.967
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Erysipelotrichaceae_UCG-004	0.103	0.967
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Faecalicoccus	0.000	1.000
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Faecalitalea	0.219	0.943
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Holdemanella	-0.149	0.943
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Solobacterium	-0.001	1.000
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Turicibacter	0.027	1.000
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Clostridium]_innocuum_group	-0.238	0.943
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__uncultured	-0.173	0.943
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Acidaminococcaceae;g__Acidaminococcus	0.006	1.000
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Acidaminococcaceae;g__Phascolarctobacterium	0.047	0.967
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Acidaminococcaceae;g__Succiniclasticum	-0.018	1.000
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Allisonella	0.052	0.967

k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Dialister	0.048	0.967
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Megamonas	0.061	0.967
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Megasphaera	0.007	1.000
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Mitsuokella	-0.223	0.943
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Veillonella	-0.018	1.000
k__Bacteria;p__Lentisphaerae;c__Lentisphaeria;o__Victivallales;f__Victivallaceae;g__Victivallis	-0.089	0.967
k__Bacteria;p__Lentisphaerae;c__Lentisphaeria;o__Victivallales;f__vadinBE97;g__uncultured_bacterium	-0.181	0.943
k__Bacteria;p__Lentisphaerae;c__Lentisphaeria;o__Victivallales;f__vadinBE97;g__uncultured_rumen_bacterium	0.079	0.967
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__uncultured	-0.077	0.967
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Mitochondria;g__	-0.135	0.967
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Parasutterella	-0.074	0.967
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella	0.174	0.943
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__	-0.219	0.943
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Oxalobacter	-0.115	0.967
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Bilophila	0.114	0.967
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	-0.096	0.967
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae;g__Succinivibrio	-0.026	1.000
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__	0.107	0.967
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus	-0.029	1.000
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;f__Synergistaceae;g__Cloacibacillus	-0.219	0.943
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;f__Synergistaceae;g__Synergistes	-0.172	0.943
k__Bacteria;p__Tenericutes;c__;o__;f__;g__	-0.040	0.969
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__Anaeroplasma	-0.160	0.943
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mollicutes_RF9;f__;g__	0.183	0.943

k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mollicutes_RF9;f__gut_metagenome;g__<empty>	-0.042	0.967
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mollicutes_RF9;f__uncultured_bacterium;g__<empty>	0.093	0.967
k__Bacteria;p__Tenericutes;c__Mollicutes;o__NB1-n;f__;g__	-0.219	0.943
k__Bacteria;p__Tenericutes;c__Mollicutes;o__NB1-n;f__gut_metagenome;g__<empty>	0.232	0.943
k__Bacteria;p__Tenericutes;c__Mollicutes;o__NB1-n;f__uncultured_bacterium;g__	0.000	1.000
k__Bacteria;p__Tenericutes;c__Mollicutes;o__NB1-n;f__uncultured_bacterium;g__<empty>	0.151	0.943
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutae_vadinHA64;f__uncultured_bacterium;g__<empty>	0.052	0.967
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia	0.044	0.967

The population used for analysis was 55 IBS patients and 28 controls.

Supplementary Table 5. Significant differences in relative abundance differences in genus level taxa between controls and IBS patients with different predominant stool patterns

First timepoint	Control	Constipation	Diarrhoea	Mixed	Unspecified
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.0955 ± 0.074[a]	0.0337 ± 0.038[b]	0.0483 ± 0.059[b]	0.0299 ± 0.031[b]	0.0716 ± 0.084[a,b]
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	0.0014 ± 0.001[a]	0.0004 ± 0.001[b]	0.0004 ± 0.001[b]	0.0008 ± 0.002[b]	0.0007 ± 0.001[b]
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__uncultured	0.0038 ± 0.003[a,b]	0.0092 ± 0.014[a,b]	0.0051 ± 0.003[a]	0.0017 ± 0.002[b]	0.0062 ± 0.004[a]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Terrisporobacter	0.0005 ± 0.002[a]	0.0019 ± 0.003[a,b]	0.0038 ± 0.005[b]	0.0005 ± 0.001[a,b]	0.0008 ± 0.001[a,b]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__uncultured	0.0041 ± 0.009[a]	0.0123 ± 0.018[b]	0.0053 ± 0.011[a,b]	0.0019 ± 0.002[a]	0.0089 ± 0.015[a,b]
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g_Turicibacter	0.0018 ± 0.007[a]	0.0021 ± 0.005[a,b]	0.0029 ± 0.006[b]	0.0010 ± 0.001[a,b]	0.0003 ± 0.001[a,b]

Second timepoint	Control	Constipation	Diarrhoea	Mixed	Unspecified
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.1021 ± 0.070[a]	0.0479 ± 0.042[b]	0.0511 ± 0.049[b]	0.0267 ± 0.024[b]	0.0665 ± 0.053[a,b]
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Barnesiella	0.0048 ± 0.006[a]	0.0119 ± 0.012[b]	0.0039 ± 0.006[a]	0.0066 ± 0.006[a,b]	0.0034 ± 0.002[a,b]
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Odoribacter	0.0001 ± 0.001[a]	0.0025 ± 0.006[b]	0.0001 ± 0.000[a,b]	0.0007 ± 0.001[a,b]	0.0007 ± 0.001[a,b]
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes	0.0079 ± 0.007[a]	0.0182 ± 0.014[b]	0.0078 ± 0.007[a]	0.0099 ± 0.012[a,b]	0.0089 ± 0.007[a,b]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales_vadinBB60_group;g__	0.0001 ± 0.001[a]	0.0023 ± 0.005[b]	0.0002 ± 0.001[a,b]	0.0000 ± 0.000[a,b]	0.0008 ± 0.002[a,b]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	0.0066 ± 0.007[a,b]	0.0137 ± 0.012[a]	0.0044 ± 0.003[b]	0.0113 ± 0.013[a,b]	0.0042 ± 0.005[a,b]
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Allisonella	0.0001 ± 0.000[a]	0.0000 ± 0.000[a]	0.0001 ± 0.000[a]	0.0007 ± 0.001[b]	0.0003 ± 0.001[a,b]
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__Anaeroplasma	0.0000 ± 0.000[a]	0.0025 ± 0.008[b]	0.0000 ± 0.000[a,b]	0.0002 ± 0.001[a,b]	0.0000 ± 0.000[a,b]

Data is presented as mean ± standard deviation. Differences were tested with unpaired Wilcoxon test.
Different superscript indicates significant differences between subgroups.

Supplementary Table 6. Mixed model analysis between fecal SCFAs and psychological and IBS characteristics

	Acetate		Propionate		Butyrate	
	Estimate (95% CI)	*p-value*	*Estimate (95% CI)*	*p-value*	*Estimate (95% CI)*	*p-value*
Psychological characteristics						
Depression	-.28 (-1.32; .76)	.589	-.01 (-.31; .28)	.920	-.02 (-.41; .37)	.919
Anxiety	-.24 (-.98; .49)	.524	-.07 (-.27; .14)	.538	-.00 (-.28; .28)	.995
Total IBS-QoL	.03 (-.15; .20)	.766	.03 (-.02; .09)	.177	-.00 (-.07; .07)	.981
IBS characteristics						
IBS-SSS	-.00 (-.03; .03)	.820	-.01 (-.01; .00)	.183	.00 (-.01; .01)	.742
Predominant stool pattern		**.000**		**.000**		**.024**
Constipation	-3.8 (-12.7; 5.2)	.406	-.9 (-2.9; 1.0)	.359	-2.5 (-5.7; 0.6)	.117
Diarrhea	14.5 (5.5; 23.5)	**.002**	3.4 (1.4; 5.4)	**.001**	.18 (-1.4; 5.1)	.267
Mixed	9.9 (-.4; 20.3)	*.059*	1.6 (-.6; 3.8)	.162	1.8 (-1.9; 5.4)	.337
Unspecified	Ref		Ref		Ref	

Mixed model analysis was done using scaled identify scale and identifying time as repeated factor and subject as random factor. SCFAs are dependent variables, psychological factors and IBS characteristics were put in the model as the independent variables as a fixed main effect. The longitudinal population was used for analysis, including 55 IBS patients and 28 controls. Abbreviations: IBS, Irritable Bowel Syndrome; IBS-QoL; IBS quality of life; IBS-SSS, IBS Symptom Severity Score; IBS-C, IBS constipation predominant; IBS-D, IBS diarrhea predominant; IBS-M, IBS alternating between constipation and diarrhea; IBS-U, IBS unspecified type.

Supplementary Table 7. Spearman correlation coefficients between short-chain fatty acids (SCFA) and dietary intake

	Acetate		**Propionate**		**Butyrate**	
	Spearman rank	*p-value*	*Spearman rank*	*p-value*	*Spearman rank*	*p-value*
Energy (kcal)	.038	.678	.026	.777	-.015	.872
Total protein (g)	.019	.835	.027	.768	-.05	.587
Plant protein (g)	.035	.707	-.002	.981	.009	.923
Animal protein (g)	.007	.937	-.026	.781	-.063	.495
Total fat (g)	.036	.693	.016	.858	-.020	.829
Saturated fat (g)	.042	.645	.038	.682	-.019	.835
Single unsaturated fat (g)	.046	.617	.017	.856	.001	.990
Multiple unsaturated fat (g)	.054	.558	.021	.818	.002	.978
Total carbohydrates (g)	.031	.736	.037	.691	.003	.974
Monosaccharides (g)	-.031	.738	-.066	.473	-.072	.436
Lactose (g)	-.135	.140	-.094	.306	-.182	**.046**
Polysaccharides (g)	.089	.329	.118	.196	.079	.389
Dietary fiber (g)	.003	.977	-.094	.306	-.027	.773
Water (g)	-.009	.922	-.137	.134	-.077	.401
Alcohol (g)	.116	.205	.077	.399	.058	.524

Data includes 91 IBS patients and 30 controls. SCFA levels at the first timepoint are compared to dietary intake.

CHAPTER 4

DEVELOPMENT AND VALIDATION OF THE FIBERSCREEN: A SHORT QUESTIONNAIRE TO SCREEN FIBER INTAKE IN ADULTS

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Abstract

Background: Health effects of dietary fibers are the topic of many studies. Eligibility criteria often include a certain fiber intake, which requires dietary screening during recruitment. However, dietary assessment methods are extensive and burdensome for both researcher and participant. Therefore, we developed and validated a short questionnaire (FiberScreen) to screen fiber intake.
Methods: The initial 5-item questionnaire assessed fruit, vegetable, whole grain, pasta/rice/potato and legume intake. The optimized FiberScreen included 18 items, which further specified intake of the abovementioned categories, and included nuts and seeds. The FiberScreen was completed during two fiber promoting interventions. In study A, participants without constipation completed the 5-item FiberScreen and a food frequency questionnaire (FFQ) during screening (n=131), and the 18-item FiberScreen and FFQ at 3-month follow-up (n=87). In study B, 29 constipated participants completed the 18-item FiberScreen at screening and FFQ during the first study visit.
Results: The fiber estimate from the 5-item FiberScreen and the FFQ was moderately correlated (r=0.356, p<.001) Importantly, the 18-item FiberScreen and FFQ, when data of both studies were combined, had a strong correlation (r=.563, p<.001). The 18-item FiberScreen had a lower fiber estimate compared to the FFQ (Δ=1.2±5.9 grams, p=.030) but the difference was relatively small. Bland-Altman plots showed a good agreement between the questionnaires. Completion time of the 18-item FiberScreen was 4.2±2 minutes.
Conclusion: The 18-item FiberScreen is a suitable short screening questionnaire to rank fiber intake of adults. The 18-item FiberScreen can help to reduce screening burden for both participant and researcher.

Keywords: Comparability; Dietary Fiber; Food Frequency Questionnaire; Functional Bowel Disorders; Questionnaire; Screening.

Introduction

The health benefits of dietary fiber have long been recognized: a high-fiber diet can reduce the risk of certain cancers, obesity, diabetes mellitus and cardiovascular diseases[1-6]. Moreover, dietary fiber can improve stool pattern by adding bulk and softening the stool, so that it passes the intestine more easily. An adequate fiber intake can therefore reduce the risk of developing stool complaints and the severity of for example constipation[7-12]. Constipation can affect a large part of the population, as the prevalence can vary between 5-20% depending on the definition used[13-15].

A daily fiber intake of 14 g/1000 kcal is recommended in the Netherlands because of these known health-promoting effects, meaning 30 grams for women and 40 grams for men[16]. In Europe, fiber intake ranges between 16-20 g/day for females and 18-24 g/day for males, which is far below the recommendations[17]. Moreover, the majority of the population is not meeting the recommended intake for fruits and vegetables, which are important sources of fiber in the European diet[18, 19]. Intervention studies have been performed to assess health effects of fiber in different study populations, or to improve intake of fiber or high-fiber food categories for prevention measures or treatment of for example constipation [8, 20-25]. Eligibility criteria for these studies often include a low dietary fiber intake, to have a window of opportunity for improvement of fiber intake towards the recommendations, which requires dietary screening in the selection process. Dietary assessment methods such as a food frequency questionnaire (FFQ) and 24hr recalls are often used during screening, but these are time consuming[26-28], expensive, and more elaborate than strictly needed for screening[29]. This places an unnecessary burden on both the participant and the researcher.

To date, several short dietary screening questionnaires for different purposes have been developed. Some screening questionnaires focus on dietary intake with respect to being at risk for a certain disease, such as obesity in children[30], malnutrition in elderly[31], or cardiovascular disease[32, 33], and are not valid for screening for an adequate fiber intake in a healthy or constipated adult population. Other screening questionnaires have only focused on fruit and vegetable intake[34-36], and thus are not capturing the complete fiber intake. One of the most frequently used screening questionnaires is the PrimeScreen, which was developed to evaluate diet quality from the assessment of several high-fiber foods such as dark green leafy vegetables, fruits and whole grain foods[37]. Although the PrimeScreen is a well-developed validated screening questionnaire to assess diet quality, it is not optimal to screen for total fiber intake as some important high-fiber food categories such as nuts and legumes are not included.

Since a lower fiber intake and fluid intake is associated with an increased prevalence of constipation[38], adults with and without constipation might have a different dietary

pattern. Both populations are of interest for fiber intervention studies. Therefore, we aimed to develop and validate a fiber-specific screening questionnaire (FiberScreen) with a short completion time for adults with and without constipation.

Methods

The development and validation of the FiberScreen was part of two previously performed intervention studies. In short, study A was a single-blind randomized controlled trial to assess the effects of a personalized dietary advice on fiber intake compared to general advice in adults without gastro-intestinal complaints. The study consisted of a 6-week intervention and a 3-month follow-up period[39], and was performed between March and September 2019. In study B, the effects of a personalized dietary advice on fiber intake and subsequent effect on constipation-related complaints in adults with constipation was investigated. The study had a pre-test post-test design, which included a 4-week run-in phase and a 4-week intervention phase, and was performed between August and November 2020. Both studies were approved by the Medical Ethical Committee of Brabant and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

The development and optimization of the FiberScreen

To develop and validate the FiberScreen, the fiber estimates from the FiberScreen were compared to those obtained from the FFQ in both study A and study B. The initial FiberScreen (study A) consisted of five items which assessed the intake of fruit, vegetables, whole grain products (for example bread, breakfast cereals, crackers), pasta/rice/potatoes and legumes of the last two weeks (Table 1; Supplementary material 1). These food categories were included since they contribute the most to dietary fiber intake in the Netherlands[19]. A scoring system was developed to score fiber intake, which was based on fiber content in the Dutch Food Composition database, and frequency and amount of consumption in a reference population as assessed in the Dutch Food Composition Survey[19, 40]. Points were summed and could range between 1-22: a higher fiber intake was reflected in higher points. Since median fiber intake of the Netherlands was estimated around 60% of the recommendation[18, 19], cut-off levels for a relatively low fiber intake were defined at ≤13 points for females and ≤15 points for males.

Based on the performance of the 5-item FiberScreen (shown in the results section), the FiberScreen was optimized to an 18-item questionnaire, which aimed to estimate fiber intake in grams instead of scoring points (Table 1; Supplementary Material 1). The optimization process was done in a qualitative practice-based manner in

Table 1. Overview of the items in the FiberScreen version 1 and 2

FiberScreen version	Food category	Number of items	Type of questions
1: 5 items	Fruit	1	Amount of fruit consumed per day
	Vegetables	1	Amount of vegetables consumed per day
	Whole grain products	1	Days per week of consumption of >2 pieces of whole grain products per day. Included whole grain bread, crackers/biscuits, bars, whole grain breakfast cereals
	Pasta, rice, potatoes	1	Whether people chose whole grain options (whole grain rice or pasta, potatoes) or refined rice or pasta
	Legumes	1	Days per week legumes are consumed
2: 18 items	Fruit	2	Amount of fruit consumed per day
			Number of days consumption of dried fruits
	Vegetables	1	Amount of vegetables consumed per day
	Whole grain products	5	For each type of bread (white, brown, multigrain, whole grain, rye); number of days consumed and pieces
		4	For each whole grain product (breakfast cereals, bran, crackers/biscuits or bars); number of days consumed and pieces
	Pasta, rice, potatoes	3	For each category the number of days consumed. Categories: 1) Refined pasta, white rice, refined couscous 2) Whole wheat pasta, whole wheat couscous, bulgur, whole grain rice, quinoa 3) Potatoes
	Legumes	2	Number of days consumed and amount of legumes consumed
	Nuts and seeds	1	Number of days consumed

Number of items reflect the amount of questions per food category. Questionnaires can be found in Supplementary material 1.

consultation with trained research dieticians and was based on the discrepancy between answers of the FFQ and 5-item FiberScreen. Whole grain, pasta, rice and potatoes, and legume intakes were further specified; such as for types of product consumed, frequency and amount of consumption. For example, the category bread now recalled the number of days and slices consumed for white, brown, multigrain, whole grain and rye bread, to obtain a more accurate estimation of bread consumption. Dried fruits, nuts and seeds were included in the FiberScreen due to the high fiber content[40], which could greatly impact fiber intake when consumed. Portion sizes were estimated using natural portions or household measures, which were the same as in the FFQ. Instead of converting answers to points, answers were now used to estimate fiber intake in grams. The frequency of consumption was multiplied by the amount consumed, and subsequently multiplied by nutrient estimates from the Dutch Food Composition database[40]. For each food category, the average fiber content in the Dutch Food Composition database was taken. For the calculation, a factor was assigned for each answer: for example ≤1 portion of fruit per day equaled a factor of 0.5, 1 portion of fruit equaled a factor of 1, 2 portions of fruit per day equaled a factor of 2 and so on. These factors were assigned for fruits, vegetables, and amount of legumes, which were then subsequently multiplied by their fiber content. For foods in which frequency answers were not continuous, factors were an estimation of number of days per week, meaning 'less than once per week' had a factor of 1/7, '1-2 days per week' had a factor of 2/7, '3-4 days per week' had a factor of 4/7 and '5-7 days per week' had a factor of 1. These factors were assigned for dried fruits, frequency of legume consumption and nuts and seeds, after which they were multiplied by the fiber content. For breads, whole grain products and pasta/rice/potatoes, no factors were assigned, as the number of days were questioned. These foods were calculated by multiplying the number of days consumed (divided by 7 to get an estimation per day) times the amount and the fiber content. The fiber estimations from each food was then summed to obtain an overall rough estimation of fiber intake.

Study design

For study A, the 5-item FiberScreen was assessed during screening (T1), after which it was optimized. The 18-item FiberScreen was subsequently applied in the same study at the 3-month follow-up (T2). The FFQ and the FiberScreen were completed during the same week at both T1 and T2. For study B, the 18-item FiberScreen was completed during screening and a FFQ was completed during the first visit of the trial (on average 33.5±12.1 days later). The FFQ was the same in both studies, but differed in mode of administration (study A: self-administered online, study B: face-to-face interview by trained researchers, Figure 1). All versions of the FiberScreen were completed online. Completion time for the 18-item FiberScreen was assessed in study B, but not in study A.

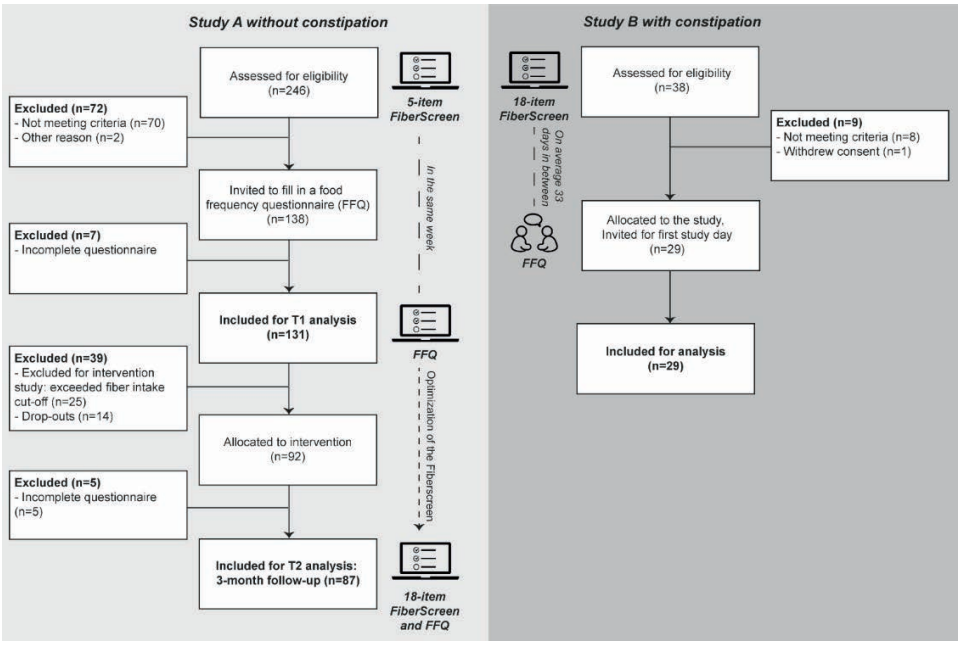

Figure 1. Design and participant flowchart of both Study A and B.

The FFQ was a 247-item semi-quantitative meal-based FFQ that recalled habitual diet of the last month, which was based on and developed using a validated FFQ[41, 42]. The same items from the validated FFQ were assessed, but due to the nature of the interventions in which we provided personalized dietary advice per mealtime to stimulate fiber intake, items of this FFQ were assessed per mealtime (breakfast, during the morning, lunch, during the afternoon, dinner, during the evening) instead of for the whole day. Selection of which item would be assessed at which mealtime was based on the Dutch Food Composition Survey[19]. Answers for each food ranged from 'never' to '7 days per week', and portion sizes were estimated using natural portions or household measures (e.g. 1 slice or 1 tablespoon). Nutrient intakes were calculated by multiplying the frequency of intake with the amount; nutrient estimates were obtained from the Dutch Food Composition database[40].

Study participants

For study A, eligible participants were older than 18 years, apparently healthy, were in possession of a computer and mobile phone compatible with applications and living in the surroundings of Wageningen (max. 50km). Participants were excluded when they had a diagnosis of any digestive tract disease or frequent bowel complaints, cardiovascular disease, diabetes mellitus, any type of cancer, renal disease, when currently following a gluten free or weight loss diet and were unable or unwilling to change, were using diuretics, antidepressants, codeine, antibiotics or fiber supplements, or currently pregnant or breastfeeding. For the intervention study,

participants were eligible when having a fiber intake <26 grams for females or <33 grams for males (≥15% below the recommendation for fiber). In the current analysis, participants with a higher fiber intake at screening were also included. As shown in Figure 1, n=246 adults were assessed for eligibility, and n=131 participants were included at T1, of which n=87 also completed the T2 measurement.

Study B had similar inclusion and exclusion criteria as study A, but differed on the following points: due to the Covid-19 pandemic, age was restricted between 18-55 years and Body Mass Index (BMI) was <30 kg/m^2, to adhere to national Covid-19 guidelines. Furthermore, eligible participants had constipation-related complaints, which were defined as being unsatisfied with their bowel habit (<6 on a visual analog scale from 1 'very unsatisfied' to 10 'very satisfied'), and had a habitual stool of Bristol stool type 1-4 and/or a stool frequency ≤4 times per week[43]. In addition to the exclusion criteria listed for study A, participants were excluded when having a depression or hypothyroidism, or using Prucalopride, Methylnaltrexone or Linaclotide laxatives. As shown in Figure 1, n=38 adults with constipation were assessed for eligibility, and n=29 participants were included in analysis.

Statistical analysis

Data are presented as mean ± standard deviation or median (interquartile range) when skewed. For the 18-item FiberScreen, analysis was performed both stratified per study and combining data of study A and B. To assess relative validity, Pearson's correlation coefficients were computed between the items of the FiberScreen and the FFQ. This was done for total fiber intake, and fiber intake per food category (fruit, vegetable, whole grain, pasta/rice/potato, legumes, nuts and seeds). Paired sample t-tests were performed to compare differences between the fiber estimates of the 18-item FiberScreen and the FFQ. Furthermore, the agreement between the 18-item FiberScreen and the FFQ was visualized in Bland-Altman plots[44], plotting the average intake versus the difference of the two questionnaires. Data was analyzed using SPSS version 25 and GraphPad Prism version 5, and a p-value of <0.05 was considered significant.

Results

The demographic data of both studies show that participants of study A at T1 were older, more often male and had a higher BMI compared to participants of study B (Table 2). Energy intake was higher in study A, but fiber intake measured by the FFQ was higher in study B. Compared to the study population at T1 of study A, the average age (48.2±21 years) was higher at T2, but BMI (24.9±4.0 kg/m^2) and the percentage of men (37%) remained similar. Completion time of the 18-item FiberScreen in study B was under 10 minutes with an average completion time of

4.2±2 minutes, which contrasts hugely to an estimated FFQ completion time of 45-60 minutes.

Table 2. Baseline characteristics of the participants included in the analysis

	Adults without constipation (study A, T1, n=131)	**Adults with constipation (study B, n=29)**
Age (years)	46.8 ± 22	33.2 ± 13
Body Mass Index, BMI (kg/m^2)	25.1 ± 4.1	22.8 ± 2.4
Gender, n (%) of males	50 (38)	5 (17)
Dietary intake based on the Food Frequency Questionnaire		
Energy (kcal)	2230 ± 680	2041 ± 425
Protein (en%)	14.7 ± 2.4	14.6 ± 2.1
Total fat (en%)	39.8 ± 4.1	37.6 ± 3.7
Saturated fat (en%)	14.0 ± 2.5	12.2 ± 2.1
Carbohydrates (en%)	39.5 ± 5.3	41.4 ± 4.8
Fiber intake (grams)	22.6 ± 8.0	24.2 ± 6.4
Meets fiber recommendation (g), n (%)*	15 (11)	4 (14)
Meets fiber recommendation (g/1000 kcal), n (%)*	6 (5)	5 (17)

Data is presented as mean ± standard deviation or n and %. BMI is self-reported. En%: energy percentage. *Recommendation according to the Dutch Health council, for males 40 grams of fiber or 14 g/1000 kcal, and for females 30 grams of fiber or 14 g/1000 kcal.

Initially we started with a 5-item FiberScreen to estimate fiber intake in study A. At T1, the average score for the 5-item FiberScreen was 8.5±3.1 points, compared to an average fiber intake of 22.6±8.0 grams estimated by the FFQ, which had a moderately strong correlation coefficient (r=.356, p<.000). For product categories, correlation coefficients were low to moderately strong (ranging between r=.126 and r=.374). Fruit showed the highest correlation coefficient and legumes the lowest (Table 3). As we were not satisfied with the performance, the FiberScreen was further developed to an 18-item questionnaire to improve agreement between the FiberScreen and the FFQ.

Fiber intake was estimated to be on average 24.2±6.0 grams by the 18-item FiberScreen at T2 of study A compared to 23.7±6.6 grams by the FFQ, which matched well (p=.138). For study B, the 18-item FiberScreen estimated fiber intake to be 17.0±3.9 grams, which was significantly lower compared to the FFQ (24.2±6.4, p<.000, Table 4). When data of the two studies were combined, the estimate of the

18-item FiberScreen was significantly lower compared to the FFQ, although the difference was relatively small (Δ=1.22±5.9 grams, p=.030). The estimate of the 18-item FiberScreen was significantly lower for all categories except legumes compared to the FFQ when the data of both studies were combined. Compared to the FFQ, the 18-item FiberScreen correctly classified 70 participants (81%) in study A, 17 participants (59%) in study B and 87 participants (75%) in both studies as having a relatively high or low fiber intake, when using the eligibility cut-off for the intervention studies (females <26 grams; males <33 grams of fiber per day).

Importantly, Pearson correlation coefficients with the FFQ were higher for the 18-item FiberScreen than for the 5-item FiberScreen. In study A, all categories at T2 had a significant correlation coefficient (p<.001) ranging between *r*=.457 and *r*=.731 between the 18-item FiberScreen and the FFQ (Table 3). Total fiber correlation was *r*=.705, p<.001. The correlation of total fiber intake between the 18-item FiberScreen and the FFQ was similar in males and females. In study B, total fiber correlation was *r*=.590, p=.001, and all categories except legumes (*r*=.178, p=.357) had a significant correlation coefficient ranging between *r*=.373 and *r*=.684, p<.05. After visual inspection, an outlier in legume intake in study B was identified (FFQ=7.95 grams, FiberScreen=0.82 grams of fiber originating from legumes). When this participant was removed from analysis, the correlation coefficient improved significantly to *r*=.454, p=.015. When data of T2 in study A and study B were combined, total fiber correlation was *r*=.563, p<.000, and correlation coefficients for the subcategories ranged between *r*=.249 and *r*=.708, p<.05, indicating moderate to strong correlations between the categories of the two questionnaires. Fruit showed the highest correlation coefficient and nuts and seeds the lowest.

The Bland-Altman plot revealed a good agreement between the 18-item FiberScreen and the FFQ including both study A and B, although the 95% limit of agreement was quite wide (-10.5 – 12.9 g of fiber, Figure 2A). The difference between the questionnaires remained stable when the average intake increased (ß=0.002±0.01, p=.980). No differences in the performance of the 18-item FiberScreen between males and females were seen ($ß_{males}$=0.07±0.16, p=.660; $ß_{females}$= -0.06±0.14, p=0.680, Figure 2B). To assess the performance of the FiberScreen for the different sources of dietary fiber, Bland-Altman plots for the individual product categories were computed. The difference between the two questionnaires was dependent for the intake of fruit (ß=0.54±0.07, p<.001, Figure 3A), vegetables (ß=0.54±0.10, p<.001, Figure 3B), and pasta, rice and potatoes (ß= -0.63±0.10, p<.001, Figure 3D). The slope for whole grains (ß= -0.09±0.10, p=.353, Figure 3C), legumes (ß=0.11±0.08, p=0.190, Figure 3E), and nuts and seeds (ß=0.22±0.12, p=0.07, Figure 3F) was stable, meaning that the difference between the two questionnaires was not dependent on intake.

Table 3. Pearson correlation coefficient between the FiberScreen and the 247-item Food Frequency Questionnaire

	Adults without constipation (study A)				**Adults with constipation (study B)**		**Adults with and without constipation (T2 study A + B)**	
	5-item FiberScreen, T1		*18-item FiberScreen, T2*		*18-item FiberScreen*		*18-item FiberScreen*	
	r	p-value	r	p-value	r	p-value	r	p-value
	n=131		n=87		n=29		n=116	
Total dietary fiber (g)	.356	**.000**	.705	**.000**	.590	**.001**	.563	**.000**
Fruit (g)	.374	**.000**	.707	**.000**	.684	**.000**	.708	**.000**
Vegetables (g)	.301	**.000**	.457	**.000**	.576	**.001**	.499	**.000**
Whole grains (g)	.241	**.006**	.603	**.000**	.587	**.001**	.593	**.000**
Pasta, rice, potatoes (g)	.144	.100	.505	**.000**	.418	**.024**	.479	**.000**
Legumes (g)	.126	.152	.731	**.000**	.178	.357	.660	**.000**
Nuts and seeds (g)	Not assessed		.469	**.000**	.373	**.047**	.249	**.007**

Values indicate Pearson's correlations coefficient and p-values. A p-value <0.05 was considered significant. For the 5-item FiberScreen, total dietary fiber and food categories received points for amount of fiber. For the 18-item FiberScreen, fiber content from each food category was tested.

Table 4. Differences between the 18-item FiberScreen and the 247-item Food Frequency Questionnaire (FFQ)

	Adults without constipation (study A, n=87)		Adults with constipation (study B, n=29)		Adults with and without constipation (T2 study A + B, n=116)	
Total dietary fiber (g)	-0.77 ± 4.8	.138	7.19 ± 5.2	**.000**	1.22 ± 5.9	**.030**
Fruit (g)	0.60 ± 1.7	**.001**	0.51 ± 1.2	**.026**	0.58 ±1.6	**.000**
Vegetables (g)	0.14 ± 1.5	.388	1.28 ± 1.5	**.000**	0.42 ±1.6	**.005**
Whole grains (g)	0.59 ± 2.9	.062	1.93 ± 3.2	**.003**	0.92 ± 3.0	**.001**
Pasta, rice, potatoes (g)	-1.60 ± 1.2	**.000**	-1.08 ± 1.1	**.000**	-1.47 ± 1.2	**.000**
Legumes (g)	0.27 ± 1.4	.078	-0.00 ± 1.7	.991	0.20 ± 1.5	.148
Nuts and seeds (g)	-5.24 ± 2.1	**.000**	0.06 ± 0.9	.709	-3.91 ± 2.9	**.000**

Results of a paired sample t-test, values indicate differences ± SD, computed as FFQ – FiberScreen. A p-value <0.05 was considered significant.

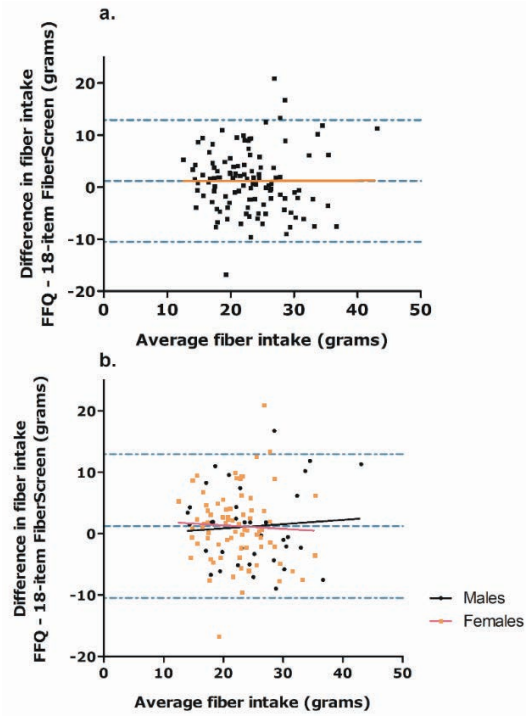

Figure 2A. Bland-Altman plot of fiber intake of both Study A and B.
Figure 2B. Bland-Altman plot of the fiber intake of both Study A and B, stratified for gender.

Both plots show the difference of the fiber estimate between the food frequency questionnaire (FFQ) and the 18-item FiberScreen on the y-axis versus the average fiber estimate of both questionnaires on the x-axis. The line represents the regression line.

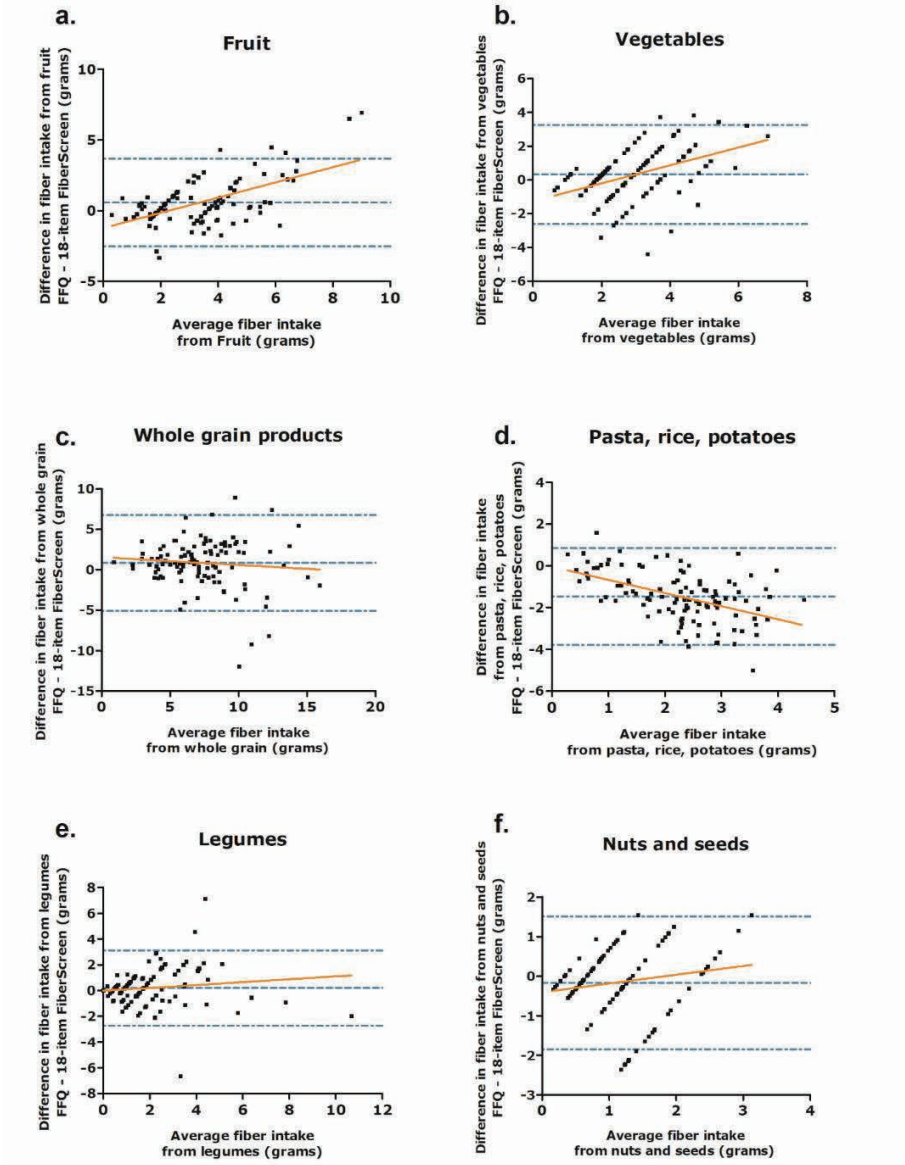

Figure 3A. Bland-Altman plot of fiber intake from fruits of both Study A and B. **Figure 3B.** Bland-Altman Plot of fiber from vegetables of both study A and B. **Figure 3C.** Bland-Altman plot of fiber from whole grain products of both study A and B. **Figure 3D.** Bland-Altman plot of fiber from legumes from pasta, rice and potatoes of both study A and B. **Figure 3E.** Bland-Altman plot of fiber from legumes of both study A and B. **Figure 3F.** Bland-Altman plot of fiber from nuts and seeds of both Study A and B.

All plots show the difference of the fiber intake from each food category between the Food Frequency Questionnaire (FFQ) – the 18-item FiberScreen on the y-axis versus the average fiber estimate of each food category on the x-axis. The line represents the regression line.

Discussion

We developed and validated a short fiber screening questionnaire, called FiberScreen, against a meal-based FFQ in Dutch adults with and without constipation complaints. Overall, we have shown that dietary fiber intake as assessed by the 18-item FiberScreen has good comparability with a meal-based FFQ, regardless of gender. The 18-item FiberScreen had a short completion time under 10 minutes, which is considerably less than the estimated 45-60 minutes for the FFQ, thus reducing the burden for both participant and researcher.

Our questionnaire adds to the existing list of short screenings for dietary intake. However, to date no specific dietary fiber screening questionnaire has been developed. Most questionnaires are developed to screen for being at risk of disease, such as malnutrition in elderly[31], obesity in children[30], or cardiovascular disease[32, 33]. Rifas-Shiman and colleagues (2000) developed the PrimeScreen, a short dietary assessment questionnaire, which has shown relatively good comparability with a FFQ in 160 healthy adults. Total fiber correlation was r=0.58, for fruit and vegetables categories this ranged between r=0.36-0.70, and for whole grain products this was r=0.51[37]. We found similar correlations for fruit and vegetables, but a stronger correlation for total fiber intake and whole grain products than PrimeScreen. Our higher total fiber correlation might be explained by the fact that PrimeScreen focuses on a short questionnaire to assess total diet quality and therefore lacks inclusion of certain high-fiber categories such as legumes, nuts and seeds, and thus not fully capturing the total fiber intake. The correlation for nuts and seeds in our study was relatively low, and the difference between the 18-item FiberScreen and the FFQ quite large. Our nuts and seeds correlation coefficient is similar to a FFQ validation study that compared with 24hr recalls[45], indicating that it is a difficult category to estimate. Previous screeners have not included nuts and seeds[30-33, 37], but due to the nutritional value and fiber content, it is an important category to include. Further work is needed to improve nuts and seeds intake estimation.

There was no significant difference in the fiber estimate between the 18-item FiberScreen and the FFQ in study A (T2), but there was a significant difference in study B. Possibly, participants in study A were better able to estimate their fiber intake at T2, as they already received a targeted high-fiber intervention and had already completed the FFQ once at T1. Moreover, due to the study design of study B, there was roughly a month between the completion of the 18-item FiberScreen and the FFQ. Participants might have changed their diet in between, especially with the prospect of having a face-to-face food interview. Research has suggested that a small dietary intervention can already instigate behavior change[46], or change responses to a self-administered questionnaire[47]. However, the FFQ recalled dietary intake from the last month, therefore it includes the time period of the 18-item FiberScreen. Furthermore, participants of study B were blinded at that time for the

goal of the intervention, namely fiber intake, thus it is unlikely that filling in the 18-item FiberScreen affected their fiber intake. It remains speculative whether this time difference could have caused the difference in performance of the 18-item FiberScreen. It seems unlikely that the difference in mode of administration caused the difference between questionnaires, since previous research found little discrepancy in dietary intakes assessed via self-administered web-based 24hr recalls versus interview-administered 24hr recalls[48]. When data of the two studies were combined and thus a larger sample size with more variation was acquired, there was a significant difference of 1.2g of fiber between the 18-item FiberScreen and the FFQ. However, this is a relatively small difference compared to the average total fiber intake of ~24g in both studies. Furthermore, since fewer items are assessed in the 18-item FiberScreen compared to an extensive FFQ, a lower estimate can be expected. Since the FiberScreen is not developed to measure absolute fiber intake, but to screen for a relatively low or high fiber intake and rank participants, researchers should keep this in mind when using the FiberScreen, as it is not suitable for a complete dietary assessment. The 18-item FiberScreen was able to accurately identify ~75% of the study population as having a relatively low or high fiber intake, based on our intervention study cut-offs. Thus, when using the FiberScreen, a larger screening sample needs to be taken into account, after which a complete dietary assessment method can be completed. This approach would result in a lower burden for more participants and researchers.

The items selected for the FiberScreen were based on the contribution of foods to fiber intake as assessed by previous literature, which has shown that cereal and cereal products (43%), vegetables (14%), potatoes and other tubers (10%), fruits, nuts and olives (11%) are the main sources of dietary fiber in the Dutch diet[19]. By assessing these food categories and including some additional high-fiber categories such as legumes, we were able to limit the FiberScreen to 18 items. Due to the item selection, the FiberScreen is validated for a Dutch adult population or population with similar dietary pattern, but needs further validation before it can be used in a population with a different dietary pattern. The same methodology can be applied, but needs to be adapted for the dietary pattern of that specific population. For example, bread or potatoes might be less consumed in other populations, and the current FiberScreen might miss important local products. Furthermore, the fiber estimate from the 18-item FiberScreen is now calculated with the Dutch Food Composition Table[40], and for usage in other countries it would be beneficial to use a local food composition tables for a more accurate estimate.

In this study, we used the FFQ as a validated comparison method, however the FFQ is not without limitations, as it can be prone to recall bias due to the longer recall period and can be susceptible for socially desirable answers. However, this is a problem for all type of dietary assessment methods, and not only specific to the

FFQ[49]. An FFQ is not validated to measure absolute dietary intake but is designed to rank intake of participants[27, 49]. Furthermore, an FFQ is strengthened by the fact that is recalls habitual diet over a longer period of time, and therefore circumvents recent changes in the diet for example due to illness[28]. Since the FiberScreen is developed to screen participants' eligibility for trials based on habitual diet, ranking participants is sufficient, and therefore the FFQ can be seen as a valid reference method for the validation of our FiberScreen. Ideally, it is best to use a biomarker as reference in validation studies, but for dietary fiber there is no valid biomarker currently known[28, 49]. Some have suggested plasma Alkylresorcinol as a biomarker for whole grain or rye intake[50-52], however it has shown poor correlations with total fiber intake and other grain sources[53], thus limiting its use.

This validation study is strengthened as it adheres to most key guidelines proposed by Serra-Majem et al (2009) regarding sufficient sample size (>100), and uses different statistics to assess validity, such as the comparison between questionnaire means, correlations and agreement by using Bland-Altman plots[54]. Furthermore, the 18-item FiberScreen was tested in two separate populations, giving a good overview regarding its validity. Therefore, even though assessment of dietary intake and the validation in the present study is not without limitations, the analyzing methods and sample size holds enough power for sufficient validation of the 18-item FiberScreen. Future studies should include further testing of the 18-item FiberScreen in different populations and include a broader range of fiber intake, to further strengthen the validation. A large advantage of the FiberScreen is the low burden for both researcher and participant. Previous research indicated that an average FFQ completion is between 30-60 minutes[28]; for our lengthier meal-based FFQ we estimated completion time to be between 45-60 minutes. When comparing the time burden to 24hr recalls, which is on average 40-45 minutes per digital recall or 20-30 minutes per telephone recall, the completion time of the FiberScreen under 10 minutes is an great advantage. Next to research, the 18-item FiberScreen could also be of value in clinical practice, which could help give a rough indication of fiber intake.

Future research needs to focus on portion size estimations, which is a major cause of measurement error in most types of dietary assessment[55]. Recent research suggested that text-based description of portion sizes seem more accurate than image-based descriptions[56], however, this was conflicting with the conclusions of a recent systematic review[57]. This indicates the complexity of portion size estimation, and the need for more research. Furthermore, sustainably increasing dietary fiber intake remains a challenge, as this is far below recommendations[17, 58]. Recently, we have shown that a digital personalized dietary advice was effective in increasing fiber intake, even 3 months after the intervention[39]. Personalized dietary advice might offer solutions for instigating long-term behavior change regarding the diet and fiber intake.

In conclusion, the 18-item FiberScreen is a valid short screening questionnaire to rank the fiber intake of Dutch adults with and without constipation. The 18-item FiberScreen can be useful questionnaire for researchers to quickly estimate fiber intake during recruitment, thus significantly reducing the burden for both participant and researcher during screening.

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Supplementary Material 1. Both versions of the FiberScreen
The Dutch version and scoring system of the 18-item FiberScreen can be provided upon request (contact email: Nicole.dewit@wur.nl). The 5-item FiberScreen and the 18-item FiberScreen were tested in Dutch language and developed for the Dutch habitual diet. For publication purposes, the questionnaires have been translated to English, however these are not the tested and validated questionnaires in the manuscript. Caution is needed for using the English translation of the FiberScreen.

The 5-item FiberScreen
Below you will find several questions regarding your diet. Please think about the average day during the last two weeks.

1. How many portions of fruit do you eat on average per day? For example, one portion can be one pear or one bowl of strawberries.
   - □ 0 portions
   - □ 1 portion
   - □ 2 portions
   - □ ≥3 portions

2. How many serving spoons of vegetables do you eat on average per day? In case of salad, one bowl of salad equals one serving spoon.
   - □ 0 spoons
   - □ 1 spoon
   - □ 2 spoons
   - □ 3 spoons
   - □ 4 spoons
   - □ 5 spoons
   - □ ≥6 spoons

3. On average, how many days per week do you eat at minimum 2 pieces of whole grain products or grains? For example, whole grain bread, whole grain crackers or biscuits, rye bread, muesli, wheat bran or oatmeal.
   - □ 0 days per week
   - □ 1 day per week
   - □ 2 days per week
   - □ 3 days per week
   - □ 4 days per week
   - □ 5 days per week
   - □ 6 days per week
   - □ 7 days per week

4. If you think about your dinner, what do you choose most often?
   - □ Refined pasta, white rice, white couscous
   - □ Potatoes, whole grain pasta, whole grain couscous, whole grain bulgur, whole grain rice, quinoa

5. How often do you eat legumes, such as beans, peas or lentils?
   - □ 0 days per week
   - □ 1 day per week
   - □ 2 days per week
   - □ 3 days per week
   - □ 4 days per week
   - □ 5 days per week
   - □ 6 days per week
   - □ 7 days per week

The 18-item FiberScreen

Below you will find several questions regarding your diet. Complete the questionnaire for your average diet in the past two weeks.

1. How many portions of fruit do you eat on average **per day?** For example, one portion can be one pear or one bowl of strawberries. This question also includes frozen fruit or fruits from cans. Dried fruit will be asked later.
   - □ I hardly eat any fruit — factor 0
   - □ Less than one portion per day — factor 0.5
   - □ 1 portion per day — factor 1
   - □ 2 portions per day — factor 2
   - □ 3 portions or more per day — factor 3

2. How many times **per week** do you eat dried fruits, such as raisins, apricots, plums, figs or Tutti Frutti? Also consider dried fruits in your desserts or yoghurt.
   - □ Less than once per week — 1/7 = 0.14 factor
   - □ 1 to 2 days per week — 2/7 = 0.28 factor
   - □ 3 to 4 days per week — 4/7 = 0.58 factor
   - □ 5 to 7 days per week — 7/7 = 1 factor

3. How many serving spoons of vegetables do you eat on average **per day?** Regarding salad or raw vegetables, 1 bowl equals one serving spoon. On the right you can see a picture of different types of spoons. The serving spoon is the most left spoon.

□ 0 spoons	factor 0
□ 1 spoon	factor 1
□ 2 spoons	factor 2
□ 3 spoons	factor 3
□ 4 spoons	factor 4
□ 5 spoons	factor 5
□ 6 or more spoons	factor 6

4. Can you indicate for **how many days per week** you choose which type of bread, and how many slices do you eat per day?

□ White bread:	___ days, ____ slices per day
□ Brown bread	___ days, ____ slices per day
□ Multigrain bread	___ days, ____ slices per day
□ Whole grain bread	___ days, ____ slices per day
□ Rye bread	___ days, ____ slices per day
□ I do not eat bread	

5. Can you indicate which types of whole grain product you eat on average **per week,** and how much you consume per day? If you do not use a product, you can indicate 0.

- □ Whole grain breakfast cereal such as oat meal or Brinta (1 bowl equals 250 grams of milk/yoghurt/quark): ____ days per week, ___ bowls per day
- □ Wheat bran (for example in milk, yoghurt or quark): ____ days per week, ___ bowls per day
- □ Whole grain crackers, crisp bread or biscuits: ___ days per week, ___ pieces per day
- □ Whole grain (muesli) bars: ___ days per week, ____ pieces per day

6. How many **days per week** do you consume the following products?

- □ White pasta (for example spaghetti of macaroni), white rice, white couscous ___ days
- □ Whole grain pasta, whole grain couscous, whole grain bulgur, whole grain rice, quinoa ____ days
- □ Potatoes (boiled, baked, mashed, fried or in stamppot) ____ days

7. How many days **per week** do you eat legumes, such as beans, chick peas or lentils? Please also consider peas in soups, such as lentil soup, brown bean soup or split pea soup. Products such as green beans, string beans and peas are not included in this category.
   - □ Less than once per week — 1/7 = 0.14 factor
   - □ 1 to 2 days per week — 2/7 = 0.28 factor
   - □ 3 to 4 days per week — 4/7 = 0.58 factor
   - □ 5 to 7 days per week — 7/7 = 1 factor

8. If you consume legumes, **how much** serving spoons do you consume? The serving spoon is the most left spoon in the picture.

   - □ 0 spoons — factor 0
   - □ 1 spoon — factor 1
   - □ 2 spoons — factor 2
   - □ 3 spoons — factor 3
   - □ 4 spoons — factor 4
   - □ 5 spoons — factor 5
   - □ 6 or more spoons — factor 6

9. How many days **per week** do you consume a handful of nuts and/or seeds? Also consider your nuts and seeds in yoghurt, quark or desserts, and nut spreads such as peanut butter on bread.
   - □ Less than once per week — 1/7 = 0.14 factor
   - □ 1 to 2 days per week — 2/7 = 0.28 factor
   - □ 3 to 4 days per week — 4/7 = 0.58 factor
   - □ 5 to 7 days per week — 7/7 = 1 factor

CHAPTER 5

INCREASING DIETARY FIBER INTAKE IN HEALTHY ADULTS USING PERSONALIZED DIETARY ADVICE COMPARED WITH GENERAL ADVICE: A SINGLE-BLIND RANDOMIZED CONTROLLED TRIAL

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Abstract

Objective: a high-fiber diet is associated with a lower risk for diseases. However, few adults meet the dietary fiber recommendation. Therefore, the effects and acceptance of an algorithm-generated personalized dietary advice (PDA) compared to general advice (GA) on fiber intake was investigated.
Design: a 6-week single-blind randomized controlled trial with a 3-month follow-up.
Setting: PDA was based on habitual intake and provided fiber-rich alternatives using a website, GA contained brochures. Dietary intake was assessed at baseline, week 1, week 6 and 3-month follow-up. Both groups evaluated their advice at week 6. All participants had access to PDA from week 7 until 3-month follow-up.
Participants: two groups of healthy adults: PDA (n=34) and GA (n=47). For 3-month follow-up analysis, participants were re-divided into visitors (n=52) and non-visitors (n=26) of the PDA.
Results: at week 6, energy intake remained stable in both groups, but fiber intake per 1000 kcal increased non-significantly in both groups (PDA=Δ0.5±2.8; GA=Δ0.8±3.1, p=.128). Importantly, a significantly higher percentage of PDA-participants adhered to the recommendation compared to week 1 (PDA=21% increase; GA=4% increase, p=<.001). PDA-participants evaluated the advice significantly better compared to GA-participants. At 3-month follow-up, fiber intake increased compared to baseline (visitors=Δ2.2±2.6, p<.001; non-visitors=Δ1.5±1.9, p=.001), but was insignificantly different between groups. Visitors had a decrease and non-visitors had an increase in energy intake (visitors=Δ-132±525; non-visitors=Δ109±507, p=.055).
Conclusion: The algorithm-generated PDA was well-accepted and stimulated adherence to the recommendations more than GA, indicating to be a suitable and cost-efficient method for improving dietary fiber intake in healthy adults.

Keywords: Dietary Fiber; Tailored; Personalized; Evaluation; Advice

Introduction

Dietary fibers play a key role in prevention of diseases. A diet high in fiber is associated with a reduced risk for developing obesity, stroke, hypertension, diabetes and colorectal cancer[1-5]. Dietary fibers have been shown to delay gastric emptying time, which reduces the postprandial glucose peak and thereby prevents the development of insulin resistance: one of the causes of many chronic diseases[6, 7]. Fibers also can increase stool weight and stool frequency, and improve stool consistency, thereby supporting a healthy stool pattern[8-13]. They are fermented by bacteria in the colon which produces short chain fatty acids (SCFA) such as butyrate. Butyrate is the preferred energy source for colonocytes and known for its anti-inflammatory properties and positive effects on gut health[11, 14-16].

Regardless of its widely known health benefits, current fiber intakes are below the recommendations. The Health Council of the Netherlands recommends a fiber intake of 14 grams per 1000 kcal, *i.e.* 30 g/day for women and 40 g/day for men[17]. In the Netherlands, median fiber intake is around 18 g/day for women, and 22 g/day for men[18, 19]. Whole grain, cereals and cereal products, vegetables, fruits, nuts and potatoes are the main food sources of fiber in the Dutch population[19].

Short-term intervention studies are often successful in increasing fiber intake, but it remains difficult to sustainably increase fiber intake in large healthy populations[20]. Moreover, successful interventions focused often on one high-fiber group such as fruit and vegetables[21-24], but did not reach the fiber recommendation, suggesting that interventions targeting single high-fiber food sources are not sufficient. Moreover, studies that use a specific -more fiber rich- diet as intervention such as the Mediterranean diet, require major changes for a population with another dietary culture, possibly making this too complex for long-term adherence[25, 26].

A personal approach based on individual needs, preferences and habitual diet may be a successful strategy towards a long-term improvement of the diet. Recently, a study among Dutch seniors found beneficial effects of personalized dietary advice (PDA) compared to general advice (GA) on body fat, waist and hip circumference[27]. Bianchi and colleagues (2020) found that computer-based tailored dietary counselling significantly improved diet quality in 80 French pregnant women, compared to general advice[28]. A large European trial, named Food4Me, found that PDA significantly improved healthy index scores and reduced red meat, salt and saturated fat intake compared to GA. However, PDA did not significantly improve dietary fiber, fruit, vegetables or whole grain intake compared to GA[29]. Possibly, this was because dietary fiber was not the sole aim of this intervention. As far as we know, the effects of a personalized high-fiber diet was only investigated in North-American children with refractory functional constipation. In that study, children received either written general dietary advice or personalized diet management by

a registered dietician. Those receiving personalized diet management showed better compliance for increasing fiber intake, water consumption, as well as energy and macronutrient intake[30]. This suggests that PDA is more effective than GA in improving dietary intake, however whether this also applies for dietary fiber intake in healthy adults is unknown.

Whereas PDA interventions may be more effective than GA, they often include supervision from a nutrition counselor, as discussed by Karagiozoglou and colleagues[30]. This can be time consuming and costly, thereby limiting the potential for large scale application. Digital interventions may form an alternative strategy, which has shown to be effective for behavior change regarding diet[31, 32]. In addition, behavioral change techniques may be incorporated, for example by recommending high-fiber substitutes for habitually consumed low-fiber products or adding high-fiber products to a meal[33]. Research has shown that substitutions within dietary subgroups can improve nutrient adequacy[34]. If participants can self-select these high-fiber substitutes or add-ons, this may increase compliance to dietary advice.

To test this approach, algorithms based on dietary guidelines were developed and incorporated in a website, that automatically generates PDA using input from participants on food intake and personal characteristics. We assessed whether this PDA website has an additional value besides GA in increasing fiber intake in a healthy adult Dutch population. Moreover, we evaluated how users perceived the PDA.

Methods

This was a 4.5-month single-blind parallel randomized controlled trial (RCT), which included a 6-week intervention period and a 3-month follow-up. The study was performed between March and September 2019. For full overview of the study, see the CONSORT checklist. To ensure blinding, participants were not informed about advices tested in the trial, and were asked not to discuss their advice with other participants. Stratified for age, gender, body mass index (BMI) and fiber intake before the study, participants were randomly allocated to either the GA or PDA group (ratio 1:1) by the research team. The GA consisted of two flyers: one of the Netherlands Nutrition Center and one of the Dutch Digestive Disease Foundation, and general information provided on the study website (www.vezelup.nl) about dietary fibers. The PDA-group also received the GA, but had additional information on the website to compose their PDA (see below). Blinding was opened after the 6-week intervention, after which both GA and PDA-participants had access to their PDA until the 3-month follow-up, to assess whether the PDA-website is feasible to use without support of research staff. Figure 1 shows the study design.

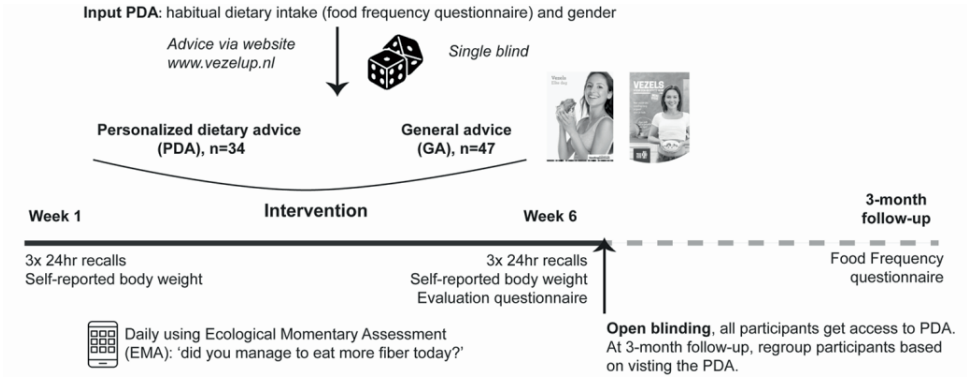

Figure 1. Overview of the study design
Questionnaires are performed online or via mobile application. General advice consisted of two flyers of the Netherlands Nutrition Center and the Dutch Digestive Foundation, and a website containing general information. The intervention group also received this information, and their personal advice.

The PDA was generated by modelling digitized personal and food data, which was implemented in a website (www.vezelup.nl). The advice was personalized based on dietary intake (assessed before study start using a meal-based food frequency questionnaire, FFQ) and gender (male/female). Generated by the algorithm and shown on the website, participants could choose high-fiber alternatives for their habitually used low-fiber products during each mealtime (breakfast, lunch, dinner and in between each meal). Prior to programming, the alternative product list was compiled by study researchers after consulting with dieticians, and included general high-fiber products without using brand names (for example whole wheat crackers). Besides replacing low-fiber foods, participants could include an extra portion of high-fiber products such as fruit, vegetables, nuts and/or legumes at each mealtime. Participants could change their PDA freely during the 6-week intervention period, by choosing different high-fiber products or including an extra portion of high-fiber products at different mealtimes. The website displayed the participant's current fiber, vegetables, fruits, nuts and legumes intake (based on the FFQ), their intake after choosing their PDA and a comparison with the recommendations, to serve as feedback. After choosing their PDA, participants were guided to make a so-called 'implementation intention'. This must help attain their goals by formulating when, where and how the goal will be reached [35]. For the PDA group, user login data were logged to evaluate the use of the website and compliance.

Study participants

Study participants were recruited using the Wageningen University & Research subject database. Participants were eligible if 18 years or older; apparently healthy; had a relatively low fiber intake (females <26 grams, males <33 grams, which is ≥15% below the recommendation, assessed using a screening questionnaire and

FFQ); were living in the Wageningen area (within 50 kilometer radius); and were in the possession of a mobile phone compatible with required applications. Participants were excluded when they had a digestive tract disorder (chronic constipation or diarrhea, Crohn's disease, Ulcerative Colitis, Irritable Bowel Syndrome, Coeliac disease); presence of Diabetes Mellitus; were currently following a strict diet and unwilling or unable to change; using laxatives, diuretics, antidepressants, codeine, antibiotics or fiber supplements; and for female participants when currently pregnant or breastfeeding. Participants filled in the questionnaires online at home. We aimed to include at least 30 participants per group, to have a power of 80%, take a 10% drop-out rate into account and have the ability to detect a difference of 5 grams per day in fiber intake[36].

Dietary assessment

Dietary fiber intake was the primary outcome of this study. During week 1 and week 6 of the intervention, dietary intake was assessed with three web-based 24hr recalls using the validated program Compl-eat[37]. To reduce bias, participants were not informed beforehand when the 24hr recalls would be performed, and to take variation into account, recalls consisted of two not consecutive workdays and one not consecutive weekend day. Before the start of the study and at the 3-month follow-up, habitual diet of the last month was assessed with a 247-item semi-quantitative meal-based online FFQ, which was based on and developed using a validated FFQ, that also included the last month as a reference period[38, 39]. The same items from the validated FFQ were assessed, however in the FFQ used for this study, the items were assessed per mealtime (breakfast, during the morning, lunch, during the afternoon, dinner, during the evening). This was done to be able to give personalized advice per mealtime. Which item was assessed for which mealtime was based on the item intake of the reference population of the National Dutch Food Composition Survey[19].

Ecological Momentary Assessment (EMA)

EMA is a structured diary technique to assess behavior, thoughts, feelings and context in daily life[40, 41]. Besides its commonly use in behavioral and social sciences, EMA has also been used to study specific dietary aspects as well as gastro-intestinal complaints[42, 43]. In the present study, smartphone-based EMA was used daily during the intervention period to answer a fixed set of questions. This included subjective fiber intake, which was assessed daily by asking 'did you manage to eat more fiber today?' on a visual analog scale (VAS) ranging from 0 'not at all' to 100 'yes, very much'. In addition, at the start and end of the intervention period the EMA app asked participants to report their fasting body weight in the morning. Notifications to answer subjective fiber intake were sent at 8pm, although participants could personalize the timing from 6pm to 10pm. Questions could be answered up to an hour after the

notification. In our study, EMA compliance was high (79.1%) during the 6-week intervention.

Evaluation of the PDA

After the 6-week intervention, participants completed an evaluation questionnaire to assess appreciation and acceptance of the PDA. Participants rated several aspects and statements regarding the advice on a 7-point Likert scale, ranging from 1 'totally disagree' to 7 'totally agree'. The evaluation included advantages and disadvantages of the advice, how positive, useful, attractive or interesting they found the advice, how much the advice helped and fitted them, whether it motivated them and whether they received enough feedback.

Statistical analysis

Data were analyzed per protocol (excluding non-compliant and drop-out participants). Due to the characteristics of the non-compliant and drop-out participants (*i.e.* elderly and lack of technological skills), this group can be seen as an inappropriate target population for this intervention, and therefore were excluded in the analysis. Continuous data is presented as mean ± standard deviation, or median (interquartile range, IQR) when not normally distributed. Categorical data is presented as counts and percentages. Differences between groups were tested using an independent t-test or Wilcoxon test when not normally distributed. Differences within groups were tested using a paired sample t-test or paired Wilcoxon test when skewed. For assessing within and between person variation for fiber intake of the 24hr recalls, we calculated a coefficient of variation (CV; standard deviation/mean*100). Regardless of the intervention group, after the 6-week intervention all participants had access to their PDA. GA-participants who visited the website after the 6-week intervention and 3-month follow-up and PDA-participants were grouped together (visitors) and were compared with GA-participants who did not visit the website (non-visitors), to assess the effect of the PDA and feasibility after 3-months. To analyze EMA data, mixed linear modelling with restricted maximum likelihood estimation using lmer was used. Participants needed to complete at least 30 of 42 (75%) of EMA days, to be included in the analysis. Treatment effects are reported using estimated least squares means and standard error of the mean (SEM). SPSS version 25 and R version 3.5 were used for testing, and a p-value of <0.05 was considered significant.

Results

Study participants

In total, 246 people were screened for the selection criteria and 106 participants were eligible, see Figure 2. During the first week of the study, 14 participants

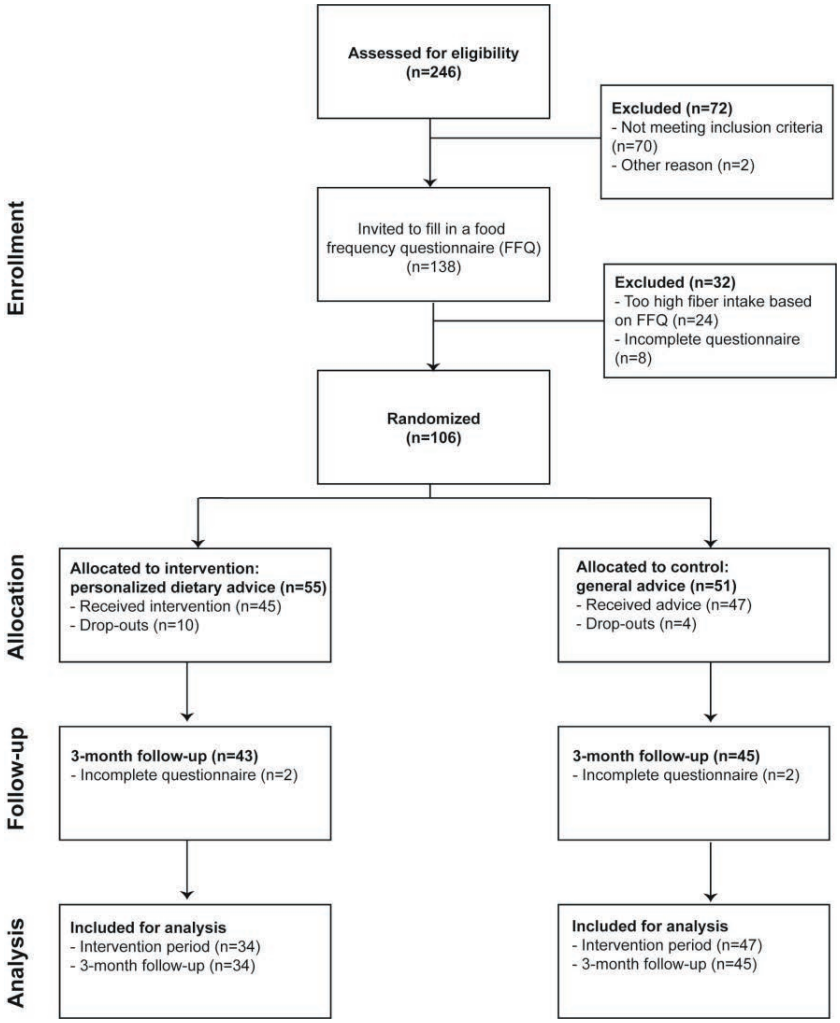

Figure 2. Study recruitment and flowchart

dropped out. Drop-outs had an average age of 64.5±15 years, a body mass index (BMI) of 25.8±4 kg/m² and 57% was male. Moreover, of 11 participants in the PDA-group, use of the website could not be confirmed by login data and therefore implementation and compliance of PDA during the intervention became uncertain. These participants were excluded from our analysis. Of these 11 participants, the median (IQR) age was 64 (47 – 68) years, they had an average BMI of 27.0±5 kg/m^2, an average fiber intake of 16.9±7 grams and 45% was male. This left 81 subjects, of which 34 in the PDA-group and 47 in the GA-group, to be included for further analyses.

Table 1 shows baseline characteristics and dietary intake based on the FFQ of the included participants (n=81). Dietary intake, median age, percentage of males and percentage of participants with a high education level was not significantly different between the groups, but BMI was. One participant in each group met the recommendation of 14 grams of fiber/1000 kcal per day, but none of the participants reached the recommendations for fiber in grams as this was an exclusion criterion.

Table 1. Baseline characteristics and dietary intake of the study population

	PDA (n = 34)	**GA (n=47)**
Age (years)	39 (21 -69)	52 (29 – 67)
Gender, n (%) males	12 (35)	18 (38)
BMI (kg/m²)	23.7±2.7*	25.6 ± 4.1*
Completed ≥ higher vocational education, n (%)	21 (62)	38 (81)
Energy intake (kcal)	2154 ± 529	2015 ± 492
Carbohydrate intake (en%)	39.5 ± 6	38.7 ± 5
Dietary fiber intake (grams)	20.9 ± 4	19.5 ± 5
Dietary fiber intake (grams per 1000 kcal)	10.0 ± 2.2	9.9 ±2.0
Water intake (mL)	2456 ±642	2553 ±625
Alcohol intake (g)	9 (2 – 20)	9 (4 – 15)

Data is presented as mean and standard deviation, or median (interquartile range). Categorical data is presented as n and %. Dietary intake is based on a Food Frequency Questionnaire (FFQ). Abbreviations: BMI, Body Mass Index; en%, energy percentage; GA, general advice; PDA, personalized dietary advice. *indicates significance between groups.

PDA usage

The 34 PDA-participants visited the website on average 5.6 times and made plans to change their diet 3.8 times during the 6-week intervention period. Based on their PDA, participants planned to increase their dietary fiber intake on average with 6.9 grams per day. There was no significant difference in visits or number of changes made in the PDA-website between males and females. From the high-fiber alternatives that could be selected on the website, 30% of the products were chosen at least once. The five most chosen high-fiber products were fresh fruit (n=139), whole wheat bread (n=134), raw vegetables (n=132), nuts (n=130) and legumes (n=90).

Body weight and dietary intake during the 6-week intervention

Body weight did not change substantially during the 6-week intervention period (ΔPDA= -0.25 kg, ΔGA= -0.05 p=.542), nor did energy intake (ΔPDA= -21.4 kcal, ΔGA= -21.1 kcal, p=.998), and these changes were not different between the

groups. Regarding dietary fiber intake, a large within (CV_{week1}=28.5%, CV_{week6}=23.6%) and between (CV_{week1}=29.6%, CV_{week6}=28.3%) person variation was observed in both groups. Both groups increased intake of fiber in grams per day (ΔPDA=1.6±6.4, ΔGA=0.8±6.6, p=.269), and fiber per 1000 kcal per day (ΔPDA=0.5±2.8, ΔGA=0.8±3.1, p=.128, figure 3A), but this was not statistically different between groups. However, importantly a significantly higher percentage of participants in the PDA-group adhered to the recommendation of 14 grams/1000 kcal after 6 weeks compared to the percentage in the GA-group (PDA=21% increase compared to baseline, GA=4% increase compared to baseline, p=<.001, figure 3B). To assess whether baseline fiber intake impacted effectiveness of the advice, data was stratified using median split based on fiber intake measured by the FFQ. The change of fiber intake during the intervention period (both in grams or per 1000 kcal) and number of participants adhering to the recommendations in week 6 was not different between participants with relatively low or high fiber intake (data not shown). Intended changes in dietary fiber intake based on the website did not correlate well with the change in dietary fiber intake measured by the 24hr recalls (*r*=-.006), although both showed an increase in fiber intake during the intervention.

Dietary fiber intake at 3-month follow-up

After the 6-week intervention, GA-participants also got access to their PDA via the website, while the PDA-group maintained their access. Of the GA-group, 19 of 45 participants visited their PDA between the end of the intervention and the 3-month follow-up. Therefore, at the 3-month follow-up participants were re-divided: visitors of the PDA-website (n=52) and non-visitors (n=26) (n=4 lost to follow-up due to incomplete FFQ data, see figure 2). Both visitors and non-visitors significantly increased their fiber intake per 1000 kcal at 3-month follow-up compared to baseline (Δvisitors=2.2±2.6, p<.001; Δnon-visitors=1.5±1.9, p=.001, see figure 5A). Non-visitors had an increased daily energy intake (Δ109±507 kcal, p=.281) compared to baseline, whereas the visitors decreased their daily energy intake (Δ-132±525 kcal, p=.075, see figure 5B). Fiber intake was not significantly different between groups (p=.239), but the difference in energy intake was close to significance (p=.055). Visitors especially increased their fiber intake via sources of fruit (Δ0.95 grams of fiber, p=.001) and legumes (Δ1.21 grams of fiber, p<.000), whereas non-visitors increased their fiber intake mainly via fruit (Δ1.53 grams of fiber, p<.001), vegetables (Δ1.16 grams of fiber, p=.009) and nuts (Δ0.52 grams of fiber, p=.001). Intake of fiber via vegetables and nuts was higher in non-visitors compared to the visitors (p=.054 and p=.052), and legumes was higher for the visitors (p=.051). For both groups the intake of dietary fibers using whole grain products did not significantly increase.

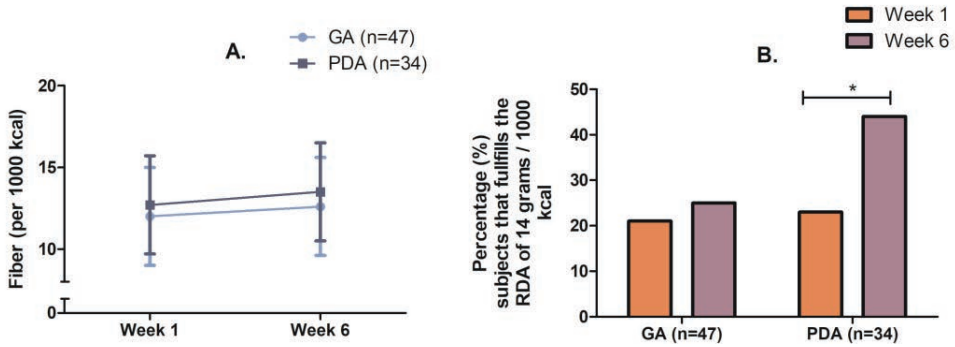

Figure 3A. Dietary fiber intake (per 1000 kcal) did not change during the 6-week intervention. **Figure 3B.** Adherence to the fiber recommendation during the 6-week intervention is higher in the intervention group. Data is based on 24hr recall recalls. Error bars represent standard error. Recommendation according the Dutch Health Council of 14 grams of fiber/1000 kcal.

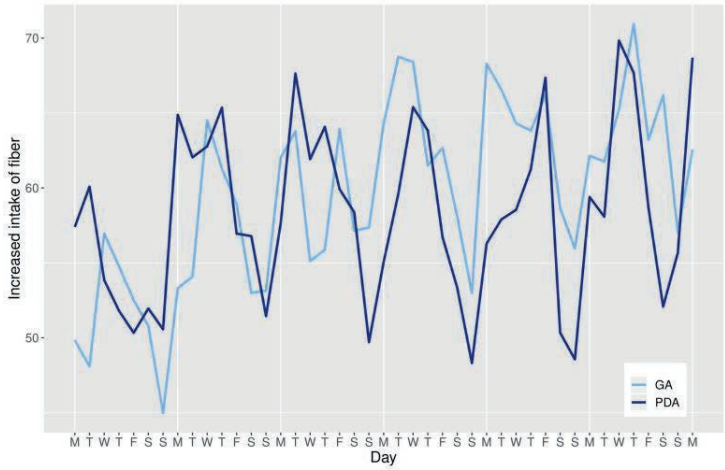

Figure 4. Answers to 'did you manage to eat more fiber today' did not differ between groups. Daily assessed using smartphone based ecological momentary assessment (EMA). Answers were rated on a visual analog scale rating from 0 'not at all' to 100 'yes, very much'.

Daily subjective fiber intake, as assessed by EMA, did not differ significantly between groups (p=.56). Interestingly, within both groups, subjective fiber intake exhibited a consistent lower score on the weekend days (p's<.001) (figure 4). The group-by-day interaction was not significant (p=.22), indicating that this weekend effect did not differ between groups. In line with EMA data, fiber intake as assessed by the 24hr recall was significantly lower on weekend days than on weekdays, both during week 1 (weekdays=24.6±7.7 g/d, Sunday=22.1±9.1 g/d, p=.032) and week 6 (weekdays=25.4±7.7 g/d, Sunday=23.7±8.7 g/d, p=.014). Again, this pattern did not significantly differ between groups (data not shown).

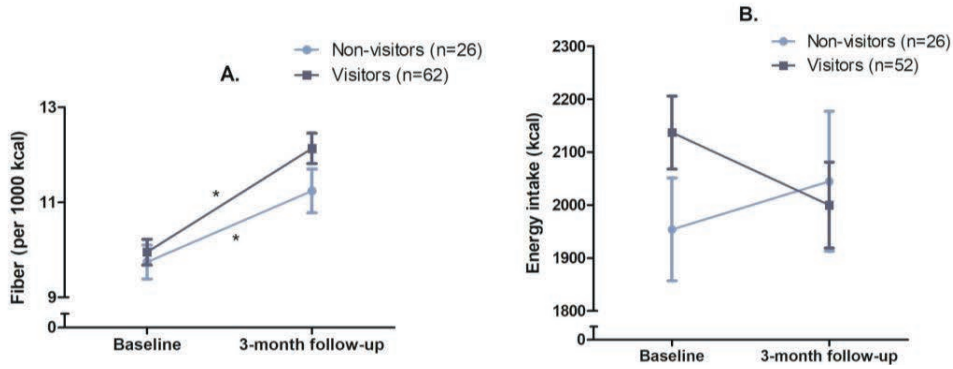

Figure 5A. Both groups increased dietary fiber intake at 3-months follow-up compared to baseline. **Figure 5B.** Visitors decreased their energy intake, non-visitors increased their energy intake (not significant). Visitors are participants in the intervention group, and control participants who visited the PDA after the intervention, non-visitors are participants who never visited the PDA website. * indicates significant difference within the group. Error bars represent the standard error. Data is based on the food frequency questionnaire.

PDA evaluation at week 6

The PDA-group rated their advice significantly better compared to the GA-group regarding the following aspects of their advice: having more knowledge on how to improve their fiber intake (PDA=5.9±0.9; GA=5.3±1.4, p=.033), liking the advice (PDA=5.5±1.4; GA=4.4±1.3, p<.001), easiness of the advice to follow (PDA=5.1±1.3; GA=4.2±1.8, p=.010), motivation to make high-fiber choices (PDA=5.2±1.4; GA=4.5±1.7, p=.055), personal fit (PDA=4.8±1.6; GA=4.0±1.7, p=.032) and working towards a goal (PDA=5.0±1.6; GA=4.1±1.6, p=.021, figure 6). Compared to GA, PDA-participants rated their advice significantly lower regarding the statement “I couldn’t do much with the advice” (PDA=3.5±1.7; GA=4.7±1.6, p=.003), which indicated they perceived the advice more positive. Although PDA-participants scored significantly higher on receiving sufficient feedback, both groups had relatively low scores (PDA=3.8±1.6; GA=2.8±1.8, p=.021). The PDA-group evaluated the advice significantly as more positive (PDA=5.3±1.1; GA=4.7±1.1, p=.014) and useful (PDA=5.2±1.5; GA=4.5±1.4, p=.048), but there were no differences between groups in ratings of attractiveness (PDA=4.7±1.2; GA=4.3±1.4, p=.187) or interestingness (PDA=4.7±1.4; GA=4.3±1.4, p=.295). There was no significant difference between the PDA and GA regarding general satisfaction of the study (PDA=5.5±0.9; GA=5.4±0.9, p=.435) or self-perceived gained knowledge about fibers (PDA=5.8±1.2; GA=5.3±1.4, p=.094).

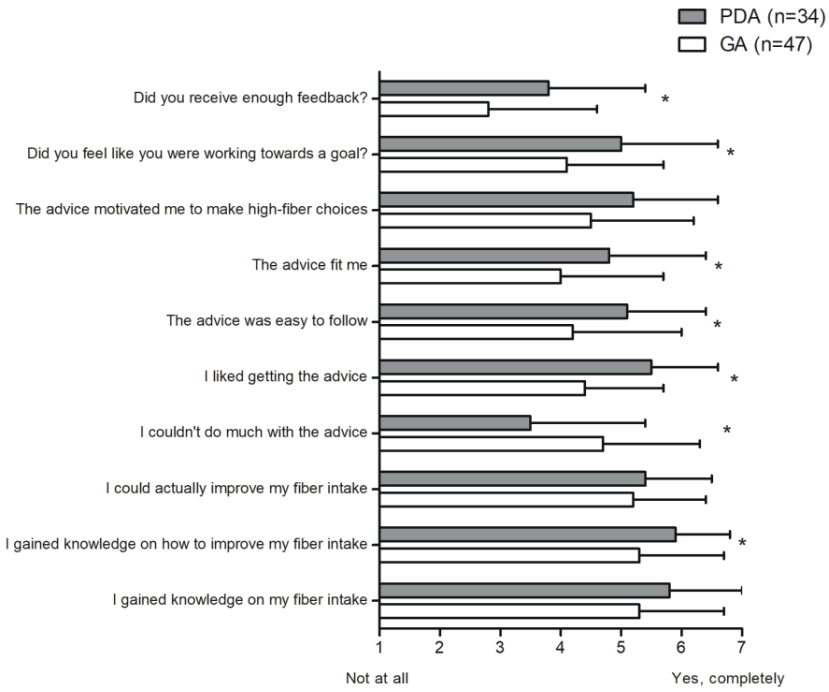

Figure 6: The intervention group rated the advice significantly better than the general advice group. The questionnaire was performed after the 6-week intervention. Statements were rated on a 7-point Likert scale. Error bars represent the standard deviation.

Discussion

In this study, we showed that a personalized dietary advice generated by an algorithm and provided by a website, has additional value compared to general advice in increasing dietary fiber intake in healthy adults. Interestingly, the absolute amount of fiber in grams per day did not increase significantly, but the percentage of people adhering to the recommendation per 1000 kcal did, indicating that people in the PDA-group ate more fiber in the same amount of consumed energy than people receiving GA. Moreover, the algorithm-generated PDA was evaluated more positively than GA, indicating that website-based PDA is well-accepted.

Several studies investigating PDA find similar positive results. Brinberg and colleagues (2000) performed a 4-arm face-to-face high-fiber advice intervention including a group that received a tailored message, general message with intake feedback, general message with no feedback, or a control group that received no message. Messages were given once at the start of the intervention, and effects were measured 6 months later. They found that participants who received a tailored message significantly increased their dietary fiber intake and dietary fiber food

knowledge, but did not find an effect on food choices, compared to the other levels of intervention[44]. Bianchi and colleagues (2020) investigated the effects of computer-based tailored dietary counseling with a dietician compared to general dietary counseling in 80 French pregnant women. The tailored advice was provided during counselling appointments with a dietician, and was generated using software that gave three options for improvement of the nutrient adequacy score. They found that the tailored advice was able to significantly increase the nutrient adequacy scores, while the general advice did not[28]. The same research group has found that substituting food items within the same subgroup improved nutrient adequacy and was moderately acceptable, indicating that food substitution within food groups is a valid method to increase diet quality and still acceptable[45]. Moreover, in a study in 86 children with refractory constipation, a face-to-face personalized high-fiber high-water intervention prescribed by a dietician was more successful in increasing dietary fiber intake compared to general written instructions from a physician[30].

The above-mentioned interventions all included face-to-face counselling, but this may not be feasible for a larger population. One of the larger internet-delivered PDA studies was the Food4Me trial, which included 1269 participants. They investigated 3 different levels of personalizing advice, namely based on (1) individual diet, (2) individual diet, anthropometry and biomarkers, and (3) as level 2 + genotype, compared to GA, aiming to improve overall diet. There was no difference between the PDA levels, but they have shown positive effects of PDA compared to GA in regards to healthy eating index scores, salt, saturated fat and red meat consumption, but not for dietary fiber intake, fruit, vegetable and whole wheat intake[29]. Possibly, this is due to that daily intakes of fiber-rich products such as fruit (378 gram), vegetables (221 gram) and whole grains (164 gram) were already high before start of the intervention, leaving little room for improvement. Moreover, dietary fiber was not the sole aim of the intervention, and fiber intake per 1000 kcal, which showed the most pronounced improvement in our study, was not reported[29].

Our study is the next step in internet-delivered PDA, since it was one of the first to use a product-level model as input, making it more feasible to reach a larger population. However, previous studies as well as ours do not show effectiveness of PDA in terms of absolute fiber intake: possibly due to small differences with general advice. Moreover, an accurate estimate of dietary intake remains challenging, partly due to the large within and between person variation.

This large within and between person variation was also found in our study (25-30%) when assessing fiber intake from 24hr recalls. We only found a subtle and non-significant increase in fiber intake during the 6-week intervention. This can partly be explained by our 24hr recall timing, since we measured a few days into week 1 of the intervention. Probably participants were enthusiastic and increased their fiber

intake already within those first days of the intervention, making it not a real baseline fiber intake measurement. This assumption is supported by the fact that fiber intake measured by the FFQ two weeks before the start of the study was lower compared to fiber intake measured by 24hr recalls during week 1 (3.9 grams). Fiber intake measured by the FFQ showed that only 2 participants met the recommendation per 1000 kcal at the start, but 24hr recall data suggested that 18 participants adhered to the recommendation of fiber. Although differences between the FFQ and 24hr recalls have been reported before, most often the FFQ had a higher estimate of fiber intake than 24hr recalls[46, 47]. This indicates that the increase in fiber during the 6-week intervention, estimated by 24hr recall data, is probably underestimated in this study. However, this is likely to apply to both the PDA and GA-group. The underestimation of the change in fiber intake based on the 24hr recalls during the intervention is further supported by our 3-month follow-up FFQ, in which both non-visitors and visitors increased their fiber intake compared to baseline FFQ. This indicates that participants did substantially and significantly increase their fiber intake during the intervention period.

At the 3-month follow-up, although non-significant, visitors had a higher fiber intake and a lower energy intake compared to non-visitors. Compared from baseline to 3-month follow-up, visitors increased fiber intake by increasing fruit intake and legumes intake, and non-visitors increased their fruit, vegetable and nut intake. However, by using the FFQ we may have missed some of the true changes the PDA-participants made. Many of the high-fiber alternatives generated by the PDA are not included in the FFQ, such as hummus (chickpea spread), quinoa, and whole grain options for rice and pasta. The FFQ used is based on the intake of the reference population from the National Dutch Food Composition Survey[19], and these products were not frequently enough consumed by the Dutch population between 2007-2010 to be included in the FFQ. Therefore, fiber intake in visitors may be underestimated, and the intervention may be more effective than we measured. It is important to note that visitors and non-visitors were not randomly allocated, and it is uncertain whether visiting the website caused the differences between these groups, or whether other factors such as higher motivation in visitors resulted in this difference. Due to our study design, we could not assess this.

Based on our experience, some factors need to be considered when designing a PDA delivered via a digital tool such as a website. As shown by our drop-outs and ‘non-login participants’, age and technological skills of the population seem to be important to consider beforehand. Most reported reasons for drop-outs were technological difficulties and time investment needed for the study (n=12, 85%). Although we provided a paper manual and instruction videos to facilitate website use, this may not be sufficient to prevent and overcome technical difficulties. However, in our study, the reported difficulty of technology may not be solely pointed

to the PDA website; a previous study in Dutch seniors >60 years did not report any issues for PDA-use via a website[27]. However, in that study less input was required because the intake and the advice were given at the more general level of food categories. This makes the advice less 'actionable'. Moreover, since we also used other technologies for study measurements (EMA application, FFQ and 24hr recall websites), a combination of several different technologies together might have been the reason to drop-out.

Limitations of this study include the self-reported outcomes such as dietary intake and evaluation which are both sensitive to variability and social desirable answers. However, currently no valid biomarker for dietary fiber intake exists. Plasma akylresorcinol is proposed as a biomarker for whole grain intake, but total fiber intake as well as other grain sources such as oats, barley, corn or rice, were not correlated with this biomarker[48]. Regarding social desirability, participants performed questionnaires online, which reduces social desirability as compared to face-to-face questionnaires[49]. However, due to these web-based platforms, we also encountered some technological problems (such as errors when logging-in) during the study, which may have influenced our results.

An important strength of this study is the single-blinded RCT design, which reduces bias. Moreover, the relatively large sample size enabled us to assess effects of the intervention. In addition, the 3-month follow-up allowed us to assess whether PDA-participants maintained a high-fiber intake after the intervention and thus whether PDA can provoke a sustainable long-term change in dietary fiber intake. To our knowledge, this is the first personalized high-fiber dietary advice study integrating personal and food data knowledge into an algorithm and thereby modeling advice to improve fiber intake in healthy adults, by allowing participants to choose their own high-fiber alternatives.

Conclusion

This study showed that algorithm-generated personalized dietary advice that was delivered via a website is an accepted method to empower people to make sustainable changes in their diet. PDA helped significantly more people to adhere to the fiber recommendation than general advice, especially as it increased fiber intake combined with a reduced energy intake after three months. Remarkably, there was significantly lower fiber intake during weekend days than on weekdays for both groups. Several aspects such as technological support and highly reproducible dietary assessment are important for effectiveness and validation of the PDA. As our study mainly included well-educated healthy adults, future studies should evaluate the effectiveness of PDA in other populations such as in participants with low-socioeconomic status, or in participants with gastro-intestinal complaints such as constipation.

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CHAPTER 6

A HIGH-FIBER PERSONALIZED DIETARY ADVICE GIVEN VIA A WEB-TOOL REDUCES CONSTIPATION COMPLAINTS IN ADULTS

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Abstract

Background: Constipation is characterized by hard stools and infrequent bowel movements, and greatly impacts quality of life (QoL). Dietary fibers can help relieve constipation, however preserving a higher fiber intake remains a challenge, and patients are often referred to fiber supplements. Therefore, we investigated the effects of a personalized dietary advice (PDA) on fiber intake and mild constipation complaints in adults with constipation complaints.

Methods: N=25 adults with mild constipation complaints were included in a 4-week observation period (week 1-4) followed by a 4-week personalized intervention (week 5-8). The PDA was based on gender and habitual diet and provided high-fiber alternatives via a web-tool. In week 1, 4 and 8, dietary intake, constipation complaints, QoL, physical activity levels and behavioral aspects were assessed. Furthermore, participants collected a fecal sample at week 1, 4 and 8 to determine microbiota diversity and composition, and short-chain fatty acids (SCFA) levels and their dynamics over time. Participants completed questions daily for 8 weeks regarding abdominal complaints, stool frequency and stool consistency. Differences over time were assessed by using mixed models.

Results: Fiber intake in week 8 was significantly higher compared to week 1 (Δ=5.7±6.7g, p<.001) and week 4 (Δ=5.2±6.4g, p<.001). Constipation severity and QoL significantly improved at week 8 compared to the observation period (p<.001). Mixed model analysis over time showed that a higher fiber intake significantly reduced constipation severity (ß= -0.031 (-0.05; -0.01), p=.001) and QoL (ß= -0.022 (-0.04; -0.01), p=.009). Stool consistency improved significantly during the intervention (p=.040), but stool frequency did not. Abdominal pain reduced significantly during the intervention (p=.030), but no changes were observed for abdominal cramps or bloating. Average microbial alpha diversity and composition, and SCFA concentrations did not change over time, but indicated individual-specific dynamics. Several SCFAs were associated with constipation complaints. Subjective knowledge (p<.001) and outcome beliefs (p=.036) increased after the intervention, and the PDA was well-accepted.

Conclusion: A PDA effectively increased fiber intake and subsequently reduced constipation complaints, indicating that guided dietary adjustments are important and feasible in the treatment of mild constipation complaints.

Keywords: Dietary Fiber; Personalized Nutrition; Constipation; Functional Bowel Disorders; Quality of Life

Introduction

Constipation complaints are characterized by straining, hard stools, and infrequent bowel movements, which can greatly impact quality of life (QoL)[1]. Moreover, constipation is associated with an increase of the risk of colorectal cancer, Parkinson's disease, cardiovascular disease and all-cause mortality, among others[2-7]. The global prevalence is estimated between 5-20% depending on the definition used, and is more often present in women[8-10]. Constipation can result from having endocrine or metabolic disorders, neurological diseases, medication use or an unhealthy lifestyle[11]. A lifestyle characterized by a low fiber intake and low physical activity level is associated with an increased prevalence of constipation complaints[12]. Dietary fibers play an essential role in supporting a healthy stool pattern, as most fibers fasten intestinal transit time and absorb water, thus increasing intraluminal volume with a positive effect on stool frequency and stool consistency[13-19]. This was also shown in two meta-analyses, in which fiber supplements were effective in increasing stool frequency[14], and inulin-type fructans improved stool pattern[20]. Fibers can furthermore influence gut microbiota kinetics by fermentation of fibers into short-chain fatty acids (SCFA). Butyrate, one of the main SCFA, is a substrate for colonic cells and known for the anti-inflammatory properties and positive effects on gut health[21-23]. Furthermore, a high-fiber diet has been associated with higher levels of microbial richness and diversity[24].

The effects of fibers from diet could also beneficially impact stool pattern in adults with constipation complaints, but this is not fully researched yet. Anti and colleagues (1998) have shown that a fiber intake of >25g/day increased stool frequency, which was more pronounced in patients who drank >2 L/day of water, after an intervention of two months[25]. A high-fiber diet of 28 g/day was also effective in improving constipation in women with pelvic floor disorders after a 42-day intervention [26]. Moreover, a high-fiber diet improved QoL of people with constipation, as was shown in elderly and patients with chronic kidney disease[27, 28]. Interestingly, medical costs associated with constipation complaints seem to reduce with an increased fiber intake[29, 30].

A fiber intake of 14g/1000 kcal, which is 30g/day for women and 40g/day for men, is recommended for adults in the Netherlands, regardless of having constipation complaints[31]. However, median current intakes are far below these recommendations, as Dutch women consume 18 g/day and men 23 g/day[32]. Personalized dietary advice (PDA) was recently suggested as a strategy to sustainably improve the diet, with promising results[33, 34]. PDA improved compliance to a high-fiber, high-water diet in children with refractory functional constipation compared to general advice[33]. However, this study used face-to-face guidance in their PDA, making it difficult to reach larger populations. In the Food4Me trial, a digital PDA was shown to be effective in improving healthy eating index scores, but

not dietary fiber intake in 1607 healthy adults[34]. However, the study population had high baseline fiber intakes, and an increase in fiber was not the sole aim of the intervention. Recently, we have shown that a digital high-fiber PDA was effective in improving fiber intake up to 3 months after the intervention in adults without gastrointestinal complaints, and this PDA was positively evaluated[35]. Therefore, we now aimed to investigate the effect of a high-fiber PDA on constipation severity, quality of life, stool pattern, and fiber intake in adults with mild constipation complaints. Furthermore, the effects of a digital high-fiber PDA on gut microbiota and SCFA, behavioral factors and acceptability were investigated.

Methods

This study had an 8-week study period consisting of one arm. The study consisted of two phases. The first phase was a 4-week observation period (week 1-4), in order to take the high within and between person variability in stool pattern, complaints and dietary intake into account[36, 37] and to serve as a control. Thereafter, a 4-week intervention period followed (week 5-8) in which participants received the PDA (Figure 1). To reduce bias, participants were unaware of the purpose of the PDA during the observation period, *e.g.* they were informed that the intervention would include lifestyle advice but not that it was focused on fiber. At the start of the intervention, participants received this information. The study was performed from August to November 2020. For full details, see the TIDieR checklist. All participants provided written informed consent. The study was approved by the Medical Ethics Committee of Brabant, and registered at Clinicaltrials.gov under number NCT04457791.

The PDA intervention

As described earlier[35], the PDA was distributed via a web-tool developed for this study and was generated by linking personal food intake to generic food data. The PDA aimed to provide high-fiber substitutes for habitually consumed low-fiber products. The advice was personalized based on gender and habitual dietary intake of the last month, as assessed by a 247-item meal-based food frequency questionnaire (FFQ). The FFQ was performed in week 1 during a face-to-face interview with trained researchers. The FFQ was validated[38, 39], except that items were not questioned for the whole day but per meal moment (breakfast, lunch, dinner, in-between meals), so that advices could be given per meal moment.

The web-tool showed a participants' habitual intake per meal moment (breakfast, lunch, diner, in-between meals) and high-fiber alternatives, which were ranked from high to low based on fiber content, to aid participants in their selection of high-fiber alternatives. The high-fiber alternative list did not use brand names but generic

product categories (for example whole wheat crackers) and was compiled by study researchers in consultation with dieticians. Participants could also include an extra portion of fruit, vegetables, legumes and/or nuts and seeds at each meal moment. In line with the Dutch recommendations, participants could not select >2 pieces of fruit and >25g of nuts and seeds per day[40], to limit sugar and calorie intake. Participants then received feedback on how much their chosen PDA increased their daily dietary fiber intake in reference with the recommendations. The final step included the formulation of implementation intentions, which can help participants to translate their intentions into behavior and achieve sustainable dietary changes into the daily routine[41].

During the first ten days of the intervention, participants were limited in their selection of meal moments to ensure a gradual increase in dietary fiber intake to prevent abdominal bloating or cramps. From day 1-3, they could select one meal moment to work on, on day 4-6 they added a second meal moment to their PDA, and so on. After 10 days, participants had access to all meal moments in the PDA, and they could freely adjust their PDA during the remainder of the intervention (Figure 1). The web-tool also stated general lifestyle tips regarding water intake and physical activity[42], and information on how to read food labels. Participants' activity on the web-tool was logged to assess compliance.

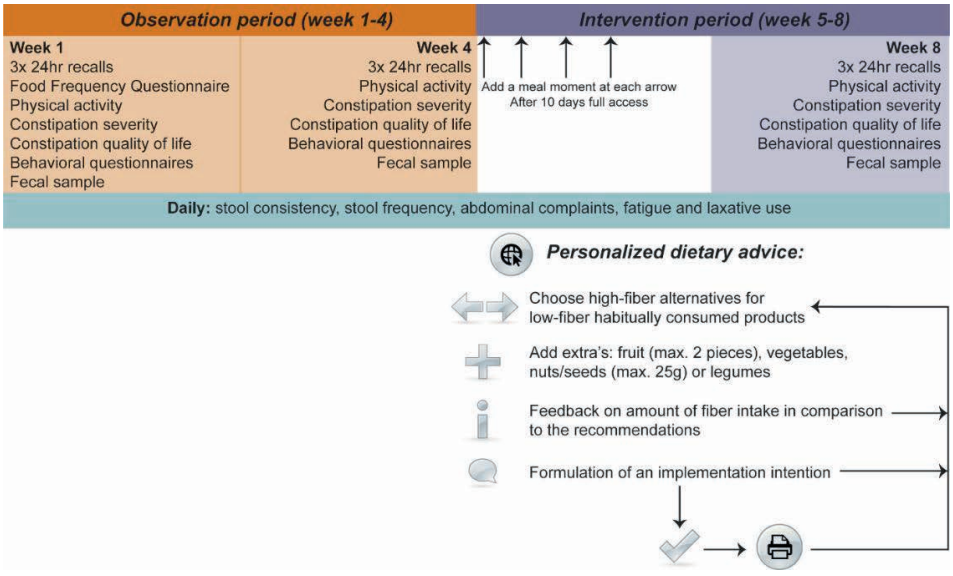

Figure 1. Study design

Study participants

Participants were recruited via the participant database of Wageningen University & Research, social media and newspaper advertisements. Participants were eligible when having constipation complaints, which was defined as being unsatisfied with

their stool pattern (<6 on a visual analog scale (VAS) from 1-10), and habitual stool form of Bristol stool type 1-4[43] and/or ≤4 defecations per week. These criteria are less stringent than the official functional constipation definition, yet were chosen for several reasons.

First, although the Rome IV criteria for constipation are validated, studies have shown a large overlap with Irritable Bowel Syndrome constipation subtype (IBS-C), and current diagnostics are unable to distinguish between both disorders[44, 45]. Second, 19-34% of the people who experience constipation complaints do not meet the Rome criteria for constipation or IBS-C, but still experience substantial symptoms and a reduction in QoL[46-48], and are frequently missed in research and treatments. Third, we expected that a mildly constipated population can benefit the most from a dietary fiber intervention, hence the main inclusion criteria were based on stool satisfaction, in combination with either a hard or normal Bristol stool type and/or a low stool frequency. Frequent loose stools and diarrhea were excluded. Self-evaluation of constipation complaints using VAS and the Bristol stool type was shown useful to determine constipation[49].

Other criteria included a restriction of age to 18-55 years and Body Mass Index (BMI) <30 kg/m^2, due to national restrictions because of the Sars-CoV-2 pandemic. Furthermore, eligible participants were living near the city of Wageningen (max. 50km) for practical reasons; had a relatively low fiber intake (females <26g/day, males <33g/day), and in possession of and able to use a computer and mobile phone. Participants were excluded when having an autonomic disorder, inflammatory bowel disease, coeliac disease, cancer, kidney disease, depression or hypothyroidism; when following a diet and unable or unwilling to change; pregnant or breastfeeding; using diuretics, antidepressants, codeine, antibiotics, fiber supplements, Prucalopride, Methylnaltrexone or Linaclotide.

We aimed to include 25 participants in the intervention period, to measure an increase in stool frequency of 1.3±1.8 stools/week with α=0.05, and 1-β=0.80[25]. We screened participants for a low fiber intake in two steps: first, a rough screening was done by using our specially developed screening fiber questionnaire[50]. Next, a second and more thorough screening based on a complete FFQ was performed. As we expected that 20% of the screened participants would have a fiber intake exceeding the cut-offs, we included 30 participants to complete the FFQ, to result with 25 participants below the cut-offs in the intervention phase.

Constipation complaints and stool pattern

Constipation severity, QoL and stool pattern were the primary outcomes. Constipation severity of the last two weeks was assessed by using the 12-item validated Patient Assessment of Constipation Symptoms (PAC-SYM)[51, 52]. This

questionnaire gives a score for total severity, and severity subscales abdominal pain, stool complaints and rectal complaints. Each score ranges from 0-4, with a high score indicating severe symptoms. The validated 28-item Patient Assessment of Constipation QoL (PAC-QOL) was used to assess the impact of constipation on daily life during the last two weeks[53]. This questionnaire computes a score for total QoL, and subscales scores for worries and concerns, satisfaction of stool pattern, physical discomfort and psychological discomfort. Scores range from 0-4; a high score indicating a poor QoL. Questionnaires were completed digitally in week 1, 4 and 8.

Abdominal complaints, stool pattern and laxative use were assessed daily during the 8-week study period by using an Ecological Momentary Assessment (EMA) app on participants' mobile phone. EMA is a structured diary technique that can take personal variation into account[54], and has previously been used to assess stool pattern in IBS patients[55]. In the present study, participants received notifications every evening (time could be personalized), and questions could be answered within one hour after the notification. Participants rated abdominal cramps, pain, bloating, flatulence and fatigue on a 100-point VAS from 'no complaints/fatigue' to 'very severe complaints/fatigue'[56, 57]. Moreover, participants reported laxative use, stool frequency as well as stool consistency, by using the Bristol stool chart, which lists stools from small pallets (type 1) to very loose (type 7)[43].

Dietary intake and physical activity

To assess changes in fiber intake and diet between week 1, 4 and 8, trained research dieticians performed 24hr recalls via the telephone. For each timepoint, three non-consecutive recalls consisting of two weekdays and one weekend day were performed to take variation into account. Participants were not informed beforehand which day the recall would take place to reduce bias. Recalls were subsequently entered in the validated program Compl-eat[58], which estimated nutrient intake by using the Dutch Food Composition Table of 2019[59]. Furthermore, high-fiber food group intake was compiled from the 24hr recall data and included whole grain bread/crispbreads, whole grain cereals and grains (*e.g.* rice, pasta, couscous), vegetables, fruits, nuts and seeds, legumes, and potatoes and other tubers. Subjective self-efficacy of eating more fiber was reported daily during the 4-week intervention via the EMA app. Participants completed the question 'did you manage to eat more fiber today' on a 100-point VAS ranging from 0 'not at all' to 100 'yes, very much'.

Physical activity was assessed at week 1, 4 and 8 by using the validated short questionnaire to assess health-enhancing physical activity (SQUASH)[60]. This questionnaire assessed commuting, leisure time, sports, household and work/school activities. For each activity a score was calculated by multiplying the metabolic equivalent of task (MET) values, derived from the Ainsworth compendium[61], by the

duration of the activity. Furthermore, a total activity score was computed by summing the score of all activities.

Gut microbiota and SCFA profiling

Participants collected a fecal sample in week 1, 4 and 8 of the study. The sample was immediately frozen at home, and participants transported the frozen sample to the research facility within 7 days by using a dedicated cooling box. Subsequently, the sample was put on dry ice, and stored at the -80 ºC freezer until further analysis.

Fecal SCFA acetate, propionate and butyrate were analyzed as previously described, with minor modifications[62]. Briefly, 0.4g of feces was used, and mixed thoroughly with 1.6mL demi water to extract the SCFA, which were analyzed by High-Performance Liquid Chromatography (HPLC, LC-2030C, Shimazu, Kyoto, Japan) with a Shodex SH1821 column (Showa Denko K.K., Tokyo, Japan). Microbiota composition was determined as previously described[63]. In short, 0.25g feces (wet weight) was used for DNA isolation with the Repeated Beating method[64]. Subsequently, PCR amplification of the V4 region of the 16s rRNA gene followed by the barcoded Illumina Hiseq2500 sequencing (150bp paired end) was performed to obtain sequencing data[65]. Afterwards, NG-Tax 2.0 was used to process the raw sequencing data for Amplicon Sequencing Variant (ASV) picking with default settings and for taxonomic assignments by using the SILVA database (version 132)[66, 67]. Sequencing data was submitted to the European Nucleotide Archive with accession number PRJEB47379.

Behavioral and PDA evaluation questionnaires

Validated behavioral questionnaires were completed to gain insight into how the PDA affected the participants and why PDA was effective or not. In week 1, 4 and 8, participants filled in a 3-item intention to eat fibers, a 2-item subjective health and a 5-item self-regulation questionnaire[68, 69]. At week 4 and week 8, participants completed a 5-item subjective knowledge and a 9-item outcome belief questionnaire regarding fibers[70, 71]. Answers were rated on a 7-point Likert scale. When filling in these questionnaires, participants were blinded for fiber in week 1, but not in week 4 and 8. Participants also received an evaluation questionnaire in week 8 to assess acceptance of the PDA. Participants rated statements on a 7-point Likert scale, which included how positive, useful, attractive or interesting they found the advice, and how much the PDA helped and/or motivated them.

Statistical analysis

Continuous data are presented as mean ± standard deviation, or median (interquartile range, IQR) when skewed. Differences over time (fixed main factor) in symptoms, QoL, diet, physical activity and SCFA were assessed using mixed models with a diagonal structure. Furthermore, mixed models were used to assess

the effects of fiber intake (main fixed covariate) on constipation severity or QoL (dependent variables). In an additional model, water intake and total physical activity score were added to assess the effects of fiber when these variables were adjusted for. Mixed model data is reported as the beta coefficient with 95% confidence intervals or the standard error. Based on the minimal important difference (MID) of total PAC-SYM, a change of ≥0.6 was considered clinically relevant[72], and responders and non-responders to the intervention were defined and compared with an independent sample t-test. To analyze EMA data (stool pattern and abdominal complaints), linear mixed models with restricted maximum likelihood estimation using lmer was used. Participants that completed ≥40 out of 56 days for EMA questionnaires were included in EMA analysis. The behavioral questionnaires were analyzed by using general linear model with repeated measures. Microbiota alpha diversity (within sample diversity) and composition were calculated at ASV level by using Phyloseq[73]. ASV richness and Shannon diversity were calculated for assessing microbiota alpha diversity, which were compared between timepoints by using a Wilcoxon signed-rank test. Principle Coordinate analysis (PCoA) based on unweighted (considering presence/absence of ASVs) and weighted (considering ASVs and their relative abundance) Unifrac distances[74] was performed for the visualization of microbiota composition. For the microbiota data, p-values for multiple pairwise tests were corrected by using Benjamini-Hochberg false-discovery rate. Microbiota and EMA data was analyzed in R version 4.0.0[75], other data in SPSS version 25 (Armonk, NY, USA: IBM Corp.). A (corrected) p-value ≤0.05 was considered significant.

Results

In total, 38 participants were screened, one participant withdrew consent before study start, and 29 participants were included in the study (Figure 2). Four participants were excluded in week 3 in line with the study protocol, resulting in 25 participants as final study population. The study population consisted mainly of young, female participants with a higher education level (Table 1). None were currently smoking nor used laxatives at the start of the study. All participants logged in on the PDA web-tool at least once, and on

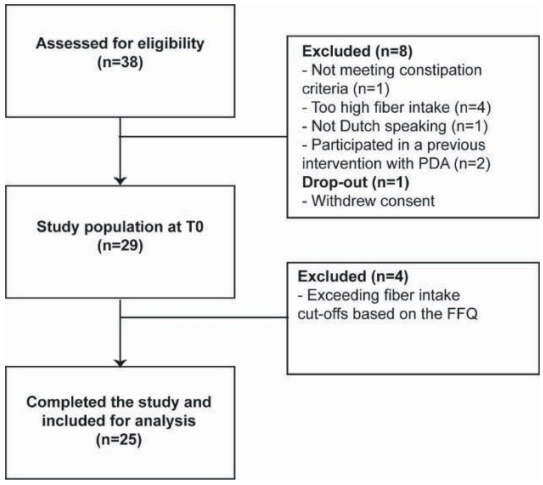

Figure 2. Study flowchart

average completed all steps on the web-tool 3.7±2.2 times during the 4-week intervention. Fruit was added to the PDA most frequently (n=14), followed by vegetables (n=10), nuts and seeds (n=8) and then legumes (n=7).

Table 1. Baseline characteristics of the study population

	Constipated adults (n=25)
Age (years)	26 (23 – 53)
Gender, males n (%)	5 (20)
Body Mass Index (BMI) (kg/m^2)	23.0 ± 2.3
Completed ≥higher vocational education, n (%)	20 (80)
Satisfaction with stool pattern[†]	3.1 ± 1.5
Stool frequency (number of stools per week)	4.2 ± 1.8
Habitual stool type[‡]	2.7 ± 1.0

Values are mean ± standard deviations or median (interquartile range) when skewed. [†]Assessed on a visual analog scale (VAS) from 1 'not satisfied' to 10 'very satisfied'. [‡]Indicated by the Bristol stool chart, which rates stools from small pallets (type 1) to very loose (type 7).

PDA increased fiber intake while other lifestyle parameters stayed stable over time

Dietary fiber intake, both in grams and g/1000 kcal, was significantly higher in week 8 compared to week 1 (Δ=5.7±6.7g, p<.001; and Δ=1.5±3.2 g/1000 kcal, p=.032) and week 4 (Δ=5.1±6.4g, p<.001; and Δ=1.9±3.2 g/1000 kcal, p=.007, Table 2), indicating that the increase in fiber intake was specifically during the intervention period. Furthermore, the percentage of participants adhering to the recommendations of fiber increased over time, with statistical significance for fiber in grams (12% to 36%, p=.023), but not for g/1000 kcal (16% to 40%, p=.148). Self-reported self-efficacy of increasing fiber intake was significantly lower during the weekend compared to weekdays (p=.004, Supplementary Figure 1). Participants significantly increased the amount of fiber from whole grain breads (p=.011) and fruit (p=.031) at week 8 compared to the observation period, but not from whole grain cereal and grains (p=.755), vegetables (p=.537), and potatoes (p=.370, Supplementary Figure 2). The fiber content from nuts and seeds (Δ=0.69±1.7 g/fiber, p=.163) and legumes (Δ=0.98±3.4 g/fiber, p=.085) increased after the PDA, albeit non-significantly. During the 8-week study period, physical activity, bodyweight, energy, water and macronutrient intake remained stable (Supplementary Table 1).

Table 2. Efficacy of the intervention and changes in lifestyle over time

	Week 1	Week 4	Week 8	p-value
Efficacy of the intervention: dietary fiber intake				
Dietary fiber (g)	21.6 ± 7.1[a]	21.0 ± 6.7[a]	26.7 ± 9.8[b]	**.025**
Adhering to fiber recommendation in grams, n (%)[‡]	3 (12)	2 (8)	9 (36)	**.023**
Dietary fiber (g/1000 kcal)	11.2 ± 2.9[a]	11.6 ± 3.2[a]	13.1 ± 3.9[b]	**.022**
Adhering to fiber recommendation per 1000 kcal, n (%)[‡]	4 (16)	6 (24)	10 (40)	.148
Dietary intake				
Energy (kcal)	1938.2±462	1848.7±446	2044.7±444	.305
Carbohydrates (en%)	42.2 ± 5.9	44.8 ± 5.7	43.2 ± 6.2	.275
Water (L)*	2.74 (2.4 – 3.5)	2.57 (2.3 – 3.0)	2.8 (2.8 – 3.2)	.829
Physical activity				
Total physical activity score[†]	5700 (2490 – 7478)	5865 (4510 – 7080)	4530 (3190 – 6525)	.271
Adhering to the recommendation, n(%)[‡]	14 (56)	13 (52)	14 (56)	.948

Values are mean ± standard deviations or median (interquartile range) when skewed. Dietary intake was assessed using 24hr recalls, and physical activity using the short questionnaire to assess health-enhance physical activity (SQUASH). Differences between timepoints were assessed using linear mixed models or chi-square when categorical, different superscripts indicate significant differences between the timepoints. The overall p-value over time is shown. Abbreviations: en% = energy percentage. [†]Calculated by multiplying the metabolic equivalent of task values per activity times the minutes per week per activity, and then summed. [‡]Recommendations for fiber are according to the Dutch Health council; 30 grams for women or 40 grams for men, or 14 grams/1000 kcal. The physical activity guideline is >30 minutes of moderate or vigorous physical activity for ≥5 days per week. *Water intake represents not only intake of liquids but also includes water in foods.

Table 3. Mixed model analysis of the effects of fiber intake on constipation severity and quality of life over time

	Model 1: fiber intake		**Model 2: fiber, water and physical activity score**	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Total constipation severity	-0.031 (-0.05; -0.01)	**.001**	-0.028 (-0.05; -0.01)	**.003**
Abdominal complaints	-0.027 (-0.04; -0.01)	**.004**	-0.024 (-0.04; -0.00)	**.014**
Rectal complaints	-0.021 (-0.04; -0.00)	**.021**	-0.021 (-0.04; -0.00)	**.028**
Stool complaints	-0.038 (-0.06; -0.01)	**.004**	-0.036 (-0.06; -0.01)	**.008**
Total constipation quality of life	-0.022 (-0.04; -0.01)	**.009**	-0.021 (-0.04; -0.00)	**.013**
Worries and concerns	-0.022 (-0.04; -0.00)	**.026**	-0.023 (-0.04; -0.00)	**.024**
Satisfaction of stool pattern	-0.041 (-0.07; -0.01)	**.003**	-0.031 (-0.06; -0.00)	**.022**
Physical discomfort	-0.033 (-0.05; -0.01)	**.002**	-0.033 (-0.05; -0.01)	**.003**
Psychological discomfort	-0.013 (-0.03; 0.00)	.121	-0.014 (-0.03; 0.00)	.075

The estimate and p-value is given for fiber intake in grams. Data is tested using linear mixed models, using a diagonal variance structure, and indicating time as repeated measures. Constipation severity of quality of life are dependent variables and lifestyle variables are added as fixed main effects to the model. Dietary intake was assessed using 24hr recalls, and physical activity using the short questionnaire to assess health-enhance physical activity (SQUASH). Physical activity is a score calculated by multiplying the metabolic equivalent of task values per activity times the minutes per week per activity, and then summed.

Dietary fiber intake significantly improved constipation complaints over time

Total constipation severity (scored from 0 to 4) improved significantly at week 8 compared to the observation period (week 1=1.49±0.6, week 4=1.48±0.7, week 8=0.99±0.6, p<.001, Figure 3A). Similar results were found for its subscales abdominal complaints (p=.003, Figure 3B) and stool complaints (p<.001, Figure 3D). Although rectal complaints did significantly change over time (p=.017, Figure 3C), pairwise comparison showed that this was only between week 4 and week 8 (p=.014). Total constipation QoL improved significantly over time (p=.001, Figure 4A), as well as worries and concerns (p=.014, Figure 4B), physical discomfort (p<.001, Figure 4C) and stool satisfaction (p<.001, Figure 4E). Psychological discomfort did not change significantly over time (p=.053, Figure 4D).

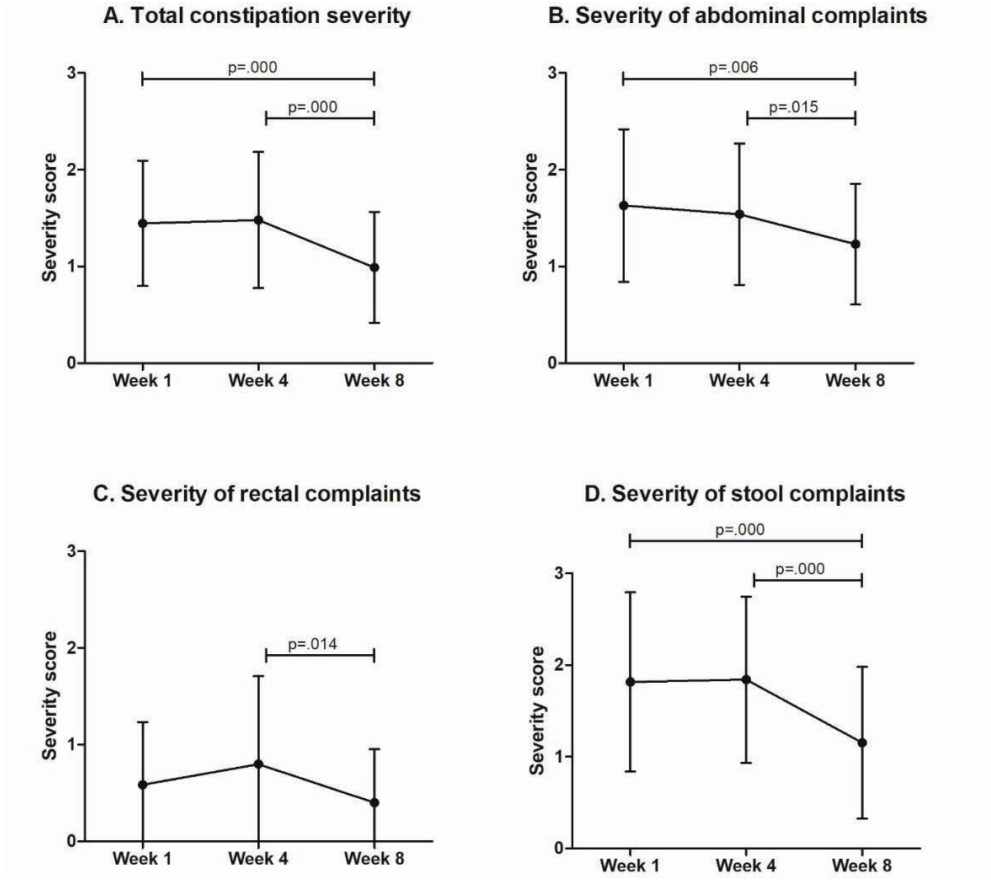

Figure 3. Changes in constipation severity over time
Measured by the Patient Assessment of Constipation severity (PAC-SYM) questionnaire. Scores range from 0-4, a higher score indicating more severe constipation. Differences over time were tested with linear mixed models. Week 1 and week 4 were observational, week 8 is after the intervention.

Mixed model analysis showed that fiber intake (g/day) significantly affected all scores of constipation severity and QoL over time, except for psychological discomfort (ß= -0.013±0.008, p=.121, Table 3). This indicates that the change in constipation severity or QoL score was dependent on dietary fiber intake over time. Results did not change after the addition of water intake and physical activity level to the model.

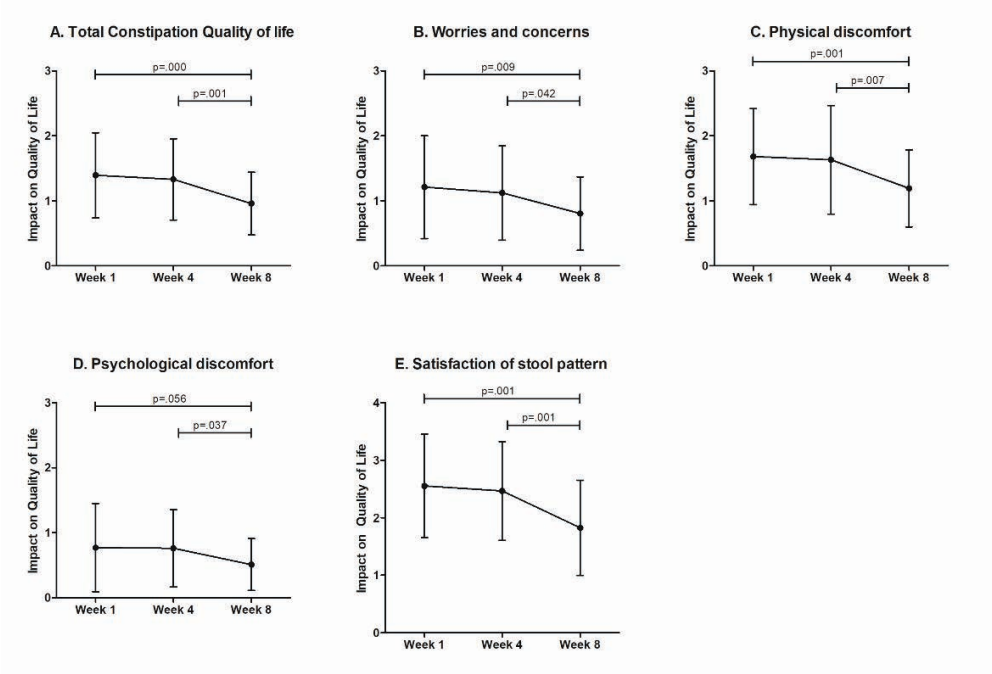

Figure 4. Changes in constipation-related quality of life over time
Measured by the Patient Assessment of Constipation Quality of Life (PAC-QoL) questionnaire. Scores range from 0-4, a lower score indicating a better QoL. Differences over time were tested with linear mixed models. Week 1 and week 4 were observational, week 8 is after the intervention.

Stool consistency and abdominal pain improved, but not stool frequency

EMA compliance was high: 85±14% of the questionnaires were completed. None of the participants reported use of laxatives during the 8-week trial. Four participants did not complete ≥40/56 days, resulting in 21 participants as study population for analysis. There was no intervention effect on the average number of stools per day (p=.795, Figure 5A), but stool consistency significantly softened during the intervention period (3.2 (95% CI=2.9-3.6)) compared to the observation period (2.9 (95% CI=2.6-3.3), p=.041, Figure 5B). Furthermore, abdominal pain significantly reduced during the intervention period (16.0 (95%CI=8.7-23.3)) compared to the observation period (21.3 (95%CI=14.0-28.6), p=.03, Figure 5C). No intervention effects were observed for fatigue (p=.238), abdominal cramps (p=.331) or bloating (p=.136), results not shown.

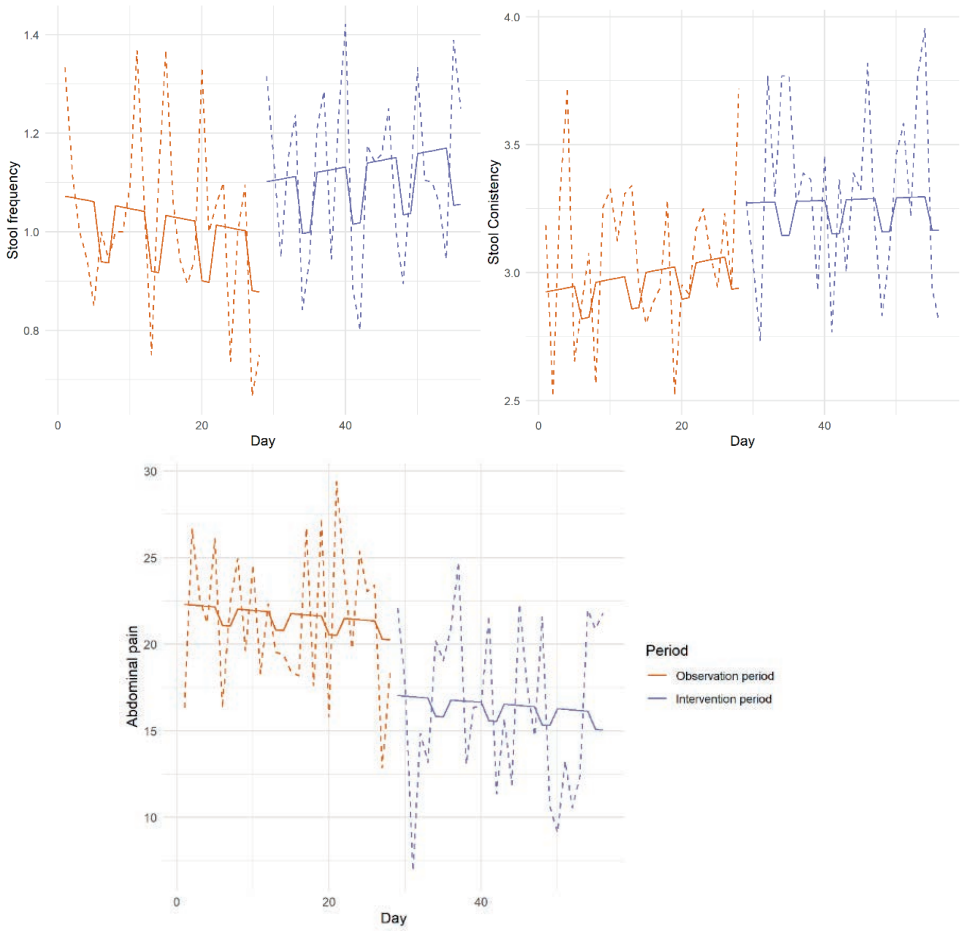

Figure 5A. Daily stool frequency, 0 indicating no stool that day. **Figure 5B.** Daily stool consistency, assessed by the Bristol stool chart, ranging from 1 'hard pellets' to 7 'loose stools'. **Figure 5C.** Daily abdominal complaints, assessed on a 100-point visual analog scale (VAS) from 0 'no complaints' to 100 'very severe'. Data was collected daily using ecological momentary assessment (EMA) application on a participants' mobile phone. The dotted line represents the group average, the solid line represents the regression line.

Gut microbiota and SCFA did not change after the intervention

Large variation in acetate (Figure 6A), propionate (Figure 6B) and butyrate (Figure 6C) was observed over time. Median levels of SCFA increased at week 8 compared to week 1 or 4, albeit non-significant. Microbial alpha diversity as shown by ASV richness (Figure 6D) and Shannon diversity (Figure 6E) did not change over time. PCoA analysis based on weighted (Figure 6G) and unweighted (Figure 6H) Unifrac distance indicated no clear separation in microbiota composition before and after the intervention. However, microbiota composition distance over time tended to be

higher between week 4 and 8 as compared to week 1 and 4, indicating that the composition changed more during the intervention than during the observation period (Figure 6F, p=.086).

Mixed model analysis showed no effect over time of dietary fiber on acetate (ß=0.45 (-0.24; 1.14), p=.197), propionate (ß=0.04 (-0.13; 0.21), p=.649), or butyrate (ß=0.17 (-0.14; 0.49), p=.281). Total constipation severity was borderline significantly associated with all three SCFA over time (Supplementary Table 2), and an increase in severity of stool complaints was significantly associated with lower levels of all SCFA. Total QoL was borderline significantly associated with propionate and butyrate. For the QoL subscales, an increase in worries and concerns was significantly associated with lower propionate levels (p=.036), while an increase in physical discomfort was significantly associated with lower butyrate levels (p=.038).

Responder/non-responder analysis

Based on the minimal important difference of the PAC-SYM, we identified 9 responders and 16 non-responders. All responders were female, and age and BMI were similar between the two groups (age responder=35.0±15.0 years, non-responder=35.0±12.9 years; BMI responder=23.4±2.5 kg/m^2, non-responder=22.4±2.0 kg/m^2). Although non-significant, responders had a lower energy intake (1843±308 kcal versus 2158±476 kcal, p=.089), and a higher fiber intake (14.2±5.0 versus 12.4±3.1 g/1000 kcal, p=.279). Furthermore, responders had a larger change in fiber intake, both in grams (7.2±7.8 versus 4.8±6.1, p=.405) and per 1000 kcal (2.64±4.8 versus 0.82±1.9, p=.302). No differences were observed for water intake, total physical activity score, gut microbiota or SCFA.

The PDA resulted in more knowledge and outcome beliefs, and was well-accepted

Participants' self-regulation and subjective health regarding diet (*i.e.,* how healthy participants find their own diet) was significantly lower at week 4, but similar at week 1 and 8 (Supplementary Table 3). Participants' subjective knowledge (p<.001) and outcome beliefs (p=.036) regarding fibers significantly increased at week 8 (4.92±1.0; 5.17±1.1) compared to week 4 (3.28±1.3; 4.78±1.0). Moreover, participants' intention to eat more fibers significantly increased at week 8 (5.8±1.22) compared to week 1 (4.28±1.3, p<.001), but not compared to week 4 (5.41±1.3, p=.106). Participants' subjective health (*i.e.,* how healthy participants find themselves) did not significantly change between the different measurement moments (week 1=5.08±1.1, week 4=4.76± 0.7, week 8=4.84±0.9).

Participants rated the PDA on a 7-point Likert scale as positive (5.6±1.1), useful (5.6±1.3), attractive (5.0±1.4) and interesting (5.3±1.4). Furthermore, participants positively evaluated the PDA regarding the following aspects: motivational to make

high-fiber choices (6.0±0.9), help to sustain these changes in dietary intake for long term (6.0±0.9), provide insight in their own fiber intake (6.6±0.8) and how to improve their fiber intake (6.3±1.0), and even though the score was slightly lower, actually improving their fiber intake (5.8±1.0).

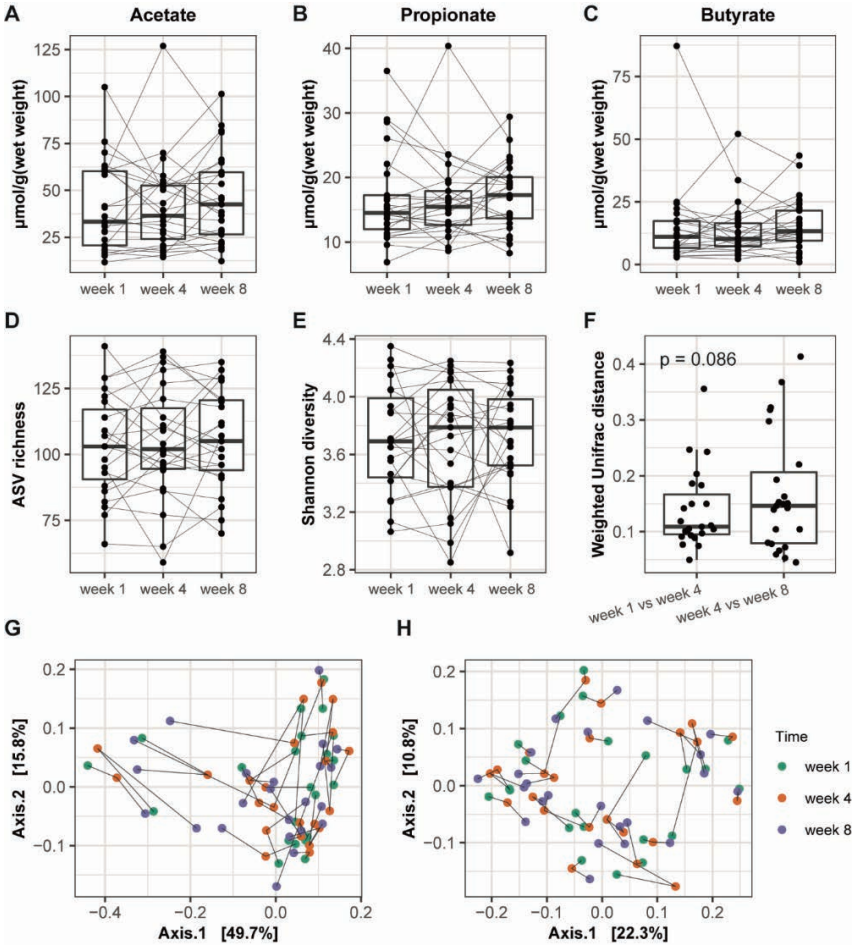

Figure 6. Analysis of short chain fatty acids and gut microbiota composition over time. Values were presented as interquartile with boxplot. Samples taken at different timepoints are connected by solid lines per subject. Week 1 and week 4 were observational, week 8 is after the intervention. No differences were observed in fecal acetate (A), propionate (B), and butyrate (C), microbiota ASV richness (D) and Shannon diversity (E) between the time points before and after intervention. A trend was observed for the comparison of microbiota composition stability based on weighted Unifrac distances between week 1 vs week 4, and week 4 vs week 8 (F). PCoA of microbiota composition based on weighted Unifrac distances (G) and unweighted Unifrac distances (H), stratification based on sampling timepoints.

Discussion

This study showed that PDA was effective in increasing dietary fiber intake, and subsequently improving constipation severity and QoL. Moreover, we observed that an increased fiber intake was associated with the reduction in constipation complaints, which remained when adjusted for physical activity and water intake. Although stool frequency did not change, stool consistency softened during the intervention. Gut microbiota and SCFA did not change significantly, but we showed an association between SCFA and several subscales of constipation severity and QoL. Questionnaires revealed that PDA increased subjective knowledge and outcome beliefs, and was well-accepted.

Our study was the first to use PDA to improve constipation complaints in adults. To our knowledge, only a few studies have used a high-fiber diet instead of fiber supplements to improve symptoms. A study from Anti and colleagues (1998) showed that a fiber intake of ≥25g/day significantly increased stool frequency[25], which we did not observe. This discrepancy might be explained by the magnitude of the change in fiber intake: even though our endpoint was similar, their baseline fiber intake was much lower around ~13g/day, therefore having a larger window of opportunity. We also saw a bigger change in fiber intake in responders. As compared to our previous high-fiber PDA intervention in healthy adults[35], a bigger change in fiber intake was achieved in this study. Possibly, adults with complaints were more motivated which resulted in more substantial changes. Furthermore, we optimized the PDA (*e.g.* user-friendliness, more high-fiber alternatives), and in contrast to the previous study, fiber intake was now attentively assessed before the start of the intervention.

Several meta-analyses have been done regarding fiber supplementation in constipation, and has been shown to be effective in improving symptoms[76-78]. However, study populations vary greatly, as the Rome criteria for constipation are far from optimal[44, 45], which is reflected in low quality evidence from these trials and large differences in response rates[76-78]. Fiber supplementations ranged between 10-22.5 g/day, which was higher than the change we achieved via the diet. However, there are substantial benefits from increasing fiber intake via the diet. By increasing intake of healthy foods such as fruits, vegetables, whole grain and legumes, not only positive effects on constipation complaints but also other health effects can be achieved. A high fruit, vegetable, legume and nut intake can reduce the risk of for example coronary heart disease[79-81] and obesity[82-84], and does not only provide fibers but also other essential nutrients. In our study, whole grain bread/crispbreads and fruit intake was significantly higher after PDA. Therefore, even though current guidelines do not distinguish between an fiber increase via diet or supplements[42], our results suggest it would be beneficial and feasible for constipation complaints and overall health to start with dietary adjustments. Furthermore, spreading fiber intake throughout the day and gradually increasing intake improves tolerability and

can prevent additional bloating and cramps that can coincide with an increased fiber intake[26].

Contradicting previous research, we did not observe a significant change in gut microbiota or SCFA and no associations with fiber intake[21, 85, 86]. However, we did observe a larger change in microbiota distance during the intervention period. Possibly, the change in fiber intake and overall diet was too small to instigate distinct changes, which needs to be larger to be reflected in the stool. Another explanation is the participant-specificity of both microbiota and change in fiber consumption (amount as well as type) making a uniform microbiota change unlikely. Furthermore, 80-95% of the SCFA are estimated to be absorbed in the gut [87, 88], which can mask the possible effects of an increased fiber intake on the SCFA production. We observed an association between all SCFA and severity of stool complaints, between butyrate and physical discomfort, and between propionate and worries and concerns over time. Supporting our results, fecal SCFA production has been associated with constipation severity before and was shown to be lower compared to healthy adults[89]. Butyrate is known for its anti-inflammatory properties and reduction of oxidative stress in the gut, and has the ability to reduce visceral sensitivity[90, 91]. Propionate has been suggested to have a beneficial effect on the blood brain barrier *in vitro*, suggesting a link with mental wellbeing[92]. However, much of the physiology remains unknown and needs further research.

The adults included in this trial had mainly mild symptoms, which was confirmed by the baseline severity score of 1.45±0.7, which is lower compared to other studies which reported a score ranging between 1.91-2.85[51, 93, 94]. We chose to target a population with mild constipation complaints as we expected the largest benefit from a dietary intervention in this group. The average change in severity score was 0.49±0.49, which is lower than the clinical relevant change threshold of 0.6[72]. This might be due to the more mild symptoms and therefore having a smaller window of opportunity. However, despite the fact that this group mainly had mild symptoms, we still achieved a clinical relevant improvement in 36% of the study population and we did see moderate to strong effect sizes for QoL scores[53], and a clear link with dietary fiber intake. This shows that our results are promising, and highlights the need for future studies with dietary interventions in a population with more severe symptoms.

An important limitation of our study is the lack of a proper placebo group. In patients with abdominal complaints, especially in IBS, the placebo effect has been well-described[95-97]. Since it was impossible to include a proper placebo group, a possible placebo or regression to the mean effect could have been present, which might drive the improvements in symptoms and QoL. However, a more objective measure such as stool consistency also significantly improved. Furthermore, the observation period was designed to correct for time or study effects. A cross-over design was not

possible due to the nature of the intervention, and including a proper placebo group is difficult in studies with dietary advice and not optimal in this population due to the large between person variability[36, 37]. Moreover, fiber intake significantly increased which aids to a healthy lifestyle. Therefore, it can be debated whether a placebo effect is a problem, or if such an intervention positively influencing diet and complaints is helpful, regardless of a possible placebo effect.

Our study is strengthened by the amplitude of measurements which aids to a more complete overview of the mildly constipated adult, including fecal material, and dietary, physical activity and behavioral assessments. Furthermore, by following participants for 4 weeks without an intervention, we were able to obtain an accurate baseline taking within person variation into account. The use of daily EMA questions increased the accuracy of our measurements, as records have shown to overreport pain and stool frequency compared to EMA in IBS patients[55]. With our study design, we were able to capture the daily variation in stool pattern and abdominal pain over time. Furthermore, we used a validated method to obtain dietary data, and included several days to take variation into account[58], which aids to estimating dietary intake more correctly.

In conclusion, our study showed that a digital PDA to increase fiber intake was effective and subsequently improved constipation complaints and QoL. Fecal SCFA was not associated with fiber intake, but was with constipation complaints and QoL. The PDA was well-accepted by study participants. Our results indicate that increasing dietary fiber intake via dietary adjustments might be a well-effective first step in treatment of mild constipation complaints. Future research is needed to assess the effects of dietary adjustments in adults with constipation complaints on a larger scale and in a more severely constipated population. Furthermore, the long-term efficacy and feasibility of PDA needs to be explored.

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Supplementary Table 1. Changes in lifestyle over time

	Week 1	Week 4	Week 8	p-value
Dietary intake				
Energy (kcal)	1938.2 ± 462	1848.7 ± 446	2044.7 ± 444	.305
Protein (en%)	14.9 ± 3.6	14.8 ± 2.6	13.9 ± 1.7	.246
Fat (en%)	36.8 ± 6.0	35.4 ± 5.4	36.7 ± 6.2	.651
Saturated fat (en%)	13.0 ± 3.2	13.1 ± 2.8	12.3 ± 3.6	.654
Carbohydrates (en%)	42.2 ± 5.9	44.8 ± 5.7	43.2 ± 6.2	.275
Water (L)*	2.74 (2.4 – 3.5)	2.57 (2.3 – 3.0)	2.8 (2.8 – 3.2)	.829
Physical activity				
Total physical activity score†	5700 (2490 – 7478)	5865 (4510 – 7080)	4530 (3190 – 6525)	.271
Adhering to the recommendation, n (%)‡	14 (56)	13 (52)	14 (56)	.948
Commuting activities score†	240 (0 – 735)[a,b]	240 (0 – 735)[a]	120 (0 – 300)[b]	**.005**
Sport score†	870 (68 – 1440)	810 (68 – 1440)	560 (0 – 1170)	.176
Leisure time activities score†	1620 (1020 – 2040)	1440 (945 – 2528)	1320 (935 – 1995)	.296
Household activities score†	720 (355 – 1470)[a]	720 (300 – 1215)[b]	810 (390 – 1350)[a]	**.000**
Work/school activities score†	2880 (0 – 4380)	3360 (840 – 4260)	2400 (165 – 4320)	.770

Values are mean ± standard deviations or median (interquartile range) when skewed. Dietary intake was assessed using 24hr recalls, and physical activity using the short questionnaire to assess health-enhance physical activity (SQUASH). Differences between timepoints were assessed using linear mixed models or chi-square when categorical, different superscripts indicate significant differences between the timepoints. The overall p-value over time is shown. Abbreviations: en% = energy percentage. †Calculated by multiplying the metabolic equivalent of task values per activity times the minutes per week per activity, and then summed. *Water intake represents not only intake of liquids but also includes water in foods.

Supplementary Table 2. Mixed model analysis of SCFA versus constipation severity and Quality of life

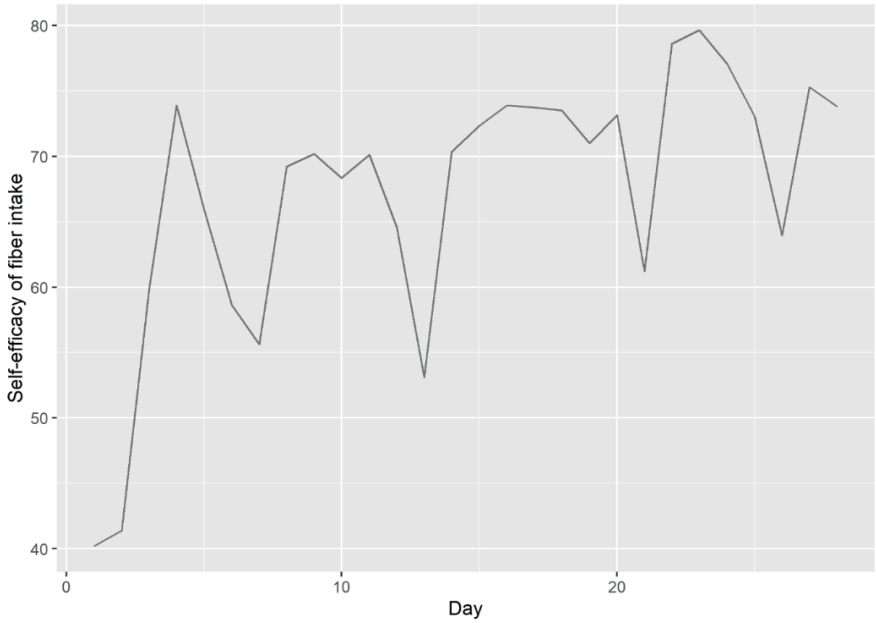
	Acetate		Propionate		Butyrate	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Total constipation severity	-7.21 (-15.5; 1.05)	*.086*	-1.96 (-4.09; 0.18)	*.073*	-3.36 (-7.12; 0.39)	*.079*
Abdominal complaints	-2.21 (-10.32; 5.90)	.589	-0.99 (-3.09 ; 1.11)	.351	-1.32 (-5.25; 2.60)	.503
Rectal complaints	-5.72 (-13.3; 1.84)	.135	-1.09 (-3.10; 0.93)	.284	-2.69 (-6.07; 0.70)	.117
Stool complaints	-6.20 (-11.9; -0.41)	**.036**	-1.58 (-3.07; -0.08)	**.039**	-2.76 (-5.44; 0.09)	**.043**
Total constipation quality of life	-6.99 (-16.3; 2.30)	.138	-2.09 (-4.49; 0.30)	*.086*	-3.78 (-8.05; 0.48)	*.081*
Worries and concerns	-6.74 (-14.64; 1.15)	*.093*	-2.18 (-4.20; -0.15)	**.036**	-2.82 (-6.52; 0.88)	.132
Satisfaction of stool pattern	-2.42 (-8.17; 3.33)	.403	-0.52 (-2.00; 0.96)	.486	-1.41 (-4.11; 1.29)	.301
Physical discomfort	-5.35 (-12.49; 1.79)	.140	-1.14 (-3.02; 0.74)	.230	-3.42 (-6.64; -0.20)	**.038**
Psychological discomfort	-4.15 (-14.03; 5.73)	.406	-1.75 (-4.31; 0.82)	.179	-3.29 (-.789; 1.33)	.160

The estimate and p-value is given for each SCFA in µmol/g. Data is tested using linear mixed models, using a diagonal variance structure, and indicating time as repeated measures. Acetate, propionate and butyrate are dependent variables and constipation severity or quality of life are added as fixed main effects to the model.

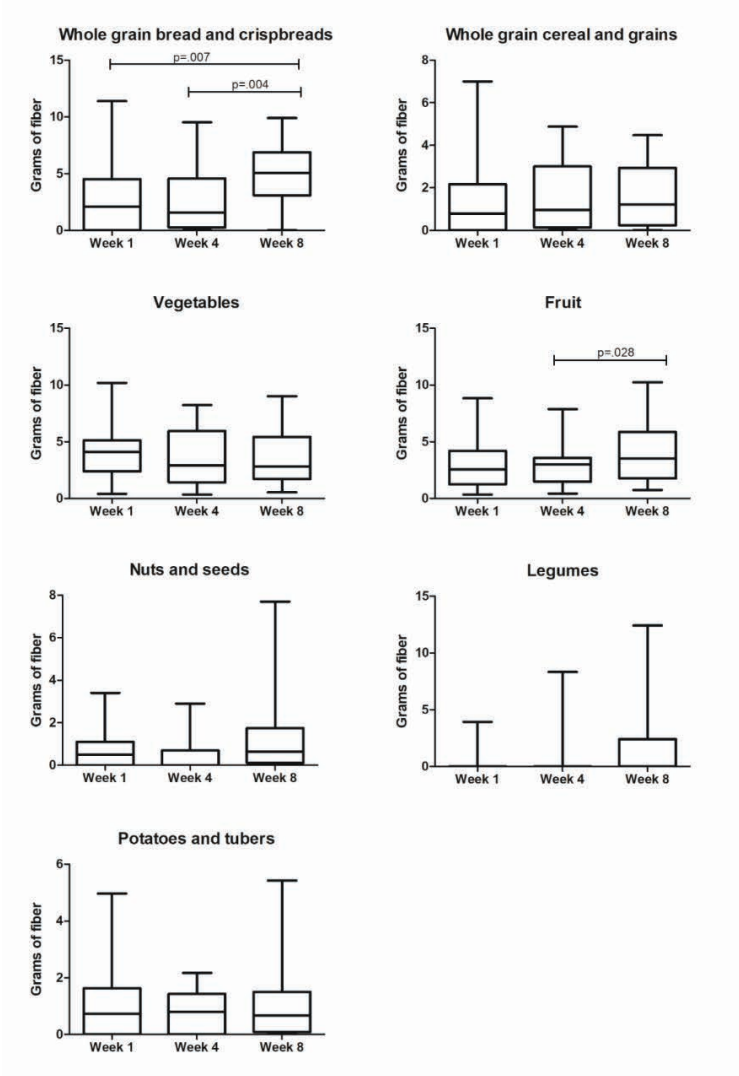
Supplementary Table 3. Changes in Psychological questionnaires over time

	Week 1	Week 4	Week 8
Self-regulation	4.72±0.78[a]	4.36±0.83[b]	4.70±0.67[a]
Subjective knowledge	Not measured	3.28±1.34[a]	4.92±1.02[b]
Outcome beliefs	Not measured	4.78±1.01[a]	5.17±1.11[b]
Intention to eat more fibers	4.28±1.30[a]	5.41±1.28[b]	5.8±1.22[b]
Subjective health regarding the diet	4.92±0.81[a]	4.48±1.01[b]	5.12±0.83[a]
Subjective health	5.08±1.12	4.76± 0.66	4.84±0.90

Values are mean ± standard deviations, items were assessed on a visual analog scale (VAS) from 1 'not at all' to 7 'very much'. Different superscripts indicate significant differences between the timepoints, no superscript indicates no significant differences over time.



Supplementary Figure 1. Self-reported efficacy of dietary fiber intake
Legend: data was collected daily using ecological momentary assessment (EMA) application on a participants' mobile phone. The line represents the group average. Self-efficacy was only measured during the 4-week intervention, with the question 'did you manage to eat more fiber today' on a 100-point visual analog scale (VAS) from 0 'not at all' to 100 'yes, completely'. On the x-axis is the day of the week shown with day 0 representing Monday. The pattern indicated a clear weekend dip in self-reported efficacy of increasing dietary fiber intake.



Supplementary Figure 2. Changes in food group intake over time

Legend: based on the data of 24hr recalls. Three recalls were performed per timepoints, which consisted of one weekend day and two weekdays. Week 1 and week 4 were observational, week 8 is after the intervention. The y-axis represents grams of fiber that was provided by that food group. Differences were tested with linear mixed models. (A) Intake of whole grain breads and crispbreads such as crackers, biscuits. (B) Intake of whole grain cereals, breakfast grains, flour, bran and other whole grains, such as rice, pasta, couscous, bulgur. (C) Intake of vegetables, including raw, frozen, cooked and canned vegetables. (D) Intake of fruits, includes raw, frozen and canned fruits (E) Intake of nuts and seeds, including natural and salted nuts, seeds or peanuts. (F) Intake of legumes, includes dried, canned and cooked legumes. (G) Intake of potatoes and other tubers, includes raw, cooked, fried and dried potatoes and other tubers.

CHAPTER 7

GENERAL DISCUSSION

Iris Rijnaarts

This thesis had as overall goal to improve GI complaints and quality of life via the diet. GI complaints and the interplay with diet and the gut microbiota was assessed via various angles. We assessed associations between GI complaints, diet, gut microbiota and SCFA in IBS patients. Furthermore, we developed and used PDA to increase dietary fiber intake in people with and without GI complaints. Although these are two separate parts, both are important sides of a coin: one assessing a more fundamental research question regarding dietary patterns related to GI complaints, and the second aiming for science for impact to develop dietary tools to improve fiber intake. Both aspects are needed to reach the overall goal of improving gut health via the diet. Furthermore, the chapters are similar as several methodological considerations are applicable to both, such as the large between and within person variation in GI complaints, dietary intake, gut microbiota and SCFA. In this final chapter, the main findings of this thesis are discussed, as well as methodological and conceptual considerations and the implications and suggestions for future research.

Main findings

Chapter 2 showed that IBS patients with different IBS subtypes or severity did not identify different self-reported dietary triggers. Greasy foods, onions, cabbage, spicy and fried foods were most often mentioned as triggering foods. IBS severity was positively associated with the severity of a response and number of dietary triggers a patient responded to, while QoL was negatively associated with the response to dietary triggers (Figure 1). No clinically relevant differences were seen in self-reported response to dietary triggers between patients with and without anxious and depressive symptoms. Only 30% of the IBS patients that made alterations in their diet was guided by a dietician. **Chapter 3** showed that IBS severity and stool pattern was highly variable over time, as well as associations between the gut microbiota composition with QoL and psychological status, even within a short period of 4 weeks. No consistent differences between IBS patients and controls or IBS severity groups were observed in gut microbial alpha diversity or composition, and SCFA levels. IBS subtypes did show differences in SCFA, with IBS-C having lower levels and IBS-D higher levels. Interestingly, the relative abundances of *Terrisporobacter* (0.2% versus 0.05%) and *Turicibacter* (0.018% versus 0.018%) were consistently higher, while the relative abundance of *Bifidobacterium* was consistently lower (4.5% versus 9.5%) in IBS compared to controls.

The development and validation of a short fiber screening questionnaire was described in **Chapter 4.** The 18-item FiberScreen assessed dietary intake of fruits, vegetables, whole grains, pasta/rice/potatoes, legumes and nuts/seeds over the past 2 weeks. The 18-item FiberScreen was shown a suitable questionnaire to screen and rank participants' fiber intake, and average completion time was 4 minutes, which is considerably less compared to a full dietary assessment method.

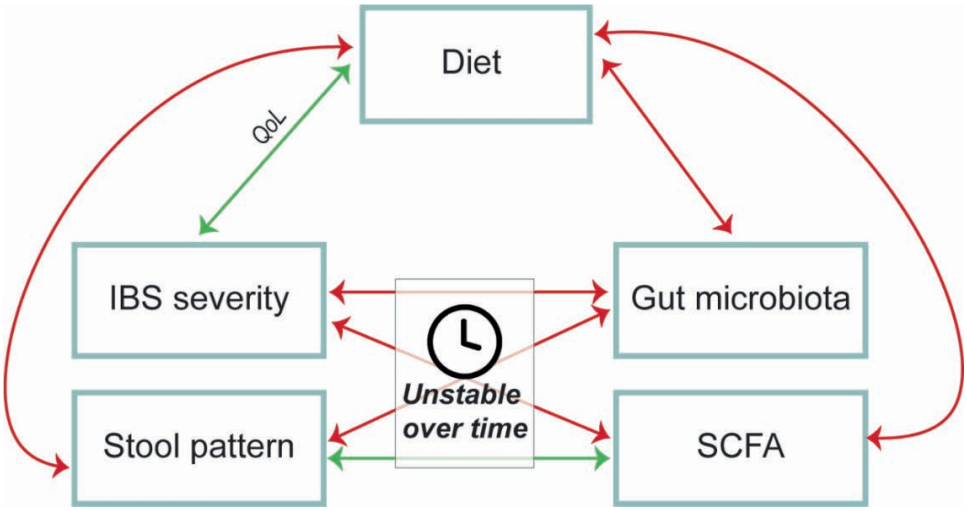


Figure 1. Overview of associations found in Chapter 2 and 3 between gastrointestinal complaints, gut microbiota and SCFA and diet in Irritable Bowel Syndrome. A red color of the arrow indicates that no association was observed while a green color indicates that associations were found. Abbreviations: IBS, Irritable Bowel Syndrome; QoL, Quality of Life; SCFA, short-chain fatty acids

In **Chapter 5,** the development and validation of a digital web-tool to provide high-fiber PDA in adults without GI complaints was described, which increased dietary fiber intake with 2.2 g/1000 kcal per day 3 months after the intervention. In **Chapter 6**, the high-fiber PDA web-tool significantly increased dietary fiber intake in adults with constipation complaints with 1.9 g/1000 kcal per day, and subsequently improved constipation severity, QoL and stool consistency. No effects of the high-fiber PDA on the gut microbiota or SCFA were observed, which may have been expected as the fiber increase was too small to see effects in the feces. The web-tool was developed so that participants could substitute habitually consumed low-fiber products for high-fiber alternatives, and add additional fruits, vegetables, legumes, nuts and seeds to their PDA. Based on the dietary choices participants made, participants formulated an action plan, a so-called implementation intention. In both chapters, the high-fiber PDA was well-accepted by the participants and improved their self-reported knowledge of fibers.

Methodological and conceptual considerations and implications for future research

The role of diet in IBS

As described in Chapter 1, diet is suggested to play a substantial role in IBS pathophysiology and treatment. However, whether the self-perceived food

intolerances are causal or a symptom from the underlying pathophysiology remains the question. Furthermore, it is unclear what causes a patient to experience symptoms after consumption of a certain food, while others do not. The large heterogeneity in type and severity of IBS symptoms would suggest differences in pathophysiology between these patients, which could be a rationale for specifying dietary treatments for these subgroups. However, the results from **Chapter 2** did not support this hypothesis. Although many IBS patients report symptoms from dietary triggers, differences were small between IBS subtypes or severity groups and therefore clinical relevance of these differences between IBS subgroups is doubtful. Furthermore, part of the proposed pathogenesis of IBS, as described in Chapter 1, are alterations in the gut microbiota, which could lead to a low grade inflammation in the gut and result in food intolerances[1-6]. However, **Chapter 3** showed that gut microbial alpha diversity and composition or fecal SCFA levels did not differ between IBS patients and controls or severity groups. This does not support the hypothesis that alterations in the gut microbiota causes differences in amount and severity of response to dietary triggers, or is responsible for the severity of IBS symptoms. Of note is that we only investigated fecal SCFA levels as marker of microbial activity, but other measures such as metabolomics were not investigated. There is an increasing interest in possible alterations in the gut microbial metabolomics in IBS, which might be of larger relevance to understand IBS pathophysiology. However, no consensus on possible alterations in gut microbial metabolomics has been reached yet, probably due to differences in methods and the large heterogeneity between IBS patients[7]. More standardized research on the gut microbial metabolomics, taking time dynamics into account, is needed to further elucidate this aspect.

Interestingly, consistent differences over time were observed in the relative abundances of *Terrisporobacter, Turicibacter* and *Bifidobacterium* between IBS and controls in **Chapter 3**. *Terrisporobacter* and *Turicibacter* are thought to regulate the biosynthesis and release of serotonin[8-11], which could be linked to the pathophysiology in IBS shown in Chapter 1. It is currently not clear whether these taxa can be altered by the diet in humans, but high baseline abundances of *Turicibacter* were found in non-responders of the FODMAP diet in children with IBS[12], suggesting a link between this taxa and the diet. The lower relative abundance of *Bifidobacterium* in IBS is also of interest as this genus is well-known to often increase after prebiotic interventions in several different populations, among which also in IBS patients[13-18]. However, this increase in *Bifidobacterium* was not always related to improvement in health outcomes, such as insulin sensitivity in obese prediabetic adults or immune function in elderly[17, 18], which is in line with the lack of association between the relative abundance of *Bifidobacterium* and IBS severity observed in **Chapter 3.** However, previous research observed that supplementation of a prebiotic galactooligosaccharide mixture increased the abundance of *Bifidobacterium* and did improve IBS symptoms[16]. Moreover, a meta-analysis has

shown that probiotic interventions can be effective in reducing IBS symptoms[19], but the optimal probiotic type is yet unclear. A different meta-analysis including 5 randomized controlled trials showed that a single probiotic strain of *Bifidobacterium infantis* did not improve IBS symptoms, while composite probiotics including *B. infantis* was effective[20]. Interestingly, one of these trials showed that supplementation with *Lactobacillus salivarius* UCC4331 was did not improve symptoms, while supplementation with *B. infantis* 35624 improved symptom severity, abdominal pain and bloating[21]. Although much is unclear, the lower relative abundance of *Bifidobacterium* in IBS patients could be a potential target for treatment in IBS, which could be enhanced via the diet (prebiotics) or supplementation of the bacteria itself via probiotics. Future studies investigating the optimal type, dosage and duration of such treatments, and the effects on IBS symptoms are needed to further advance in this field.

Studies have suggested that the response to a low FODMAP diet in IBS can be predicted via the gut microbiota[22-24] and thus can identify responders and non-responders, but this has also been contradicted recently[25]. These studies are however limited in their evidence due to the short FODMAP intervention of 4 weeks without including the reintroduction phase, and blinding is often not possible, thus results might be influenced by a large placebo effect which has been described before in IBS[26]. Furthermore, outcome evaluations are often based on a single measurement before and after the intervention. As described in **Chapter 3,** already a large within person variation was observed in IBS patients within 4 weeks of observation time, and changes over time and the associated predictions could be based on coincidence. Moreover, there are concerns about the long-term safety of the FODMAP diet as it might alter the gut microbiota due to the exclusion of many foods, which could also cause nutrient deficiencies[27-29]. Identifying responders and non-responders while not knowing the underlying mechanisms associated with changes in the gut microbiota remains thus a black box for treatment of IBS patients. The safety of the FODMAP diet and the longitudinal stability of these predictions needs further investigation before identification of responders based on the gut microbiota can be applied.

In approximately 50% of the IBS population atypical food allergies presented by alterations in the intestinal mucosal response and a dysfunction in the intestinal barrier have been shown. Exclusion of these allergenic foods (wheat, milk, soy and/or yeast) from the diet resolved much of the symptoms[30], suggesting that these food allergies are part of the pathophysiology for a proportion of the IBS patients. What separates these patients from other IBS patients not presenting atypical food allergies remains unclear. It is known that the majority of IBS patients alters their diet to reduce symptoms[31, 32], which we also observed in **Chapter 2.** Unfortunately, only a fraction of the IBS patients does so under supervision of a dietician, increasing the

risk for nutrient deficiencies, which have been observed before in IBS populations[33, 34]. A personalized dietary treatment plan in consultation with a dietician is recommended for IBS, which is in line with IBS guidelines, that state that "dietary advice should only be given by a healthcare professional with expertise in dietary management"[35]. Indeed, most studies that found positive effects of for example the FODMAP diet were dietician-led[36], and a trial including 65 IBS patients that all received dietary advice from a dietician besides the management of symptoms by a doctor, reported positive effects on symptoms[37]. In contrast, in clinical practice 79% of medical doctors reported to provide lifestyle or dietary advice such as leaflets or website tips to IBS patients without the inclusion of a dietician[38]. The low prevalence of dietetic supervision is worrisome, as IBS patients may only adhere to certain aspects of a diet promoted online or in flyers that appeal to them, increasing the risk for nutrient deficiencies and sub-optimal dietary treatments. Furthermore, it is increasingly recognized that a proportion of IBS patients is portraying avoidant of restrictive food intake disorders[39-41], which requires additional dietary and psychological care. It is therefore strongly recommended that a dietician should be included in the management of IBS symptoms. Most optimal would be a multidisciplinary approach including a psychologist or mental health professional to reduce stress, anxiety, depression or food intake disorders, which is associated with an exacerbation of symptoms[42, 43].

Study population selection for research in bowel disorders

Although the search of biomarkers for bowel disorders as IBS has been ongoing for years[44, 45], currently diagnosis and study population selection is based on the symptom-based Rome criteria[46]. Even though these criteria have been validated, they lack a discriminatory ability from other bowel disorders, as described in Chapter 1[47, 48]. This may affect study participant characteristics**,** which can further increase the heterogeneity between different studies and hamper study agreement. Indeed, individual and cohort-specific characteristics have shown to impact the gut microbiota and associations with health[49], for which large heterogeneity in different IBS studies was observed[50-55].

This thesis included both IBS patients and adults with constipation, and different inclusion criteria were applied for the different chapters. In **Chapter 2 and 3**, all IBS patients who either fulfilled the Rome criteria and/or had a diagnosis of a physician were included, while in **Chapter 6**, the Rome criteria were not applied but inclusion was based on self-perceived constipation and dissatisfaction with stool pattern. The lack of a clear diagnosis may have impacted the study populations and results of these trials. Adults with mild constipation complaints were investigated in **Chapter 6**, but presence of IBS was not excluded. It is possible that within the constipated population adults with IBS-C were also present. However, a previous meta-analysis indicated that a high-fiber diet was not effective in improving IBS symptoms[56], while

we did observe a reduction in complaints. If a large proportion of IBS-C patients were present in the population, the positive effects of the high-fiber PDA that were observed in **Chapter 6** may be underestimated, and would even be more effective in a "truly" constipated population. A large presence of IBS-C patients in this study population is therefore not expected. Similarly, we cannot exclude that patients with other functional bowel disorders than IBS were selected as study population for **Chapter 2 and 3**, which might have influenced the results. However, in **Chapter 2**, no clinically relevant differences between IBS subtypes and dietary triggers were observed in a cohort of 1601 participants. The difficulty in distinguishing bowel disorders is mostly due to the overlap in type of complaints (*e.g.* IBS-C and functional constipation, IBS-D and functional diarrhea)[57], thus if dietary triggers would be associated with a certain stool pattern or GI symptom, this would have shown in the analysis.

The effects of the different study populations and inclusion criteria on the research questions in these chapters are estimated to be small. However, the results from **Chapter 3** showing the large within person variability over time further highlights the diagnostic issues in this population and hampers study agreement in populations with IBS and constipation. It is therefore questionable whether the Rome (or similar) criteria are useful and practical in these heterogenous and unstable populations. It might be more clinically relevant to include people when they report to experience substantial GI complaints. Alternatively, eligibility of participants with GI complaints should be assessed by a medical doctor to asses IBS or constipation complaints and exclude other possible diagnoses, which increases the clinical relevance but further complicates the recruitment and screening process.

Box 1. Comparing the study populations characteristics between chapters

When comparing the study populations of **Chapter 2, 3** (IBS patients) **and 6** (constipation complaints), it shows that BMI and gender were relatively similar, while age was lower in adults with constipation compared to IBS (Figure 2). Energy, macronutrient and water intake was similar in IBS and constipated adults. Only dietary fiber intake was slightly higher (~3g) in IBS compared to the baseline intake of adults with constipation. These differences are probably a result of the different inclusion criteria regarding age and fiber intake. Strikingly, gut microbial stability over time, shown by weighted Unifrac distance of the gut microbiota composition between timepoints, is higher in patients with constipation complaints than in IBS patients and controls. This larger distance is already present within the observation period, but further increases after a high-fiber PDA intervention, indicating that the gut microbiota composition in constipation complaints changed more over time compared to IBS patients or controls. Microbial alpha diversity does not seem to differ between these populations. Interestingly, acetate levels are relatively similar, while propionate and butyrate levels are lower in IBS-C

compared to adults with constipation. Adults with constipation had similar SCFA levels as controls in Chapter 3. Furthermore, severity of stool complaints and different QoL subscales in constipation were associated with SCFA, while none of these SCFA were associated with IBS severity. There is no clear baseline characteristic that differs between these populations that might explain these observations. It seems unlikely that the small difference in dietary fiber intake can causes these differences, as in both Chapter 3 and Chapter 6 dietary fiber intake was not associated with the gut microbiota composition or SCFA. Possibly, the study population of Chapter 6 with constipation complaints is more heterogenous than the IBS patients from Chapter 3, as the Rome criteria were not used for study selection, and adults with more mild symptoms without a chronic nature could be included in the study design. However, this needs further investigation.

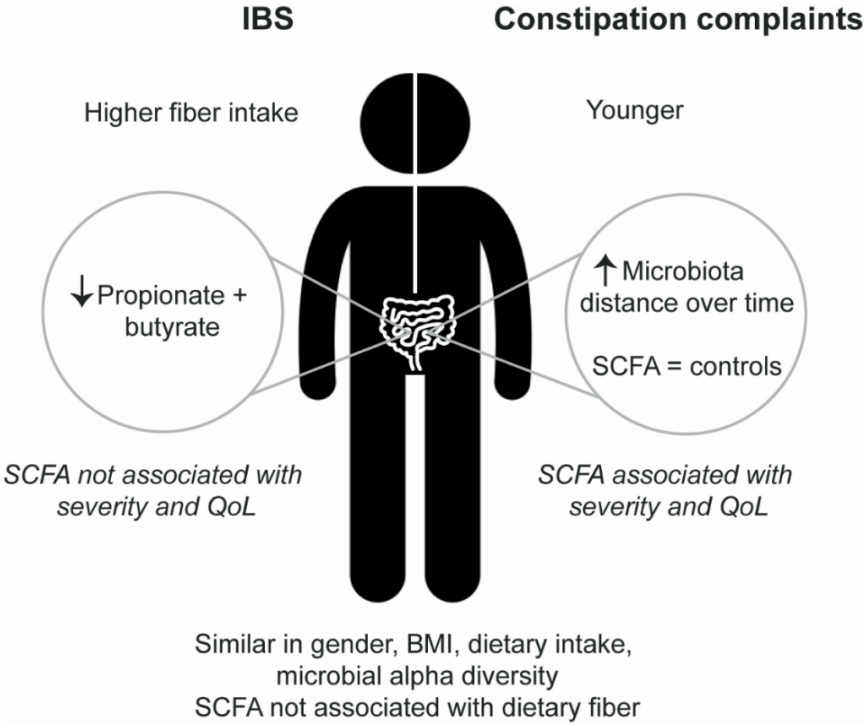


Figure 2. Differences in characteristics, gut microbiota and SCFA between IBS and constipation complaints measured over time.

There is a need for a large longitudinal trial including adults with 1) different complaints which would fit IBS and/or constipation, and 2) people who fulfill and not fulfill the Rome criteria but still experience GI symptoms, to assess feasibility of these criteria in such populations and possible differences between these groups. Of interest are possible differences in gut microbiota composition and SCFA over time, as well as type and severity of symptoms and mental wellbeing, and associations

between these factors. Furthermore, as described in Chapter 1, diet-gut microbiota associations were more linked to foods than nutrients[58]. This has not been investigated in this thesis, but would be of interest to further understand possible associations with the gut microbiota. Understanding the differences between these populations could help to identify and distinguish them, which could reduce study heterogeneity and can aid to consensus between studies.

Outcome assessment in bowel disorders

Most outcomes in GI complaints are self-reported via questionnaires such as the IBS-SSS, PAC-SYM and the IBS-QoL[59-61], which were used in **Chapter 2, 3 and 6**. Although these are validated and currently the best option as biomarkers are lacking, they can be prone to bias. Especially in IBS patients, an average placebo response of 40% has been found in a meta-analysis[26]. For dietary triggers, currently there is no validated method to objectively assess whether patients respond to a food or not. Therefore, it is difficult to state whether the 5 most triggering foods described in **Chapter 2** are truly dietary triggers. Previous research has found alterations in the intestinal mucosal response to certain foods and improvement in symptoms after exclusion of these foods from the diet[30]. However, this method assessing the intestinal mucosal response needs further validation, and is quite invasive due to the endoscope and confocal laser endomicroscopy which are needed. Another objective measurement in IBS is a rectal barostat assessing visceral hypersensitivity. A lowered rectal pain threshold has been shown in IBS patients compared to controls[62], and has been shown relatively stable over time[63]. Ludidi and colleagues (2012) have found rectal barostat cut-offs indicating hypersensitivity, but also conclude that differences with controls were mostly seen at group level and no conclusions can be made yet for individual patients[64]. This shows promise for the development of an objective outcome in IBS which is essential for the further understanding of IBS pathophysiology, but it will not be usable for all, as visceral hypersensitivity prevalence ranges between 18-63% in the IBS population[64-66]. Furthermore, as it can be a painful procedure, rectal barostat measurements can be a burden for IBS patients. It also remains essential to acknowledge the self-perceived food intolerances and complaints. A thorough complaint interview is thought to be a prerequisite for effective reassurance[67], and reassurance has been shown to improve QoL in IBS[68], and therefore has an important role in IBS treatment.

One of the major challenges for assessing GI complaints, diet and the gut microbiota, is the large within person variation. Furthermore, the between person variation in the IBS population might be even larger, which may complicate IBS research even more. Large heterogeneity between IBS patients was observed in type and severity of symptoms, as well as psychological status in **Chapter 2.** In **Chapter 3**, we have shown that IBS symptoms, stool pattern and the gut microbiota are not stable over time. These fluctuations in IBS symptoms and the gut microbiota have also been

shown before in IBS[69-73]. In **Chapter 6**, also a large within and between person variability was observed in for example fecal SCFA levels. To take the large heterogeneity in an IBS population into account, trials with larger sample sizes are needed, or contrarily studies should focus on specific subgroups to reduce part of this variation. Including several measurements is a good method to study time dynamics and to obtain a more accurate baseline[74], which has been done in **Chapter 3, 5 and 6.** One of the dangers of these large natural fluctuations is that they can be mistaken for placebo effects in experimental trials including a placebo group[74], as it is difficult to distinguish natural variation from a placebo effect. One possible solution for assessment of time fluctuations in GI complaints and stool pattern is to apply EMA technology for daily questionnaires, which was done in **Chapter 5 and 6**. EMA has been shown to reduce retrospective bias and overestimation of symptoms by diaries compared to frequent questionnaires[75-77]. Much work in the IBS field includes cross-sectional studies, but the strength of evidence from these studies is limited due to these fluctuations and should be interpreted with caution as cross-sectional associations can be driven by chance, which has been recognized already before[78]. When studying populations or outcomes that are known to fluctuate over time, cross-sectional studies can provide a helpful first glance, but cannot differentiate between cause, consequence or coincidence, and results need confirmation in longitudinal and experimental designs. Similarly, the association between dietary triggers and IBS severity in **Chapter 2** was observed in a large cohort which took the large between person variation into account, but these findings need to be confirmed in a longitudinal study to assess the stability over time.

The importance of psychological status and QoL

As shown in Chapter 1, psychological status in patients with GI complaints is of importance, as QoL is reduced[79], and the prevalence of anxiety and depression is high[80] which is negatively associated with the gut microbiota[81-83]. In this thesis, we confirmed the reduced QoL and high prevalence of anxious and depressive symptoms in IBS patients in **Chapter 2 and 3**. However, we did not find a clinically relevant difference in self-reported response to dietary triggers between patients having anxious or depressive symptoms versus not, contradicting previous research[84]. Furthermore, we did not observe a consistent association over time between the gut microbiota and QoL or psychological status in IBS patients in **Chapter 3**. Fecal SCFA and several QoL subscales were associated over time in adults with constipation complaints **(Chapter 6)**. Although a clear association between psychological status, dietary triggers and the gut microbiota in IBS patients was not observed in our studies, it remains an important aspect to consider when assessing this population. Reassurance and recognition of self-perceived complaints has a considerable role in IBS treatment and shown to improve QoL[67, 68]. Furthermore, depression itself is associated with a different gut microbiota composition[85], which is proposed to be part of the IBS pathogenesis. Psychological

treatments have been suggested to be effective in reducing symptoms which has been shown in a meta-analysis[86], however, the effect was mainly driven by studies that included a wait-list control group or similar inactive treatments. Possibly, it is not the psychological treatment but the attention and reassurance, linked to a possible placebo effect, that is responsible for symptom improvement. Nevertheless, it remains important for practitioners to acknowledge the impact of GI symptoms on QoL and psychological status, as this acknowledgement can already improve symptoms.

Dietary assessment

Accurately assessing dietary intake is challenging, as current dietary assessment methods are prone to recall bias and misreporting due to socially desirable answers[87, 88]. Furthermore, correct estimation of portion sizes can greatly impact dietary assessment, but is prone to measurement error[89]. Ideally, dietary intake of fiber measured in **Chapter 4, 5 and 6** would be measured using biomarkers. Plasma Akylresorcinol has been suggested as a marker for whole grain and rye intake[90-92], but has poor correlations with total dietary fiber intake[93]. Plasma beta-carotene or vitamin C has been suggested as biomarkers for fruits and vegetables, but showed limited results and lack a dose-response[94, 95]. Therefore, the search of a biomarker for dietary fiber intake is still ongoing.

It is plausible that dietary intake measured throughout the chapters of this thesis is prone to some error. In **Chapter 3,** the habitual dietary intake of IBS patients and controls was assessed by using a FFQ recalling the last month. Possibly, an association between the gut microbiota and diet was not found due to measurement error or recall bias. Furthermore, IBS patients are known to adjust their diet according to their symptoms[31, 32]: for example lactose and gluten avoidance is frequently reported[96]. Although the FFQ used in Chapter 3 did contain several additional questions regarding dietary adjustments and the FODMAP diet, the FFQ was not validated for large alterations in the dietary pattern of IBS patients such as lactose-free dairy substitutes or gluten free bread, which could have influenced the results. However, currently only two FFQs globally have been developed to assess the FODMAP diet either in Brazilian IBS patients[97] or Australian adults without GI symptoms[98], and to date no specific IBS FFQ has been developed. Since only a minority of the population in Chapter 3 followed the FODMAP diet, a FODMAP FFQ would not be optimal for most. Furthermore, a FFQ is validated for ranking individuals based on their habitual intake[99, 100], and if gut microbiota profiles in IBS would be linked to the habitual diet, these probably would have shown.

In **Chapter 5 and 6**, we have estimated habitual dietary intake via a meal-based FFQ to compute the PDA, and the effects of the PDA were determined by 24hr recalls. To reduce bias and optimize the dietary assessment, several recall days

were performed per timepoint and included weekend and weekdays to account for daily variation. Furthermore, participants were not informed beforehand when recalls would take place. Although these estimates might be prone to an underestimation or overestimation of the fiber intake, these errors are expected to be consistent over time and stable within the individual, and thus would still show an improvement in dietary fiber intake after PDA intervention. The meal-based FFQ to estimate a participants' habitual diet was feasible in a study set-up, but the lengthiness of the questionnaire (1-1.5 hour per participant) limits the use in daily practice. In order to make such dietary advice tools broadly available for the general public, shorter dietary assessment methods are needed.

In **Chapter 4**, we describe and validate the 18-item FiberScreen versus a FFQ. As mentioned before, ideally dietary intake questionnaires need to be validated against an objective marker, but for fiber intake this is unknown. As the overall aim of the 18-item FiberScreen is to screen participants for having a relatively low or high fiber intake, a small error can be expected, but a true estimate of the fiber intake is not the goal of the questionnaire. As it substantially reduces both researcher and participant burden, it is a practical tool that helps including volunteers for fiber intervention studies with limited screening burden.

Box 2. Comparing dietary intakes between the chapters

Energy intake was similar in controls of **Chapter 3** and adults without GI symptoms in **Chapter 5**, and lower in IBS patients of **Chapter 3** (measured by a FFQ) and constipation complaints of **Chapter 6** (measured by 24hr recalls). Dietary fiber intake was lower in **Chapter 5 and 6** compared to both IBS patients and controls of **Chapter 3**, however a habitually low fiber intake was part of the inclusion criteria of these chapters. The different methods could lead to different estimates, as shown in the validation study of a Dutch 24hr recall method Compleat compared to a FFQ, which found a ~2g in fiber intake between methods[101]. Although there are some differences between these populations and methods differ, average fiber intakes were still below the Dutch recommendations[102]. Habitual dietary intake of an constipated population was not assessed before, but **Chapter 6** showed too low intakes of fruits, vegetables, nuts/seeds and legumes, all categories advised to consume by the Dutch Health council[103]. This is however not much different from Dutch adults without GI complaints[104]. Even though dietary assessment methods are far from optimal, these results indicate the need for dietary improvement to increase adherence to the national guidelines. Much research is performed to optimize dietary assessment strategies, but this remains a challenge.

Methods of personalizing dietary advice

In **Chapter 5 and 6** of this thesis, the dietary advice was personalized based on gender and habitual dietary intake, and in Chapter 5 PDA was shown effective to increase dietary fiber intake up to 3 months after the intervention. Although PDA is still in its infancy, several PDA trials have been done that showed promising results on dietary intake, metabolic markers and wellbeing[105-115]. Furthermore, PDA seems effective in instigating long-term dietary behavior change[108, 116], which is of importance to sustainably improve the health of individuals and reduce the risk for diseases. The type of PDA interventions (duration, mode of distribution, aim) and on what characteristics the advices were personalized differs greatly between studies, as shown in Chapter 1. Several studies have personalized the advice based on serum biomarkers and fecal gut microbiota[106, 113, 114]. Indeed, it has been shown that postprandial glucose response can differ between individuals[113], and that diet-gut microbiota associations are personalized[69]. However, the Food4Me trial including n=1269 adults from 7 different European countries showed benefit from PDA compared to general advice, but no difference between the different types of PDA (based on diet, diet and phenotype, or diet, phenotype and genotype)[106]. Furthermore, Pavlidis and colleagues (2015) have shown in a meta-analysis that commercially available nutrigenomic tests lack specific and consistent associations between the diet and 38 identified genes that have been related to dietary intake or nutrition-related pathologies before, and conclude that solid scientific evidence is currently lacking[117]. In contrast, Zeevi and colleagues (2015) have successfully lowered postprandial glucose responses via PDA based on a predictive algorithm, taking postprandial glucose levels and the gut microbiota among others into account[113]. However, they did not compare the predictive PDA algorithm to measures of diagnosis of normoglycemia such as oral glucose tolerance tests or fasting glucose, but only to carbohydrate and energy intake. Furthermore, they only compared the PDA with a diet opposite of the PDA (*e.g.* a personalized sub-optimal diet), but not to standard treatment such as a lower carbohydrate intake or improving dietary glycemic indexes, which have also shown effective[118]. Lastly, they report a large within person variability, but lack to acknowledge the large between person variation; possibly the variation observed is within the normal curve of the postprandial glucose responses of a population[119]. Although the research of Zeevi and colleagues is novel and provides much interesting leads, more research is needed to assess the effectiveness of such predictions. For now, it seems too early for PDA based on genetics, gut microbiota or serum biomarkers to have additional value besides personalization on habitual diet and behavior.

One of the conditions to further develop PDA for the general population is to better understand the mechanisms between serum and fecal markers and the diet. Regarding dietary fiber, some observed that a high-fiber diet was associated with higher microbial richness[120], and that fiber interventions can alter the gut microbiota

or SCFA[121, 122]. A study from O'Keefe and colleagues (2015) observed that a high-fiber intervention of two weeks did not alter gut microbiota composition, but did change fecal SCFA levels[123]. Furthermore, a meta-analysis including 64 randomized controlled trials assessing the effects of dietary fiber via supplements or foods (fiber range: 1.2-50 g/day) in healthy adults found that dietary fiber interventions did not change gut microbial alpha diversity, but did increase the abundance of *Bifidobacterium* spp. and *Lactobacillus* spp., as well as fecal butyrate levels[124]. We did not observe an association between the gut microbiota and dietary fiber intake in IBS patients **(Chapter 3)**, and no significant alterations in the gut microbiota or SCFA levels after a high-fiber PDA in adults with constipation **(Chapter 6)**. In both studies, we took the within and between person variation and time dynamics into account. Possibly, since the participants in **Chapter 6** substituted habitually consumed low-fiber products for similar high-fiber alternatives, the change in fiber intake was too small to be reflected in the stool. Indeed, recent work has shown that diet-gut microbiota associations were more dependent on type of foods than nutrients in the food[58]. Furthermore, these studies assessing the effects of fibers on the gut microbiota often use single fiber supplements or single foods which does not reflect the whole diet[125], which could also lead to differences in the impact on the gut microbiota and SCFA.

It has been suggested that the gut microbiota in different individuals could be separated based on three clusters dominated by either *Bacteroides, Prevotella* or *Clostridiales*[126], which have been linked to different diets across the globe[127-129]. These clusters could be the basis for the development of a PDA based on the gut microbiota, but have several limitations. Firstly, these clusters have been shown unstable over time[130]. Secondly, in a meta-analysis including 5 studies and 747 fecal samples, it was shown that clusters of *Bacteroides* and *Prevotella* do not represent consistent microbial communities, indicating that no other bacterial taxa correlates with these clusters[131]. Lastly, the abundances of *Bacteroides* and *Prevotella* have the greatest range but are also inversely related to each other, thus the clusters could be driven by a statistical artifact and not by personalized biological mechanisms[131]. Scientific rationale to base PDA on these gut microbiota clusters seems lacking, and further investigations of individualized diet-gut microbiota associations are needed before any PDA can be based on this.

These interactions are already diverse in the general population, but even less evidence has been gathered for PDA in disease. Recently, a multi-center consortium has started the development of PDA for patients with inflammatory bowel disease[132]. The authors indicate that before any trials can be performed, first the effects of diets and dietary triggers on disease outcomes need to be determined, after which predictive models and biomarkers to identify responders/non-responders need to be developed[132]. These challenges also apply for PDA development in constipation

complaints and IBS patients. As mentioned before, objective outcomes and diagnosis in these populations are already difficult, and as shown in **Chapter 2**, no patterns of dietary triggers in subgroups of the heterogenous IBS population could be identified. Furthermore, as shown in **Chapter 3,** the gut microbiota and the associations with GI complaints are not stable over time in IBS, complicating predictions of associations and assessment of responders/non-responders. It seems therefore too soon to develop PDA for IBS patients, in which too much is unknown to develop such algorithms. For adults with constipation complaints, increasing dietary fiber intake has been shown effective to reduce symptoms before[133, 134]. We have shown that a PDA based on habitual diet and gender is effective in improving fiber intake in Dutch adults with and without constipation, even up to 3 months after the intervention, and subsequently reduced constipation complaints (**Chapter 5 and 6**). These results indicate that for adults with and without constipation complaints a PDA based on phenotypic aspects with the goal to increase dietary fiber intake is sufficiently effective, and can lead to a more long-term change in dietary behaviors. For further personalization of the advice based on serum or fecal markers, a deeper understanding of the associations and validated markers are needed.

Study population selection for digital health interventions

Digital PDA interventions may not be suitable for all in the general population, as described in the discussion of **Chapter 5**. Digital health interventions require a certain level of technical skills, which have shown insufficient in three quarters of the elderly population[135], resulting in drop-outs or ineffective interventions. When participants of all ages could join the digital PDA intervention in **Chapter 5**, 14 participants dropped out or did not log in onto the webtool, in contrast to the 0 drop-outs and non-compliance in **Chapter 6,** of which age was restricted ≤55 years. Although participants from **Chapter 6** had three face-to-face visits during the trial and had GI complaints which could influence motivation and adherence and which participants in **Chapter 5** did not have, it seems plausible that age and the related technological skills does influence the effectiveness of digital health interventions. Research showed that 63% of elderly were willing to use e-health applications, but that this was highly related to their self-perceived ease of use of digital health technologies[136]. Recently, a nutritional telemonitoring e-health intervention specifically developed for Dutch elderly has been shown effective[137]. But also in this study, drop-outs were older, had lower cognitive and physical functioning and were more care-dependent[138]. Interestingly, in **Chapter 2,** an online survey was performed of which 25% of the study population was >60 years, suggesting that filling in an online questionnaire does not pose major issues for an elderly population. This however might be influenced by an increased motivation to participate in research due to the experience of GI complaints. Taking the abovementioned points into account, it might be advisable for digital health interventions targeting the general population to exclude elderly and develop separate digital health

interventions for an elderly population which has more attention for their technical skills. Furthermore, the most frail elderly might not be a suitable population for digital interventions at all, and for this population health behavior change might still be best instigated using normal face-to-face health care.

Conclusion

Based on the findings of this thesis, the following can be concluded: 1) time dynamics and large within and between person variation need to be taken into account when assessing associations between GI complaints, diet, the gut microbiota and SCFA. Associations between these aspects found in cross-sectional studies can be based on coincidence, and conclusions based on these studies need to be reconsidered or confirmed in longitudinal and experimental designs. 2) Digital high-fiber PDA based on habitual dietary intake and gender is effective in increasing fiber intake in adults with and without constipation complaints, even up to three months after the intervention. Digital health interventions designed for the general population may not be suitable for elderly, which need further tailoring to meet their technological assistant needs. 3) It is possible to screen dietary fiber intake with a short fiber screening questionnaire (the FiberScreen). This reduces the burden for both participants and researchers. 4) Psychological status and QoL are important factors to consider when assessing associations in patients with GI complaints, due to the high prevalence of anxiety and depression in IBS, and the associations between fecal SCFA and QoL in adults with constipation.

The work in this thesis contributes to new insights of the role of diet in gut health in and more specifically adds important knowledge to the field of IBS and personalized nutrition. This thesis showed that GI complaints, the gut microbiota and SCFA are capricious and can change within a short period of time, which was larger than expected beforehand. These fluctuations do not seem to be associated with each other, as a change in fiber intake or GI symptoms was not associated to changes in the gut microbiota or SCFA. Changes in GI complaints are not associated with SCFA levels over time in IBS patients, but are in patients with constipation. Furthermore, dietary triggers are highly personalized. Therefore, the interplay between GI complaints, the gut microbiota and diet is complicated in IBS, and it is too soon to develop a PDA for IBS patients. Objective outcomes, biomarkers and longitudinal and experimental designs are highly needed to advance in this field. PDA is effective to increase dietary fiber intake in the general population, and subsequently reduces GI complaints in adults with constipation.

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APPENDICES

ENGLISH SUMMARY

Even though functional bowel complaints are not life-threatening disorders, they can have an immense impact on quality of life (QoL), social functioning, and can co-exist with chronic fatigue, anxiety and depression. Fully understanding these complaints and exploring the potential of the diet to reduce symptoms is therefore pivotal for a large group of people, as functional bowel disorders, of which Irritable Bowel Syndrome (IBS) is the most well-known, can occur in up to one fifth of the global population. The majority of IBS patients report that symptoms can be induced by specific foods, so-called dietary triggers, but these differ largely between patients, and it is unclear what is causing these differences. It has also been suggested that the gut microbiota in IBS patients is altered, however a specific microbial signature remains elusive. This may be due to the cross-sectional designs of most studies that do not take instability of the gut microbiota and IBS symptoms into account. This is the focus of **Chapter 2 and 3** of this thesis, which includes the investigation of associations between diet, IBS symptoms, psychological status, and the gut microbiota and short-chain fatty acids (SCFA).

In **Chapter 2,** a large online cross-sectional survey was performed, which included 1601 IBS patients. In this survey, associations between self-reported dietary triggers and IBS symptoms such as severity and subtypes were investigated. No differences were found between IBS patients of different subgroups (*e.g.* different IBS subtypes or severity groups), indicating that there is no difference in self-reported dietary triggers between these groups. Greasy foods, onions, cabbage, spicy and fried foods were most often mentioned as triggers. IBS severity was positively associated with the severity of a response and number of dietary triggers a patient responded to, while QoL was negatively correlated with the response to dietary triggers. No differences were seen in self-reported response to dietary triggers between patients with and without anxious and depressive symptoms. Only 30% of the IBS patients that made alterations in their diet was guided by a dietician, showing a clear need for dietetic supervision in this population.

Next, a longitudinal study including two timepoints with 4 weeks in between was performed, which included 91 IBS patients and 30 matched controls **(Chapter 3)**. This study investigated the dynamics of gut microbiota and SCFA levels in different IBS severity groups, and the association between stool pattern, diet, depression, anxiety and QoL over time. We observed large time-dynamics, as already 36% of the IBS patients changed severity group and 53% had a different stool pattern within 4 weeks of time. No consistent differences between IBS patients and controls or IBS severity groups were observed in microbial alpha diversity or composition, and SCFA levels. IBS subtypes did show differences in SCFA levels, as constipation-predominant IBS had lower levels and diarrhea-predominant IBS had higher levels of SCFA. The relative abundances of *Bifidobacterium* was consistently lower in IBS compared to controls while that of *Terrisporobacter* and *Turicibacter* were higher.

These genera could be potential targets for future microbiota-mediated treatment strategies in IBS. Importantly, Chapter 3 shows that longitudinal studies are crucial to discriminate consistent associations between different datasets from coincidental observations. Due to the large within-person variation over time, biological interpretation from cross-sectional studies is thus very limited and may partly explain the inconsistency between studies.

Constipation is another frequently occurring bowel disorder, which is characterized by hard stools and infrequent bowel movements Constipation can reduce QoL and increase the risk for several diseases. Constipation can be the result of an unhealthy lifestyle characterized by low physical activity levels, and a low fiber and fluid intake. A high fiber intake is considered positive for health, regardless of having constipation or not, as this is associated with a reduction of risk of diseases such as diabetes mellitus, cardiovascular disease and colorectal cancer. A fiber intake of 30 g/day for females and 40 g/day for males are recommended in the Netherlands, but Dutch median intakes are currently 18 and 23 g/day for females and males, respectively. Sustainably increasing dietary fiber intake remains a challenge, and tools are needed to assist people in achieving this change in diet. Personalized dietary advice (PDA) has been suggested as a promising method to sustainably improve dietary intake, which is able to reach large groups of people due to its digital applications. A successful recruitment and screening is essential for such high-fiber interventions, but this has been shown difficult as current dietary assessment methods are extensive and include large questionnaires and interviews, which are more elaborate than strictly needed for recruitment. This places an unnecessary burden on both participants and researchers.

The development and validation of a short fiber screening questionnaire was described in **Chapter 4.** The 18-item FiberScreen assessed dietary intake of fruits, vegetables, whole grains, pasta/rice/potatoes, legumes and nuts/seeds over the past 2 weeks, and was compared with the results of a food frequency questionnaire (FFQ) in 87 adults without gastrointestinal complaints and 29 adults with constipation complaints. The 18-item FiberScreen had a good correlation with the FFQ, and differences between the estimates were relatively small. Average completion time was only ~4 minutes, compared to 45-60 minutes of the FFQ. The 18-item FiberScreen was therefore shown to be a suitable questionnaire to screen for a relatively low fiber intake in Dutch adults.

In **Chapter 5 and 6**, the development and validation of a digital high-fiber PDA in 81 adults without gastrointestinal complaints and 25 adults with constipation complaints was described. The PDA was based on a participants' habitual diet and gender, and participants could substitute habitually low-fiber products for high-fiber alternatives, and could add additional fruits, vegetables, legumes, nuts and seeds to the PDA to

meet the recommendations. Based on the dietary choices participants made, participants formulated an action plan, a so-called implementation intention. In **Chapter 5**, it was shown that a digital high-fiber PDA was effective in increasing dietary fiber intake, even up to 3 months after the intervention when still given access to the PDA. In **Chapter 6**, the further optimized high-fiber PDA increased fiber intake, reduced symptoms and improved stool consistency and QoL in adults with constipation complaints. No effects of the high-fiber PDA on stool frequency, gut microbiota or SCFA levels were observed. In both chapters, the high-fiber PDA was well-accepted and improved self-reported knowledge of fibers.

In conclusion, this thesis adds important new knowledge regarding associations between diet, GI symptoms and the gut microbiota in IBS patients and developed practical tools for the screening and stimulation of dietary fiber intake. The results from this thesis show dietary triggers for IBS complaints are highly personal. Furthermore, bowel complaints and the gut microbiota are highly fluctuating over time and do not seem associated with each other or with a change in fiber intake. PDA based on habitual diet and gender is effective in increasing dietary fiber intake in the general population, and can subsequently reduce complaints in adults with constipation. The interplay between bowel complaints, the gut microbiota and the diet is complicated, indicating that it is too soon to develop a PDA for reducing complaints IBS patients.

APPENDICES

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APPENDICES

ABOUT THE AUTHOR

Curriculum Vitae

Iris Rijnaarts was born on the 10th of October 1992 in Nijmegen, the Netherlands. After completing secondary school at the Nijmeegse Scholengemeenschap Groenewoud in Nijmegen in 2009, she followed her passion for human biology and nutrition and enrolled as a student in nutrition and dietetics at the Hogeschool van Arnhem en Nijmegen. She followed a minor in clinical nutrition and had a clinical dietetic internship in Rijnstate Hospital in Arnhem. In 2015, she proceeded to the master Nutrition and Health at Wageningen University. During her masters, Iris spend 6 months at the Center for Translational Research in Aging and Longevity (CTRAL) in Texas, USA, where she studied the protein metabolism and body composition in congestive heart failure patients. She completed her thesis on the association between vitamin K intake and cognition in healthy adults.

After graduation, Iris worked as a junior researcher on the Healing Gardens project within Wageningen University & Research. In this project, she conducted a pilot study to assess the feasibility of vegetable gardening on dietary intake, physical performance and quality of life in cancer survivors. In 2017, she got the opportunity to proceed as a PhD Candidate at the Division of Human Nutrition and Health, Food and Biobased Research, and Laboratory of Microbiology under the supervision of Professor Ben Witteman, Nicole de Roos, Nicole de Wit and Erwin Zoetendal. The research was part of two consortia, namely the IBSQUtrition and Personalized Nutrition & Health – living lab more fiber, of which the results are presented in this thesis. Iris enjoyed the multidisciplinary work within these consortia, and the collaboration with many partners. Furthermore, Iris supervised 9 MSc and 3 BSc students and contributed to teaching within several MSc courses throughout her PhD. In 2021, she won the James Lind Prize in the category applied science, and had a poster of distinction at the Digestive Disease Week.

List of publications

This thesis

Rijnaarts, I., de Roos, N. M., Wang, T., Zoetendal, E. G., Top, J., Timmer, M., Hogenelst, K., Bouwman, E. P., Witteman, B. J. M., de Wit, N. J. W. (2022). A high-fiber personalized dietary advice given via a web-tool reduces constipation complaints in adults. Accepted in *Journal of Nutritional Science.*

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Other publications

Kirschner, S. K., Deutz, N. E., Rijnaarts, I., Smit, T. J., Larsen, D. J., & Engelen, M. P. (2021). Impaired intestinal function is associated with lower muscle and cognitive health and well-being in patients with congestive heart failure. *Journal of Parenteral and Enteral Nutrition,* 46(3), 660-670.

Botros, N., Rijnaarts, I., Brandts, H., Bleumink, G., Janssen, I., & de Boer, H. (2014). Effect of carbohydrate restriction in patients with hyperinsulinemic hypoglycemia after Roux-en-Y gastric bypass. *Obesity surgery*, *24*(11), 1850-1855.

Overview of completed training activities

Discipline specific activities

Name	Organizer	Location	Year
Seminar personalized Nutrition & Health	TNO	Wageningen	2018
International Dietary Fiber Conference*	International Cereal Consortium, TNO, WUR	Rotterdam	2018
Annual gut day*	Laboratory of Microbiology, WUR	Wageningen	2018
Granen en Chronische aandoeningen	Nederlands Bakkerij Centrum	Wageningen	2019
Conference of Personalized Nutrition and Health*	Personalized Nutrition and Health Consortium, WUR, TNO	Wageningen	2019
International Conference of Diet and Activity Methods (ICDAM)*	Division of Human Nutrition and Health, WUR	Online	2021
Digestive Disease Week (DDW)**	DDW	Online	2021
Good Clinical Practice course	GCP central	Online	2021
ESPEN conference*	ESPEN	Online	2021

*Poster presentation, **poster of distinction

General courses

Name	Organizer	Location	Year
Supervising and teaching thesis students	WGS	Wageningen	2018
PhD week	VLAG	Baarlo	2018
Research Data Management Part I and II	WGS	Wageningen	2018
Introduction into R	VLAG	Wageningen	2019
Applied statistics	VLAG	Wageningen	2019
Chemometrics	VLAG	Wageningen	2019
Reviewing a scientific paper	VLAG	Wageningen	2019
Career perspectives	WGS	Online	2021

Other activities

Name	Organizer	Location	Year
Preparation of research proposal	VLAG	Wageningen	2017
Member of PhD committee	Division of Human Nutrition and Health, WUR	Wageningen	2018-2021
PhD tour	Division of Human Nutrition and Health, WUR	East coast Canada	2019
Food for thought*	Alliantie Goede Voeding, Hospital Gelderse Vallei	Ede	2019
Young Food Supplement Professionals Science day*	NPN	Wageningen	2021
Winner James Lind Prize category Applied Science – Vezel-UP research	NPN		2021
Biweekly scientific group meetings Nutrition and Disease	Division of Human Nutrition and Health, WUR	Wageningen	2017-2022
Weekly scientific group meetings	Food and Biobased Research, WUR	Wageningen	2017-2020
Weekly scientific group meetings	Laboratory of Microbiology, WUR	Wageningen	2020-2022
Rothman Lunches	Division of Human Nutrition and Health, WUR	Wageningen	2020-2021
Bi-annual scientific project meetings*	IBSQUtrition consortium	Various locations	2017-2022
Quarter annual scientific project meetings*	PNH - Living lab more fiber	Various locations	2017-2021

*Oral presentation

Colophon

The research described in this thesis was performed within the IBSQUtrition consortium (Chapter 2 and 3) and the PNH living lab more fiber consortium (Chapter 4, 5 and 6). The IBSQUtrition consortium was supported by a Public Private Partnership grant from the Top consortium for Knowledge and Innovation Agri & Food by the Dutch ministry of Economic affairs (TKI-AF-16012) and co-funding was received from participating companies (Winclove, Ingredia, Ingredion, Nexira, Naturex, BioIberica, Wecare, Roquette and Darling Ingredients). The PNH living lab more fiber consortium was funded by the Ministry of Economic affairs and the Maag Lever Darm Stichting (MLDS), and co-funding was received from participating companies (Sensus, Kelloggs, Bolletje, Sonneveld, Roquette and Nederlands Bakkerij Centrum).

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