Prevalence of dysbiosis and microbiotic effect of the low FODMAP diet in coeliac disease patients with IBS-like symptoms

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Ida Serine Melhus Strindmo

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Abstract/summary

Background and aim: A subgroup of coeliac disease patients have IBS (irritable bowel syndrome)-like symptoms despite following a gluten free diet (GFD). We wanted to compare the microbiota in these patients with an IBS and a healthy population, and look at changes during a low FODMAP (fermentable oligo-, di-, monosaccharides and polyols) diet versus a stricter GFD. We also wanted to look at the effect of diet on symptom relief in these patients.
Methods: 40 celiac disease patients with IBS-like symptoms confirmed by the Rome III-criteria and IBS-SSS (symptom severity scale)>75 were compared to Norwegian IBS and healthy cohorts, and randomized into two groups. Group A followed a stricter GFD, whilst patients in group B reduced FODMAPs in their GFD. Both groups followed their diet for six weeks. Faecal samples were collected at baseline and 6 weeks and IBS-SSS at baseline, 3 and 6 weeks. Hydrogen breath test was performed at baseline and after six weeks. The faecal samples were analysed by Genetic Analysis for bacteria and Dysbiosis Index (DI) 1-5, where DI>2 is considered clinically relevant. Statistics: T-test, Mann-Whitney U, RM one way ANOVA, Fisher's linear discriminant analysis.
Results: FODMAPs were reduced from 11.5 to 1.6g/day (p=0.0001) in group B, and IBS-SSS score improved in both group A (p=0.0022) and group B (p=<0.0001). 45% of the patients had dysbiosis at baseline, compared to 73% in IBS (p=0.0091) and 16% in healthy controls (p=0.0007), with a mean score of 2.5±1.1 versus 3.0±1.0 and 1.7±0.7, respectively. Several bacterial genera were significantly altered at baseline compared to healthy controls, including *Bacillus* and *Prevotella*. In group A (18F/2M, age 39±15), dysbiosis stayed constant on diet, but more patients had severe dysbiosis (DI>3), 15% vs. 25% (p=0.85). In group B (15F/5M, age 44±12), fewer patients had dysbiosis after diet, 60% vs. 50% (p=0.79). There was a statistically significant reduction in the genus *Bacteroides* after the LFD compared to the stricter GFD (p=0.024). Responders to low FODMAP diet (IBS-SSS score reduction >100) had a distinctive microbiota pattern with less *Lactobacilli* and *Firmicutes (Clostridia)*, and more *Atopobium* at baseline. There were no reduction in the AUC for hydrogen after six weeks on a low FODMAP diet (p=0.926).
Conclusion: Celiac disease patients with IBS-like symptoms had less severe dysbiosis than an IBS-population, but more than healthy controls. This study give evidence for the effect of the low FODMAP diet for symptom relief in these patients. We found that the level of *Lactobacilli, Firmicutes (Clostridia)* and *Atopobium* predicted response to the low FODMAP diet.

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AUC – area under curve

CD – coeliac disease

DI – Dysbiosis Index

ENS – enteric nervous system

FGID – functional gastrointestinal disorders

FMT – faecal microbiota transplantation

FODMAP – fermentable oligosaccharides, disaccharides, monosaccharides and polyols

FOS – fructooligossaccharides

GFD – gluten free diet

GOS – galactooligosaccharides

HLA – human leucocyte antigen

IBD – inflammatory bowel disease

IBS – irritable bowel disease

IBS-C – irritable bowel disease with constipation

IBS-D – irritable bowel disease with diarrhoea

IBS-M – irritable bowel disease with a mixed bowel pattern

IEL – intraepithelial lymphocytes

IgA TTG – IgA tissue transglutaminase antibodies

IgG DPG – IgG deamidated gliadin peptide

IQR – interquartile range (25 and 75 percentiles)

LFD – low FODMAP diet

NRCD – non responsive coeliac disease

PI IBS – post infectious IBS

RCD – refractory coeliac disease

REC – regional committee for medical and health research ethics

rRNA – ribosomal RNA

SCFA – small chain fatty acids

SIBO – small intestinal bacterial overgrowth

1 INTRODUCTION

1.1 Coeliac disease

1.1.1 Background

Coeliac disease (CD) is described as a systemic autoimmune disease, where ingestion of gluten will cause damage to the small intestinal mucosa in genetically predisposed individuals. Gluten ingestion will initiate an immune reaction, and can cause villous atrophy, crypt hyperplasia and chronic inflammation in the mucosa [1]. The signs and symptoms of CD can vary substantially, but some of the common ones are malabsorption symptoms such as weight loss, diarrhoea, iron deficiency anaemia and growth retardation in children [2].

Gluten is a collective term for storage proteins found in wheat, barley and rye. Gluten is composed of two different proteins named glutenin and gliadin, and it is the gliadin protein in wheat that triggers an immune reaction. In barley and rye, these protein fractions are called hordeins and secalins, respectively. Another term for these proteins is prolamins, which refers to alcohol soluble proteins rich in proline and glutamine [2].

The prevalence of coeliac disease is 1 in 100 individuals, affecting more women than men [2, 3]. CD was previously thought of as a rare disease occurring only in children, but we now know that it can develop at any age, and have many different manifestations. Some can have diarrhoea and serious classical malnutrition symptoms, whilst others have very diffuse or no symptoms. More atypical symptoms are joint pain, fatigue, constipation and abdominal distension [4, 5].

1.1.2 Diagnosis

Diagnosis in adults is made by serological testing and small bowel biopsies. The gold standard at present is IgA tissue transglutaminase antibodies (IgA TTG), in combination with a small intestinal mucosal biopsy. It is recommended to measure total IgA as well, in order to identify those with IgA-deficiency [6]. If IgA is deficient, IgG deamidated gliadin peptide (DPG) is the preferred test to IgA TTG. In addition to positive serology, biopsy findings will essentially confirm the diagnosis [7]. It is important that a gluten containing diet is followed weeks before and during testing for CD. The histological findings are graded by the Marsh classification, a system ranging from Marsh I to Marsh III A-C and Marsh IV, referring to

amount of intraepithelial lymphocytes (IEL) per 100 enterocytes, and degree of crypt hyperplasia and villous atrophy [8]. Marsh I refers to normal or minimal histological findings and intraepithelial lymphocytosis, which can also be found in other conditions such as Helicobacter Pylori-infection. Thus, this finding is not necessarily consistent with coeliac disease. In Marsh II, crypt hyperplasia is also present. Marsh III is the most common finding in CD-patients, and includes some or complete villous atrophy [9]. Marsh IV is rare and describes complete villous atrophy and no lymphocytes. This has been seen in non-responders [8].

Table 1: Marsh classification system

Oberhuber et al. *Eur J Gastroenterol Hepatol*, 1999 [10].

Marsh Type	IEL / 100 enterocytes – jejunum	IEL / 100 enterocytes - duodenum	Crypt hyperplasia	Villi
0	<40	<30	Normal	Normal
1	>40	>30	Normal	Normal
2	>40	>30	Increased	Normal
3a	>40	>30	Increased	Mild atrophy
3b	>40	>30	Increased	Marked atrophy
3c	>40	>30	Increased	Complete atrophy

IEL, intraepithelial lymphocytes

Coeliac disease mainly affects the proximal small intestine. Damage to the small intestine causes malabsorption due to a smaller absorption surface and reduced amount of digestive enzymes, which in turn can lead to weight loss and malabsorption of micro-nutrients such as fat soluble vitamins A, D, E and K, iron, zinc, folate and vitamin B12. If a deficiency is present, it will usually normalize when gluten is excluded from the diet [5].

1.1.3 Etiology

A crucial factor for disease development is gluten exposure. Besides this, a major genetic risk factor is predisposition with genes that code for human leucocyte antigen (HLA)-DQ2 or DQ8 proteins. Around 99% of CD patients carry this; the majority carry HLA-DQ2, whilst a smaller fraction HLA-DQ8. However, these HLA-types is present in about 30% of the general population, and most of these do not develop coeliac disease [11]. Thus, HLA-typing can be

an effective test for exclusion of coeliac disease, but does not give any confirmation of the disease [12, 13]. Studies on monozygotic twins reveal 75-80% concordance compared to 20% in dizygotic twins, and sibling relative risk of 20-60, implying a strong genetic component in CD development. HLA genes have shown to account for parts of this, while non-HLA genes and environmental factors accounts for the rest. This is less studied, but non-HLA genes, epigenetics and gut microbiota have all been proposed to be involved in CD development [3, 14]. Individuals with type 1 diabetes, Down's syndrome or Turner's syndrome and autoimmune thyroid disease are at higher risk of developing CD, due to shared genetic risk factors [15].

1.1.4 Immunopathology

In coeliac disease, an abnormal immune response to gluten proteins causes intestinal damage. It is mainly the adaptive immune system that is active in coeliac disease. Because of the high content of proline, gluten is difficult to digest and is only broken down to gliadin polypeptides. These peptides cross the enterocytes into lamina propria via para- and transcellular routes, where they are deaminated into negatively charged glutamic acid by the enzyme tissue transglutaminase (TTG). This increases the affinity for gliadin binding to the pockets of HLA DQ2/8, expressed on antigen presenting cells (APCs). The binding of gliadin on APCs leads to the activation of gluten-specific CD4+ T-cells, which in turn produces proinflammatory cytokines such as IL-21 and IFN-ɤ. These cytokines lead to the damage of the epithelial cells such as villous atrophy and crypt hyperplasia [16-18]. TTG is also able to crosslink with gliadin, which together with inflammatory cytokines are thought to activate B-cells that produces antibodies, including autoantibodies towards TTG, and antibodies towards deamidated gliadin peptide (DPG) and other molecules [16, 19].

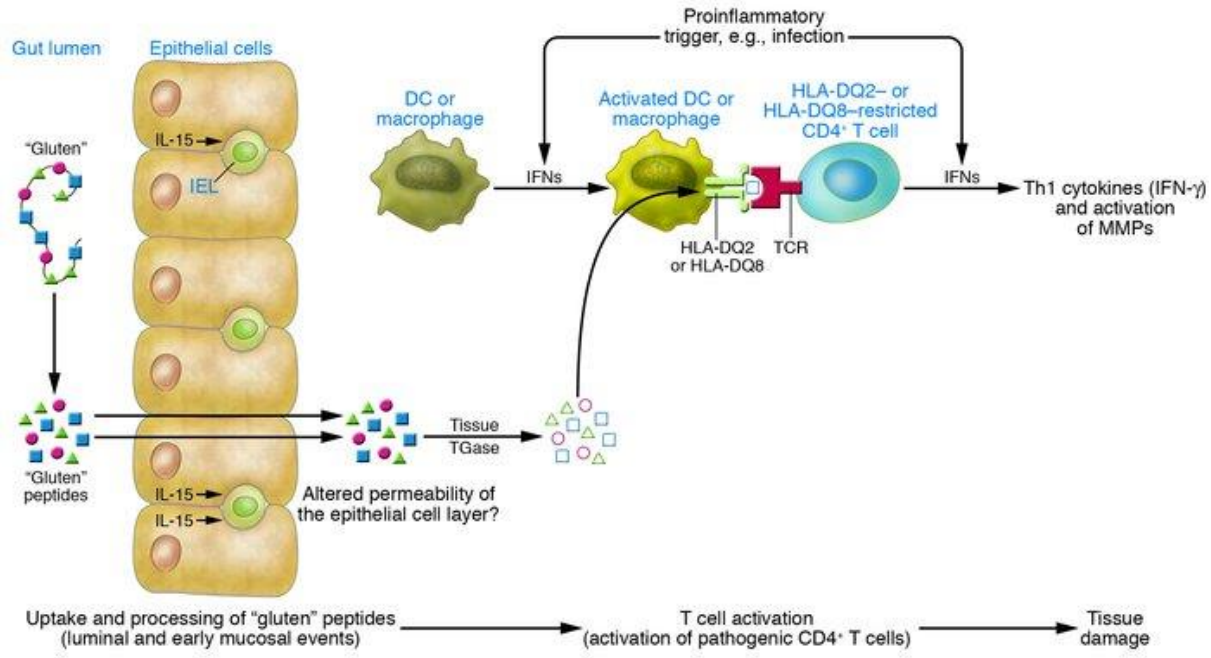


Figure 1: Immunopathology in coeliac disease

Ingested gluten is degraded to gluten peptides, which are able to cross the enterocytes into the lamina propria. Here the peptides are deaminated by TTG and binds to HLA DQ2/8 found on APCs (denditric cells or macrophages). This binding leads to the activation of CD4+ T-cells which produce proinflammatory cytokines that cause intestinal tissue damage. B-cells are also activated and produce antibodies. APC; antigen presenting cells. Kagnoff MF, Celiac disease: pathogenesis of a model immunogenetic disease. J Clin Invest. 2007 [16].

1.1.5 Treatment

The only treatment of coeliac disease today is through life long diet. By excluding all sources of gluten in the diet, most individuals will experience full mucosal healing and symptom relief. Gastrointestinal symptoms seem to resolve quickly and considerably after commencing a gluten free diet (GFD) [20]. The serology can take longer to normalize; a study by Bürgin-Wolff et al. found IgA TTG to normalize within a year for patients in Switzerland and Germany [21]. A histological normalization can take years [22].

A GFD in general refers to a diet as low in gluten as possible, as some contamination is inevitable. To follow a gluten free diet can be challenging, due to factors such as a higher cost, gluten contamination in production, storage or cooking and limitations in situations such as dining out. Some have minimal symptoms when ingesting gluten, which can make the adherence more difficult [2]. However, strict adherence to a GFD can reduce mortality risk in CD patients, as well as improve quality of life [23, 24].

1.1.6 Non-responsive coeliac disease

Some patients experience persistent or recurring symptoms despite following a GFD. This is often referred to as non-responsive coeliac disease (NRCD) [25]. The literature reports prevalence of NRCD varying from 7% to around 30% [22, 25, 26]. NRCD is defined as persistent or recurrent symptoms or signs and/or histological findings after 6 months or more on a GFD [26-28]. The definition varies some in the literature, where some use 12 months as a limit for making this diagnosis, and some definitions require abnormal histological findings [29-31]. It is reported that the major cause of NRCD is gluten contamination in the diet, either unintentional or voluntarily, accounting for 35-50% of the cases [28]. It can also be caused by several other coexisting conditions such as the irritable bowel syndrome (IBS), other food intolerances, small intestinal bacterial overgrowth (SIBO), pancreatic insufficiency, or refractory coeliac disease (RCD) [29]. The American College of Gastroenterology (ACG) Guidelines advises to reconsider the initial CD diagnosis through evaluating the biopsy results and serology as the first step towards determining the cause of NRCD. If the diagnosis is certain, a thorough assessment of the diet can identify any gluten contamination or other possible food intolerances. If diet can be excluded as a cause, a new biopsy is recommended, which can help with further determining the ethology [28].

1.1.7 Refractory coeliac disease

For 1-2% of coeliac disease patients, villous atrophy and symptoms of malabsorption will continue or come back, resulting from refractory coeliac disease (RCD). We distinguish between type I and type II RCD. Both types are characterized by lymphocytosis as seen in untreated coeliac disease. Type II differs from type I because of abnormalities in the T-cells, which is responsible for a much poorer prognosis, as clonal expansion of these cells can develop into enteropathy-associated T-cell lymphoma. Type I is often treated symptomatically, and steroids or immunosuppressive agents can be used additionally in serious cases. Type II is treated much the same way, but response to treatment is often poor. The symptoms in type II are also more severe, and nutritional treatment might be necessary [28, 32].

1.1.8 Associated conditions and complications of coeliac disease

There is an increased risk of developing osteoporosis in coeliac disease, which may be caused by several different factors. This includes malabsorption of calcium before diagnosis, persistent villous atrophy after diagnosis, low intake of calcium or lactose intolerance amongst other things [8]. There is also a link between coeliac disease and other conditions, such as type I diabetes, autoimmune thyroiditis and other autoimmune diseases, abnormal liver function and dermatitis herpetiformis, which is the skin manifestation of coeliac disease [8, 33].

1.2 Irritable bowel syndrome

1.2.1 Background

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder, characterized by abdominal discomfort, bloating and altered bowel habits [34]. It is recognized as a functional gastrointestinal disorder (FGID), describing a group of digestive disorders where diagnosis is based solely on clinical symptoms and absence of structural abnormalities. We distinguish between different subtypes of IBS based on whether constipation (IBS-C) or diarrhoea (IBS-D) is predominant, or a mix of both (IBS-M). The severity and frequency of IBS varies amongst individuals, from tolerable to severe and periodic to continuous symptoms [35, 36].

The global prevalence of IBS is between 10%-20% [37]. A Norwegian cross-sectional survey performed in 2001 shows a prevalence of 8% in Norway [38]. IBS affects more women than men, and more women seek medical attention than men. [39-41]. IBS is not associated with increased mortality, but it has significant negative influence on quality of life [42]. These patients have impaired social life, lower self-esteem and are more often absent from work [43]. Additionally, IBS sufferers generate large economic costs due to their medical care visits and investigations and increased sick leave. IBS is associated with other conditions such as fibromyalgia, fatigue and anxiety [42, 44].

1.2.2 Diagnosis

The Rome III diagnostic criteria can be used to diagnose IBS (see Methods), but are more commonly used in research than a clinical setting [45]. As there is neither biomarker nor any structural or physiological abnormalities that can identify IBS, the elimination of any organic disease is crucial before diagnosis is set [43]. This refers to the elimination of symptoms such

as unexplained weight loss, anaemia, nocturnal symptoms, rectal bleeding or family history of organic diseases, as well as other features. Colonoscopy is recommended for adults over age 50 years [34, 45].

1.2.3 Pathophysiology

The pathophysiology of IBS is multifactorial and still not fully understood, but increasing evidence show visceral hypersensitivity, a disturbance in the gut-brain-axis, chronic low grade inflammation, abnormal gut motility, intestinal microbiota, abnormal gastrointestinal endocrine cells and psychosocial factors to potentially be involved [34, 46-49]. Family and twin studies have also shown a possible genetic factor and parent-child interactions to be a part of the development of IBS [45].

Visceral hypersensitivity have for a long time been seen as a central mechanism in IBS. Abdominal pain is one of the most common symptoms, and is thought to be explained by this. It is in fact shown that IBS patients are hypersensitive to rectal distension, [50] and also the sensation of bloating is linked to hypersensitivity [51]. Gut microbiota may play an important role in the development of IBS, and a changed faecal and mucosal microbiota has been observed in IBS. This may in turn have an effect on the innate immune response, gut permeability and regulation of the enteric nervous system (ENS) [52]. There is reported faster gut transit in IBS-D and a reduced transit in IBS-C in some patients with IBS, supporting that gut motility is changed in at least a subgroup [53]. Abnormal endocrine cells found in IBS patients can be responsible for visceral hypersensitivity, disturbed gastrointestinal motility, and abnormal gut secretion [48].

1.2.4 Treatment

No clear pathophysiology makes it difficult to develop potent treatments for IBS, but several approaches have been made, targeting both host and environmental factors of the pathophysiology.

Pharmacological treatments include serotonin receptor agonist and antagonists targeting motility, sensation and secretion. Fibre, laxatives and antidiarrheal agents have been tested for IBS-C and IBS-D, respectively [45]. Small intestinal bacterial overgrowth (SIBO) is seen in some with IBS [54], and therefore antibiotics have been tested for a shorter period and shown

to be beneficial for some. However, there is still not a large quantity of evidence, and there are several uncertain factors such as dosage, efficacy and the concern of antibiotic resistance [52]. A Cochrane review from 2005 concluded that evidence for the effects of drug therapy in IBS is weak [55]. Probiotics have been tested as a way of altering microbiota. They are defined as live microorganisms that can provide a health benefit, when given in sufficient amount [56]. Different types and mixtures of probiotics, including *Bifidobacteria* and *Lactobacilli,* are tested in several clinical trials and have had some effect on different symptoms associated with IBS, such as bloating, abdominal pain and flatulence [57]. Faecal transplantation has shown to be successful in treatment after *Clostridium Difficile*-infection, and has been tested as a possible treatment in IBS [58]. Also psychological treatments have been tested, such as cognitive behavioural therapy and hypnotherapy [45, 59, 60].

Many IBS patients report food as a symptom trigger, especially food containing carbohydrates and fats [61, 62]. Gluten has been considered to cause symptoms, but this has no convincing evidence [63, 64]. Traditional IBS dietary advice include regular meals, moderate fibre and fat intake, reduction of caffeine and avoidance of gas-producing foods such as cabbage, onion and beans. This successfully reduced symptoms in one randomized, controlled trial [46]. The most effective dietary treatment today is the elimination of FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) [65].

1.2.5 Coeliac disease and IBS

Several symptoms in coeliac disease and IBS overlap, such as diarrhoea, abdominal pain and bloating, making misdiagnosis possible. The prevalence of CD amongst those already diagnosed with IBS have been studied, and found the prevalence to be around 4-5% [66-68]. Such studies have led to the recommendation to test for CD before diagnosing IBS [45].

The number of CD patients that fulfil the Rome criteria for IBS is reported to be between 15-23.3% [25, 26, 69-71]. A study by Usai et al. in 2007 found a much higher rate, where 55% of CD patients had IBS-like symptoms [72]. A pooled prevalence of 38% was found was found in a meta-analysis by Sainsbury et al. [73]. These findings suggest a link between CD and IBS, but there is not much data to support this theory yet. It might be possible to have both CD and IBS at the same time, but O'Leary rather propose that these symptoms arise from a continued mucosal inflammation in treated CD patients, predisposing for IBS symptom development [69]. Also Barbara et al. airs the idea that continued immune activity and

mucosal permeability despite following a GFD might be involved [74]. However, a link between CD and IBS needs to be determined. What might support this idea is the prevalence of IBS after an infectious gastroenteritis, named PI-IBS (post infectious-IBS), and in patients with IBD in remission. A pooled prevalence of 10% develop IBS after an infectious gastroenteritis and a prevalence of 33-57% is reported in IBD patients in remission [74, 75]. Although there is no certainty, studies have found mechanisms that can possibly explain some of the pathophysiology in these patients. Failure to downregulate immune cells is shown in those with PI-IBS, which could alter gut function, such as motility and permeability, thus creating symptoms. A genetic predisposition to produce less of the anti-inflammatory cytokine interleukin-10 has been seen in PI-IBS [76].

There is also overlapping symptoms between IBS and IBD, and a high prevalence of IBS-like symptoms in IBD patients in remission with no evidence of inflammation has been reported. A meta-analysis found a pooled prevalence of 35% [77]. This has raised questions on whether this is a very mild level of IBD, coexisting conditions or if IBD somehow predisposes for IBS-like symptoms [78]. A proposed management of these patients is to use inflammation markers such as C-reactive protein and calprotectin to exclude active IBD as the cause of the symptoms. If patients are confirmed to be in remission, but still have symptoms, symptom relieving therapy such as probiotics and soluble fibres should be tested [77].

1.3 The low FODMAP diet

Many with IBS can relate their symptoms to food ingestion, thus many have excluded different foods they expect to cause problems from their diet in order to improve symptoms. This has also been the basis for the dietary approaches made to reduce symptoms in these patients [46, 79].

1.3.1 Rationale for the low FODMAP diet

FODMAP is a grouping of short-chain carbohydrates with chains up to 10 sugar units. FODMAPs are generally poorly absorbed in the small intestines due to little absorptive capacity, lack of digestive enzymes or lack of absorptive pathways [80]. Instead of being degraded and absorbed in the small bowel, they travel to the colon where they are fermented by bacteria, which produce hydrogen, carbon dioxide and/or methane gases and small chain fatty acids (SFCA). Because of their small size, these molecules are osmotically active and

will draw water into the lumen of the small and large intestines. Increased volume of water and gas will lead to luminal distension, which are mechanisms tested in previous trials [81, 82]. Increased water volume can also cause diarrhoea, as it has a laxative effect [83]. Luminal distension is thought to be responsible for many of the symptoms seen in IBS, such as abdominal pain and bloating [84]. The SCFA produced are acetate, propionate and butyrate, which are an important energy for the colonocytes, but can also affect the gut motility. Based on this, the reduction of FODMAP could be beneficial for IBS patients [84]. Importantly, FODMAPs are equally poorly absorbed in healthy individuals as in IBS patients, emphasizing the fact that the diet can improve symptoms, but not treat the cause of IBS.

The evidence for symptomatic effect of a low FODMAP diet is accumulating, and randomized controlled trials are showing promising results. There is also evidence outside Australia, where the diet was first developed and tested [85-88]. A very recent follow-up study on IBS and IBD patients following a low FODMAP diet also show a long term efficacy of the diet on managing symptoms [89]. This study does however have several limitations, such as possible selection bias and a retrospective design, which calls for more studies confirming the long-term effects.

1.3.2 Application of the diet

Oligosaccharides refers to fructans and galactans, monosaccharides to fructose in excess of glucose, disaccharides to lactose, and polyols to sorbitol, isomalt, maltitol, mannitol and xylitol.

Fructose is a monosaccharide found in fruits and honey. It is absorbed in the small intestines via two different pathways; by GLUT-2 carriers in co-transport with glucose and by GLUT-5 carriers. In excess of fructose over glucose, the absorption of this “free fructose” is dependent of GLUT5-carriers, which only offer a low capacity transport. This results in malabsorption of some fructose, which will be fermented by bacteria. Fructose malabsorption is as present in healthy individuals as in FGIDs or coeliac disease [90].

The disaccharide lactose is found in milk and dairy products. We are dependent of the brush border enzyme lactase in order to digest the sugar before absorption, and the lack of expression of this enzyme leads to malabsorption. However, lactose malabsorption does not seem to be more present in IBS than a healthy population. Additionally, though prevalent in

Asian and African countries, malabsorption in Nordic countries is rare [91, 92]. Secondary hypoclactasia can occur in coeliac disease or other conditions where intestinal damage is present, causing less expression of lactase. This condition will normally pass when the intestines are healed [93]. Some IBS patients still reports symptoms related to lactose without having lactose malabsorption, but the mechanisms of this is not investigated [94].

Oligosaccharides refer to fructans and galactans. Fructans can also be referred to as fructo-oligoshaccarides (FOS). Important sources of fructans in our diet are foods such as onion, garlic and wheat. These foods do not contain a large amount of fructans, but will represent a large part of the fructans in our diet due to a large consumption. Galactans or galactooligosaccharides (GOS) is found in legumes, pistachios, beans and lentils. Humans are not able to digest fructans and galactans due to lack of hydrolases, and they are therefore only fermented by bacteria in the distal small intestine and colon [94].

Polyols are sugar alcohols found naturally in different plants, fruits and vegetables. More commonly, they are commercially prepared and added to sweets, chewing gum and other food products because of its sweetening properties. They can be found naturally in foods such as avocado, apples, pears, peach, champignons and cauliflower. Polyols can only be absorbed passively through diffusion in the pores of the epithelium. The absorption relies on molecular size as the pore size along the small intestine varies, and causes malabsorption of some polyols. Transit time will also be of importance [80, 94].

The low FODMAP diet requires the elimination of carbohydrates with prebiotic effects, which can have an effect on gastrointestinal health long term. This elimination also increases the risk for nutritional deficiencies, as many different foods are cut for the diet. Most patients react to some, but not all FODMAPs, and the individual tolerance for different FODMAPs will also vary [95]. It is therefore important to start reintroducing FODMAPs after the elimination phase, in order to determine what FODMAPs you react to and to find individual tolerance [96]. Previous studies on the LFD have found that adherence to the diet is crucial for it to be effective [97-99].

1.4 Intestinal microbiota

Microbiota has become a popular field of research, as improved analysing methods have allowed us a greater understanding of the relation between microbiota and health. The human gut microbiota includes a higher number of microorganisms than cells in the body, as well as more genes than the human genome, and is often referred to as an organ itself [100]. Bacteria are found along the gastrointestinal tract, and the greatest abundance is found in the colon. Bacteria live in symbiosis with the host organism, and it appears that gut microbiota can be linked to many important functions in the human body [101]. This include the maturation of the immune system, protection towards pathogens, digestion and nutrient utilization, vitamin production, and may also be of significance in different diseases, including gastrointestinal disorders.

Our first contact with bacteria may already happen *in utero*, as it is discovered bacteria present in the placenta, umbilical cord, amniotic fluid and meconium [102-106]. Studies on epigenetics and neonatal nutrition have also shown that diet during pregnancy might impact the gut microbiota in the offspring [107]. What is certain is that colonization starts immediately at birth, where the route of delivery plays an important role. Those born vaginally have more *Lactobacillus*, reflecting the bacteria found in the mother's vagina, while those born with caesarean section have bacteria from the mother's skin [108]. Other important factors for the composition of the microbiota are gestational age, feeding regime, use of antibiotics and exposure to different environmental factors. By the second to third year of life, the gut microbiota starts to stabilize and resembles an adult-like composition with a more rich and diverse microbiota [109]. The microbiota is classified into kingdom, phylum, class, order, family, genus and species. The adult microbiota is to a large degree dominated by the two phyla Bacteriodetes and Firmicutes, whilst Actinobacteria, Proteobacteria and Verrucomicrobia are also present, but less abundant. There has been proposed that the gut microbiome is divided into three different "enterotypes" where different bacteria predominate, namely *Bacteroides, Prevotella* and *Ruminococcus* [110]. Around 60-70% of the microbiota remains stable through life, whilst the remaining 30-40% can be altered by different environmental factors such as diet, stress, age, diseases, use of medication and antibiotics and genetics. This gives a large inter-individual variation in the microbiota [52, 108, 109, 111].

1.4.1 Gut microbiota and diet

Diet is one of the environmental factors that affect microbiota the most. It is important for microbiota establishment, but also later in life. Studies have shown that individuals on a diet based on plant-derived carbohydrates have microbiota predominated by *Prevotella,* whilst diets high in protein and fat results in microbiota predominated by *Bacteroides* [112]. Proteins also serves as substrate for bacteria, as 10% of ingested proteins will reach the colon and be degraded by proteolytic bacteria, such as *Streptococcus*, *Bacillus* and others. While the fermentation of carbohydrates gives metabolites considered beneficial for the host, the degradation of proteins can give products that are less beneficial and some potentially harmful. Also some fat are fermented by bacteria, and the fermentation of the macronutrients results in different bacterial metabolites with different physiological functions [108].

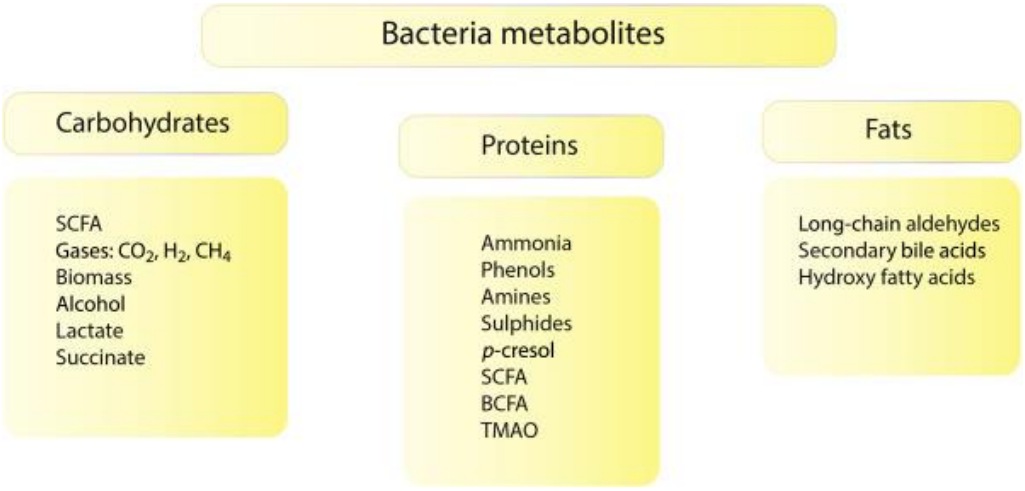


Figure 2: Different metabolites from the colonic fermentation of carbohydrates, proteins and fats

BCFA; branched-chain fatty acid, SCFA; short-chain fatty acid, TMAO; trimethylamine N-oxide. Kashtanova et al. Association between the gut microbiota and diet: Fetal life, early childhood, and further life. *Nutrition,* 2016 [108].

A low FODMAP diet involves restriction of fermentable carbohydrates, which in turn might affect the gastrointestinal health on a long term basis by affecting the bacterial composition [113]. Many colonic bacteria have fermentative abilities and uses fibres and undigested FODMAPs and proteins as their main source of energy. This fermentation results in metabolites such as SCFA, gases, ammonia and amines. The bacteria also participate in the production of vitamins and lignans. A reduction in indigestible carbohydrates may lead to a decrease in these bacteria and their fermentation products [114]. The different SCFA

produced function as a source of energy for the colonocytes, as signalling molecules in the lipid and glucose metabolism, have anti-inflammatory effects, and can affect gut motility [101]. SCFA also stimulate colonic salt and water absorption, directly and indirectly by expression of transporters [52].

There are done some studies on low FODMAP diet and its effect on the microbiota. Staudacher et al. found a significant reduction in *Bifidobacteria*, but no change in the SCFA as an effect of the low FODMAP diet [114]. A lower absolute abundance of total bacteria was found by Halmos et al. This involved less butyrate producing bacteria and less *Akkermansia muciniphila* compared to an Australian diet. This study did not find the same decrease in *Bifidobacteria* [115]. A recent study on a low FODMAP diet in patients with inactive Crohn's disease did not find a change in bacterial abundance or *Bifidobacteria*, but more butyrate producing bacteria and *Akkermansia muciniphila* and less *Ruminicoccus torques* on an Australian diet compared to a LFD [116]. Also McIntosh et al. concluded that low FODMAP diet could affect the microbiota long term [86]. A study by Chumpitazi et al. found that gut microbiota may predict response to the low FODMAP diet in children with IBS, as responders of the diet had more saccharolytic baseline bacteria from different taxonomic levels, such as *Bacteroides, Ruminococcacae* and *Dorea* [117]. There are not done any studies on the long term effect on microbiota and the LFD, so we do not know whether an effect on the microbiota persist after reintroduction of FODMAPs.

1.4.2 Methods for studying microbiota

Before the advancement of today's DNA techniques, many studies on gut microbiota have been based on culture-dependent techniques. Culturing bacteria has its advantages, as it allows us to study live bacteria and their physiological properties, and it is also a cheap and reproducible technique. However, a major disadvantage is the fact that somewhere between 40-90% of our gut bacteria cannot be cultivated in the lab, making it an insufficient technique. Still, it has brought a lot of knowledge on the importance of gut microbiota [118, 119].

Culture-independent techniques have been developed, using DNA sequencing in order to identify bacteria. This is called "high-throughput sequencing", and even newer methods are referred to as "next-generation sequencing" [120]. In the majority of these techniques, the 16S ribosomalRNA (16S rRNA) gene is used to identify and classify bacteria. This rather small gene is found in all bacteria, and their 16S rRNA sequence allows us to differentiate between

species and strains [119]. These molecular methods have given us a lot more information on gut microbiota, especially the diversity of it, and allows us to determine sequences quickly and at low costs. There are now many different high throughput sequencing technologies and there are constantly new developments [118-120].

1.4.3 Dysbiosis

Dysbiosis can be defined as any change in the bacterial composition compared to that found in a healthy population. It can involve loss of diversity, increased growth of potential pathogens and loss of beneficial bacteria [121]. Due to many inter-individual differences in the microbiota, it is not quite clear what can be determined as a healthy microbiota. Differences in diet, age, location, method of analysis and other environmental factors in studies have also made it more difficult to identify which species characterize a healthy microbiota [101].

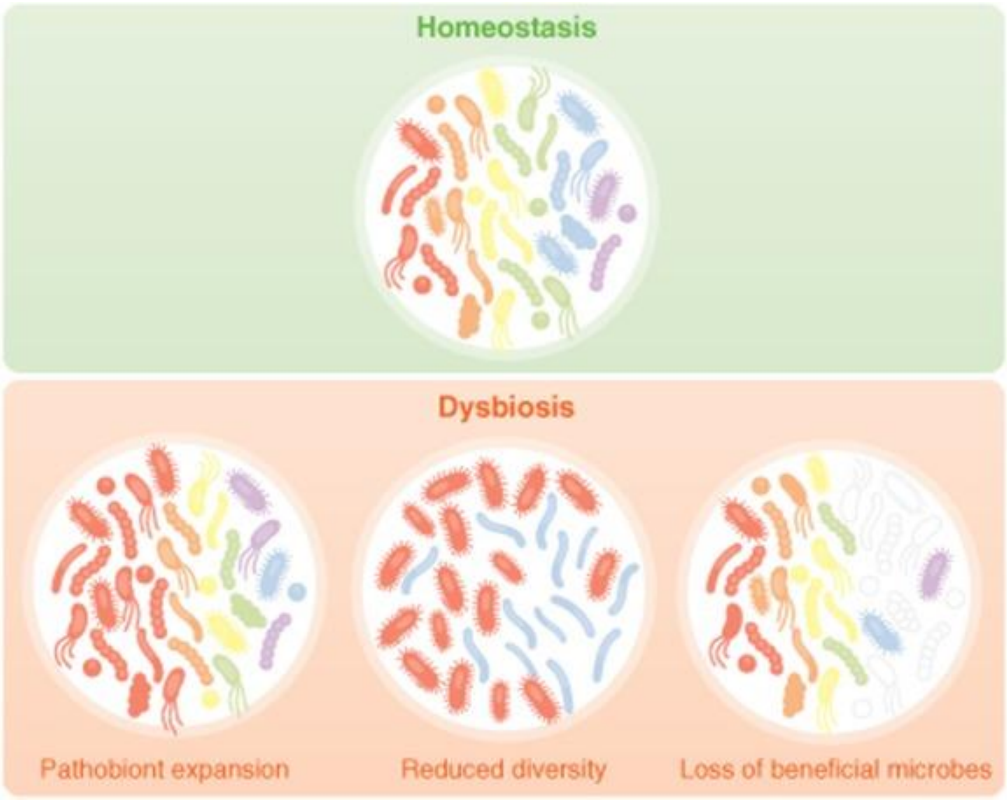


Figure 3: Illustration of dysbiosis

Homoeostasis of the gut microbiota can be disrupted by expansion of pathogens, a reduction in the diversity of microbes or loss of beneficial microbes. This state is called dysbiosis.
Petersen et al., Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol. 2014* [121].

Dysbiosis have been seen in different diseases and conditions, such as coeliac disease, inflammatory bowel disease (IBD), IBS, type I diabetes, obesity, asthma and allergy [101]. It is thought that the change seen in the microbiota might be involved in either the development or the persistence of diseases, but it is hard to determine whether it is a cause or a consequence of the disease.

The intestinal microbiota in IBS has been found to differ from that in healthy populations [122-125]. Microbiota has been linked to the pathogenesis of IBS because treatment approaches towards microbiota have shown results and because bacterial and viral infections have caused IBS [123]. Also possible pathogenic mechanisms in IBS such as low grade inflammation, immune and gut barrier dysfunction can be linked to the gut microbiota [126]. However, there is not found a specific microbiota signature amongst IBS patients.

There is also detected dysbiosis in individuals with coeliac disease both in children and adults in faecal and duodenal samples, but there is not found any clear bacterial patterns in these patients [127-129]. There does seem to be a higher abundance of *Firmicutes* and *Bacteroidetes*, but there are also contradicting findings [128]. Most studies are done on children with coeliac disease, which might be different from microbiota composition in adults. The role of gut microbiota in coeliac disease is not determined, but it has been linked to gut barrier and immune response, which is central in the CD pathogenesis. There is also data suggesting that GFD might not restore gut microbiota completely, and also that the nature of the diet itself could lead to dysbiosis [129].

1.5 Breath testing

A H_2-breath test is a simple, non-invasive and inexpensive test used for different purposes, such as testing for sugar malabsorption, SIBO and oro-coecal transit time. A breath test measures the amount of hydrogen and methane gas in expired air, which correlates to the amount of gas produced from fermentation by colonic bacteria. It is mainly non-digestible carbohydrates that work as substrates for gas production [130]. Oral administration of the sugars lactulose and glucose is commonly used when testing for SIBO. An early rise in hydrogen reflects fermentation by bacteria in the small intestines and SIBO, defined as an overgrowth of bacteria or abnormal bacteria present in the small intestines [54]. Glucose will be completely absorbed in the small intestines, and a peak reflects fermentation by present

bacteria. Lactulose is a synthetic non-absorbable sugar, and an early peak reflects bacterial fermentation in the small intestines or increased transit time and thus fermentation by colonic bacteria [130]. However, breath tests are not validated, and there have been discussion on error rates, different cut off values and interpretation of the tests [131]. Oral administration of lactose, fructose and sorbitol can be used to determine carbohydrate malabsorption, in example before commencing a low FODMAP diet [79, 130, 132].

Hydrogen breath tests have previously been used in studies on the low-FODMAP diet to determine carbohydrate malabsorption before conducting the diet or to determine degree of fermentation and adherence to the diet [95]. Halmos et al. found that patients following a low-FODMAP diet had significantly less production of hydrogen compared to those following an Australian diet [85]. Ong et al. found the same for a low FODMAP diet compared to a high FODMAP diet [133]. Methane production has shown to be associated with constipation in IBS-patients on several occasions, although not the only cause of constipation [134, 135]. Methane production have also been associated with a slower transit time, possibly explaining its link to constipation [134, 136]. However, there have been some studies not finding the same associations. [137, 138].

1.6 Rationale

The primary aim of this project was to look at the microbiota profile in coeliac disease patients with IBS-like symptoms, and to compare it with an IBS- and a healthy population. We also looked for any change in microbiota and degree of fermentation, as well as effect on IBS-like symptoms after six weeks on diet. Additional aims were to look at baseline microbiota patterns as predictor for diet response.

Also health-related quality of life was assessed in this study. Quality of life and symptoms are thoroughly presented and discussed in another Master thesis by Kamilla Nuland, 2016.

2 SUBJECTS AND METHODS

2.1 Subjects

To recruit patients we asked the question "Do you have coeliac disease, follow a gluten free diet, but still suffer from bothersome abdominal symptoms?"

We only wanted to include patients with a confirmed coeliac disease diagnosis, which entails positive intestinal biopsy results. It was also important that the patients had followed a GFD properly, and six months was considered as a long enough period to ensure this.

An age limit of 18-60 years was set. We did not want to include patients under 18 years of age, as this study did not have a paediatric focus. An upper age limit of 60 years was set in order to ensure best possible adherence to the diet. The intervention requires time, motivation and the ability to study and understand the diet, and individuals under the age of 60 years were considered more capable of following this intervention. Two of the patients included were over 60 years, but were considered very motivated and capable of doing what was required in the study.

Table 2: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
- Confirmed coeliac disease diagnosis for at least 6 months - IBS-symptoms confirmed by the Rome III-criteria - Score >75 on the IBS-Symptom Severity Scale (IBS-SSS) - Subjects between 18-60 years of age	- Subjects with therapy-resistant coeliac disease - Recent biopsy with abnormal findings - Already following a low FODMAP diet

2.2 Recruitment

Patients were mainly recruited through announcements of the study on relevant Facebook pages and web sites, amongst previous and upcoming patients of the Polyclinic for coeliac

disease at Haukeland University Hospital and amongst participants of coeliac disease or IBS education courses organized by the hospital. Newspaper notices, posters and word of mouth were also used. This creates a mixed group of patients both seeking healthcare for their symptoms and some who had not. In total 45 patients came to the first inclusion meeting, in which 44 patients fulfilled the inclusion criteria.

2.3 Randomization

The participants included were randomized through small and variable sized blocks into group A or group B. Block randomization is a technique used in studies with a small number of subjects, when equal sample sizes are desired [139].

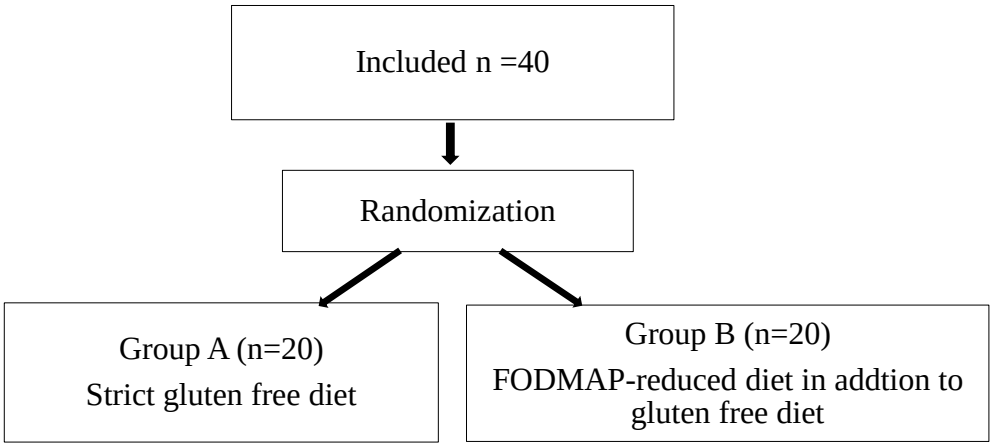


Figure 4: Randomization process

2.4 Intervention

Group A

Group A was assigned to follow a strict gluten free diet for 6 weeks. This involved excluding all wheat starch and trace amounts of gluten in their diet, even found in gluten free products. The patients were also instructed to be thorough with their gluten free diet in general, especially with kitchen hygiene. The patients received a list with an overview over some of the most common gluten free products they had to avoid or could use during the intervention period, as well as a brochure on gluten free diet in general.

Group B

Group B was assigned to follow a low FODMAP diet in addition to their gluten free diet. This involved eating only foods classified as low FODMAP for 6 weeks. The patients were thoroughly instructed in the diet and the scientific background for it before commencing. They also received a booklet with instructions to the diet, of what foods to avoid during the intervention period and alternatives to these foods, which they could use as guidance in the beginning. There were also some recipes in this booklet. They were encouraged to start the diet the very same day as the baseline tests were performed.

Study timeline

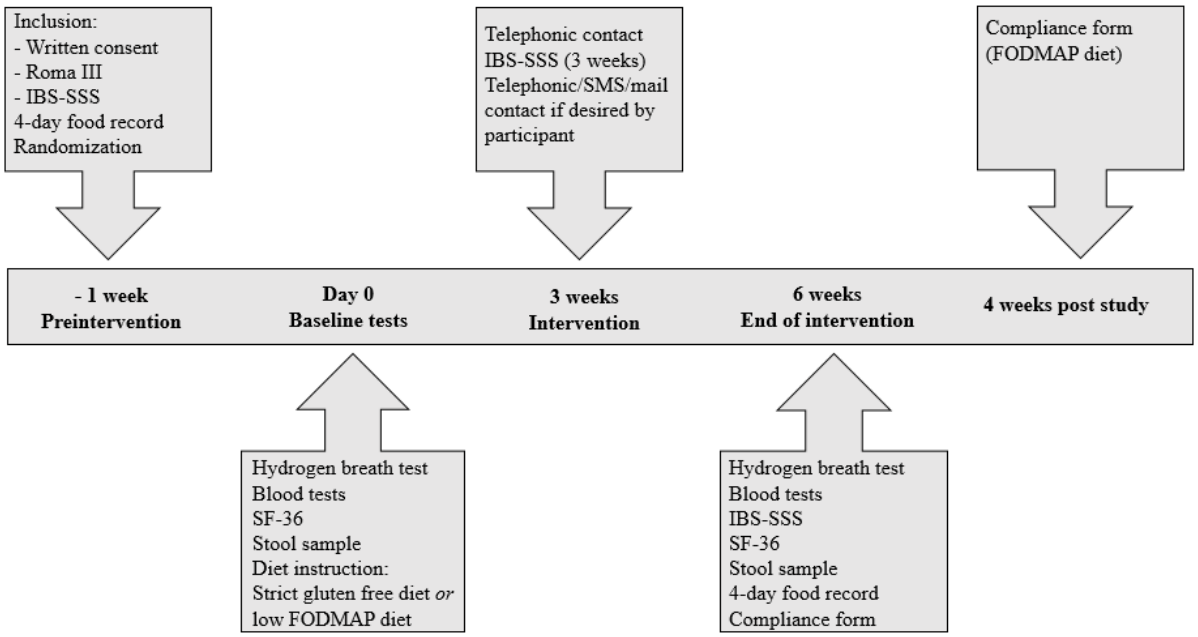


Figure 5: Timeline showing the course of the study

Visit 1: Screening. During the first meeting, the patients received detailed verbal and written information about the study and gave written consent. Following that, the patients filled out the forms IBS-SSS and Rome III to determine whether they were suitable for inclusion. Because of long travel distance, some patients received these forms by mail or e-mail. However, the majority of patients came to Haukeland University Hospital at three different occasions. The patients were informed that they would be randomly assigned into either group A or B.

Visit 2: Intervention start. The patients performed a breathing test and filled out the form SF-36. They also received a thorough introduction to the diet they were to follow for the next six

weeks by a master student in clinical nutrition. The patients were told to start the diet the same day.

After three weeks, all participants filled out the form IBS-SSS for the second time. We had telephonic contact with all participants at this stage, to make sure they filled out the form and were following their diet. It also gave the patients an opportunity to ask any questions they might have and to talk about how they were doing. The participants were encouraged to make contact by e-mail, SMS or by phone if they had questions of any sort during the intervention period.

Visit 3: End of intervention. After six weeks, all patients included came back and took a second breathing test and filled out IBS-SSS and SF-36. A compliance form was also filled out. Additionally, the diets and experiences during the intervention period were verbally evaluated with the patients. All patients in group A were offered an introduction to the FODMAP-diet after the end of intervention. Three patients came back for the last visit about 3 weeks after scheduled time, because travelling was not possible at the scheduled time (n=1), and due to job obligations (n=2). These three patients all followed their assigned diet for three more weeks before coming in to the last visit.

Four weeks after end of intervention, all patients in group B filled out an additional compliance form to evaluate the reintroduction phase of the diet.

Blood tests and stool samples were collected at baseline and after 6 weeks of diet in both groups. The stool samples were stored at -20 Celsius until analysis.

All patients kept a 4-day prospective food record before start of the intervention and at the end of the intervention. They were told to record all intake of foods and drinks for three working days and one weekend day, and to register as detailed as possible. The food records were used to calculate the total intake of FODMAPs before and at the end of the intervention, in addition energy, carbohydrate, fibre, fat, protein and calcium intake.

2.5 Questionnaires

2.5.1 Rome III-criteria

The Rome III diagnostic criteria for irritable bowel syndrome was used to confirm IBS-like symptoms. They were the following:

"Recurrent abdominal pain or discomfort at least 3 days a month in the past 3 months, associated with two or more of the following:

- Improvement with defecation
- Onset associated with a change in frequency stool
- Onset associated with a change in form (appearance) of stool

Symptom onset greater than 6 months prior to the diagnosis, with the above criteria fulfilled for the past 3 months" [140].

2.5.2 IBS-SSS

Severity of IBS-like symptoms were measured using the standardized and validated IBS-SSS [141]. The form has five questions concerning abdominal pain, distension, bowel habits and IBS' impact on life in general, where a visual analogue scale (VAS-scale) ranging from 0 – 100 mm is used for scoring. A score of 0 implies satisfaction and a score of 100 dissatisfaction. One question asks for number of days with abdominal pain from 0-10; this question is multiplied by 10 to give a score from 0-100, similar to the other four questions. Together, they create a score from 0-500, that classify IBS in remission (≤75), mild IBS (75-175), moderate IBS (175-300) or severe IBS (>300). A reduction of ≥ 50 is considered a successful improvement [141]. Using the score from IBS-SSS at baseline, three weeks and after the intervention allows assessing change over a short period of time. The form also has ten supplementary questions concerning additional symptoms that can be seen in IBS, also scored 0-100 on a VAS-scale.

2.5.3 SF-36

To measure health-related quality of life, the questionnaire Short Form Survey (SF-36) was used. This form includes 36 questions divided into 8 subcategories; physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), general health perceptions (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE) and mental health (MH). These subcategories can be summarized into two main categories:

Physical component summary score (PCS) and mental component summary score (MCS). The higher score, the better health-related quality of life. The undercategories contribute to the main scores in varying degrees, where some categories are more impontant for MCS and vice versa, but there is a correlation between all the eight subcategories [142, 143].

2.5.4 Dietary compliance during intervention

Adherence to the diet during the intervention period was assessed in both groups using compliance forms which were filled out at the end of study for both group A and B. This forms included a combination of VAS-scales and questions with answer options. This included questions about satisfaction, self-reported compliance and satisfaction with diet instructions given in forehand.

2.5.5 Dietary compliance 4 weeks after end of study

Group B also received a compliance form to fill out 4 weeks after end of study, with questions on whether the patients were still following the diet and whether they had started the reintroduction of different FODMAPs.

2.6 Gut microbiota analysis

The faecal samples were analysed by Genetic Analysis AS (Genetic Analysis AS, Nydalen, 0401 OSLO, NORWAY) using the GA-map™ Dysbiosis Test (GA-map Dysbiosis Test, Genetic Analysis AS, Oslo, Norway), a novel test specifically developed to identify and define clinical dysbiosis. It is a high throughput test that uses 54 probes to target variable regions (V3-V9) within the 16S rRNA gene, where signals and signal strength from these probes are used to characterize a microbiota profile, as well as a Dysbiosis Index (DI). The 16S rRNA gene is used because it is unique for bacteria. DNA is isolated form faecal samples and amplified using an 1180 basepar primer and PCR, and then labelled with probes. Signals from the probes are then identified and quantified. The probes used can discover bacteria from the phyla: *Firmcutes*, *Proteobacteria*, *Bacteriodetes*, *Actinobacteria*, *Tenericutes* and *Verrucomicrobia*, 10 bacterial classes and 36 genera. DNA of some bacteria is easier amplified than others, thus we cannot compare probe signals directly with each other. An algorithm is used to assess bacterial abundance and deviation in the microbiota from normobiosis, which results in a Dysbiosis Index. Dysbiosis Index from 1-5 is used to present the degree of dysbiosis, where a DI > 2 is defined as a clinical relevant dysbiosis. The higher

the index is above 2, the more it differs from the defined normobiosis, and DI-score of 5 has no defined limit. The test is based on faecal samples from a Scandinavian reference population of 211 healthy individuals to determine a normobiotic profile. After analysis, a DI-score and a microbiota profile was given for each patient before and after the intervention. The specific bacteria presented were chosen by Genetic Analysis AS due to their relevance for dysbiosis and gastrointestinal diseases.

The validation cohort for this test included faecal samples from healthy (n=43) individuals and IBS (n=109) and IBD (n=135) patients from a Scandinavian population. Amongst the healthy individuals, 16% were dysbiotic versus 73% in the IBS cohort and 74% in the IBD cohort. The healthy and the IBS cohort serve as controls in this study when looking at dysbiosis index, and the healthy cohort serves as control when looking at microbiota profile. The GA-map test is compared to Illumina deep sequencing and found strong correlations in detecting many bacteria and accordance in determining dysbiosis [122].

2.7 Hydrogen breath test

A breath test was performed in both groups A and B at baseline and after the end of study. The breath tests were collected using the AlveoSampler^{TM} collection kit and analysed using Quintron Model SC MicroLyzer (Quintron Instrument Company, Milwaukee, Winsconsin, USA). The instrument measures the amount of hydrogen, methane and carbon dioxide in the inserted breath sample. We performed a 60-minute breath test, measuring breath at 0, 15, 30, 45 and 60 minutes. The patients were told to inhale, and exhale instantly into the collection bag. When they felt they were nearly out of breath, they gave a sign so we could collect the alveolar air. The instrument uses a correction factor to give a truer estimate of alveolar air, due to possible sampling errors and contamination of room air into the sample. The level of CO_2 in alveolar lies stable at 5,5%, and this value is used to normalize the hydrogen and methane values so they are more accurate to those in alveolar air. The rationale behind the breath test is the assumption that the amount of breath hydrogen correlates to the amount of gas produced by colonic bacteria [130]. The gases produced in the intestines will diffuse from the intestinal lumen to the circulation, be exhaled from the lungs and quantified in the breath. In this study we measured the degree of fermentation based on the patients' habitual diet and after 6 weeks of assigned diets. The patients had fasted for at least 10 hours before the test

was performed. The total hydrogen and methane gas production was found by calculating area-under-the-curve (AUC) using the trapezium rule and given in parts per million (ppm).

2.8 4-day dietary registration

The dietary registrations were analysed using Kostholdsplanleggeren, an online application designed to calculate nutrient intake (Kostholdsplanleggeren 2014. Mattilsynet og Helsedirektoratet. www.kostholdsplanleggeren.no). In order to calculate FODMAP-content, we used published data on the subject. We preferably used Norwegian data, but substituted with Danish and Australian data where needed. The majority of published data on FODMAP-content per today is Australian, and accounted for most of the data we used. [83, 144] All data on lactose content was Norwegian and collected from Opplysningskontoret for Meieriprodukter [145]. We created our own FODMAP database using the free computer software Dietist Net Gratis (Kost och Näringsdata, Bromma, Sweden) and Fabrikanttabellen,, where we added all published data for total FODMAP-content for different foods. For composite dishes, we used standard recipes from the website www.matprat.no, a website containing a collection of food recipes created by Opplysningskontoret for egg og kjøtt, to calculate FODMAP-content and other nutrients. Standard portions from Kostholdsplanleggeren were used. We highlighted the sources of FODMAP in the recipe, and calculated total FODMAP-content, which we divided into lactose and non-lactose (referring to the amount of fructose, fructans, galactans and polyols).

The cut off values in gram per portion for the different FODMAPs are [146]:

Oligosaccharides (grains, legumes, nuts and seeds):	<0.3
Oligosaccharides (vegetables, fruit and other products):	<0.2
Polyols: sorbitol or mannitol	<0.2
Total amount of polyols	<0.4
Excess fructose	<0.15
Lactose	<1

2.9 Blood tests

A coeliac disease panel were taken at baseline and after end of intervention. The panel included IgA TTG, IgG DPG, Hb, Ferritin, Calcium, Vitamin D, Vitamin B12 and others. The

tests and analysis were performed by the Laboratory of Clinical Biochemistry at Haukeland University Hospital.

2.10 Ethical considerations

The study was approved by REC Sør-Øst (regional committee for medical and health research ethics, Sør-Øst) in June, 2015. All participants gave written consent, and a copy was held both by the participant and a research study assistant. All personal data was handled in a confidential manner. The study was voluntarily and the participants could withdraw from the study at any point without providing any justification. There was no risk of harm in this study.

2.11 Economics

Travel costs, including public transportation and parking fees, were covered for all participants. Accommodation was covered for participants travelling from other parts of Norway.

2.12 Statistical analysis

All data was plotted into Microsoft Excel ® in order to create a database. GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com, was used to perform statistical analysis.

D'Agostino & Pearson omnibus normality test was used to test for data normality. Data were presented as mean ± SD, or as median with IQR (interquartile range) when not following a normal distribution. Paired t-test was used to compare means of two sets of data, and an unpaired t-test to compare means of two groups. If data was not following a normal distribution, Wilcoxon matched-pairs signed rank and Mann-Whitney U test was used, respectively. For categorical variables, a chi-square test was performed or a Fischer's exact test if not following a normal distribution. Repeated measures one-way ANOVA was used to compare IBS-SSS-scores at baseline, three weeks and six weeks, followed by Tukey's multiple comparisons test. Here, Friedman test was used if the data was not following a normal distribution. A p-value of 0.05 or less was considered significant.
*$P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

3 RESULTS

3.1 Recruitment

The majority of patients were recruited through advertising on the Norwegian Coeliac Disease Society's web page and their Facebook pages. A large group was also recruited after we made contact because they had been or were going to the Polyclinic for coeliac disease or the coeliac disease or IBS education course organized by the hospital. Newspaper notices, posters and word of mouth were also means used to recruit patients.

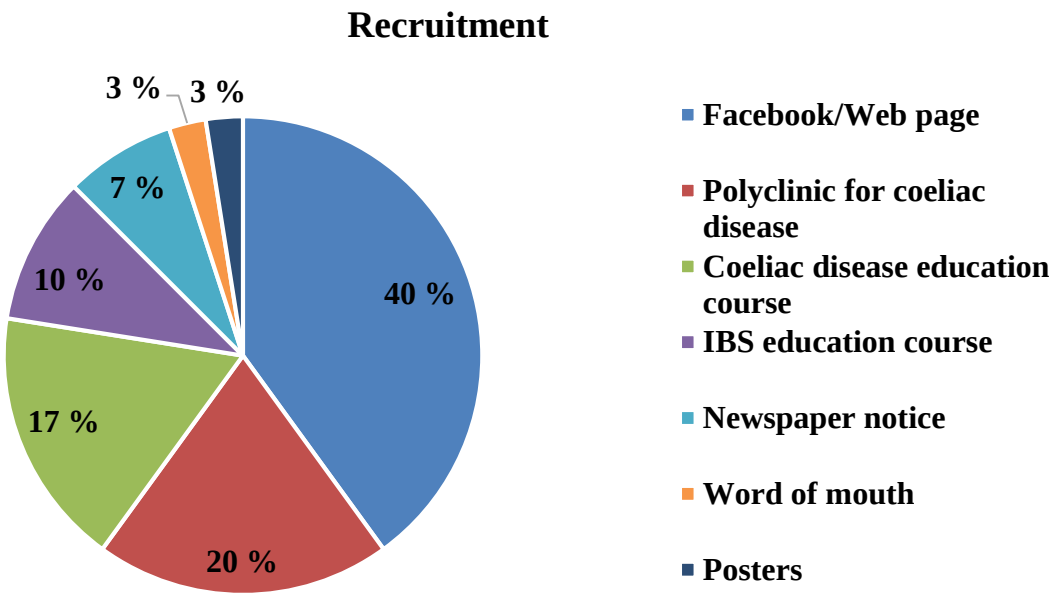


Figure 6: How patients were recruited to the study

In total four patients dropped out of the study. The reasons for withdrawal was lack of time and motivation (n=2) and wish to be in the other intervention group (n=1). These patients were excluded before intervention start. One patient from group B was excluded after three weeks because the diet had purposely not been followed. 40 patients in total completed the study.

3.2 Demographics

Baseline demographics for the whole study population show a majority of women and a mean age of 41 years. The majority of patients had IBS SSS scores equivalent to moderate IBS and more patients had constipation as their predominant stool pattern.

Table 3: Demographics for the total study population (n=40) at baseline

Included (n)	40
Female/male (n)	33/7
Age, mean ± SD	41 ± 14
BMI (kg/m^2), median (IQR)	23.3 (21-26,2)
IBS-C, IBS-D, IBS-M, n, (%)	17 (42.5), 11 (27.5), 12 (30)
IBS-SSS, mean ± SD	261.5 ± 79.2
IBS-subtype; remission, mild, moderate, severe, n (%)	0, 6 (15), 22 (55), 12 (30)
Dysbiosis index, mean ± SD	2.48 ± 1.09

Table 4 show that there were no statistically significant differences between group A and B in baseline characteristics.

Table 4: Demographics and p-values for group A and group B at baseline

	Group A (n=20)	**Group B (n=20)**	**P-value**
Females, n (%)	18 (90)	15 (75)	0.408
Age, mean ± SD	39 ± 15.1	43 ± 11.7	0.308
BMI (kg/m^2), median (IQR)	23 (21.5-27.3)	23.3 (20.3-26.1)	0.654
IBS-C, n (%)	9 (45)	8 (40)	0.331
IBS-D, n (%)	7 (35)	4 (20)	
IBS-M, n (%)	4 (20)	8 (40)	
IBS-SSS, mean ± SD	259.8 ± 89.6	263.3 ± 69.5	0.889
Dysbiosis Index	2.45 ± 0.94	2.50 ± 1.24	0.947
Total FODMAP, g, median (IQR)	14.5 (10.9-21.6)	11.5 ± 9.4	0.079
Hydrogen, ppm, median (IQR)	423.8 (165.0-1223)	266.3 (64.6-879.4)	0.291
Positive IgA TTG (>14,9 U/mL), n (%)	5 (25%)	1 (0.5%)	0.182
Positive IgG DPG (>14,9 U/mL), n (%)	2 (10%)	3 (15%)	1.000

Data are shown in n (%), mean ± SD and median (IQR).

Unpaired t-test (age, IBS-SSS), Mann Withney U test (total FODMAP, hydrogen, Dysbiosis index, BMI), Chi square test (IBS subtype), Fischer's exact test (IgA TTG, IgG DPG, gender).

3.3 Diet intervention

The diet intervention in group B was successful, with a total FODMAP-reduction from 11.5 to 1.6g (p=0.0001). There was no significant reduction in total FODMAP intake in group A (p=0.5217).

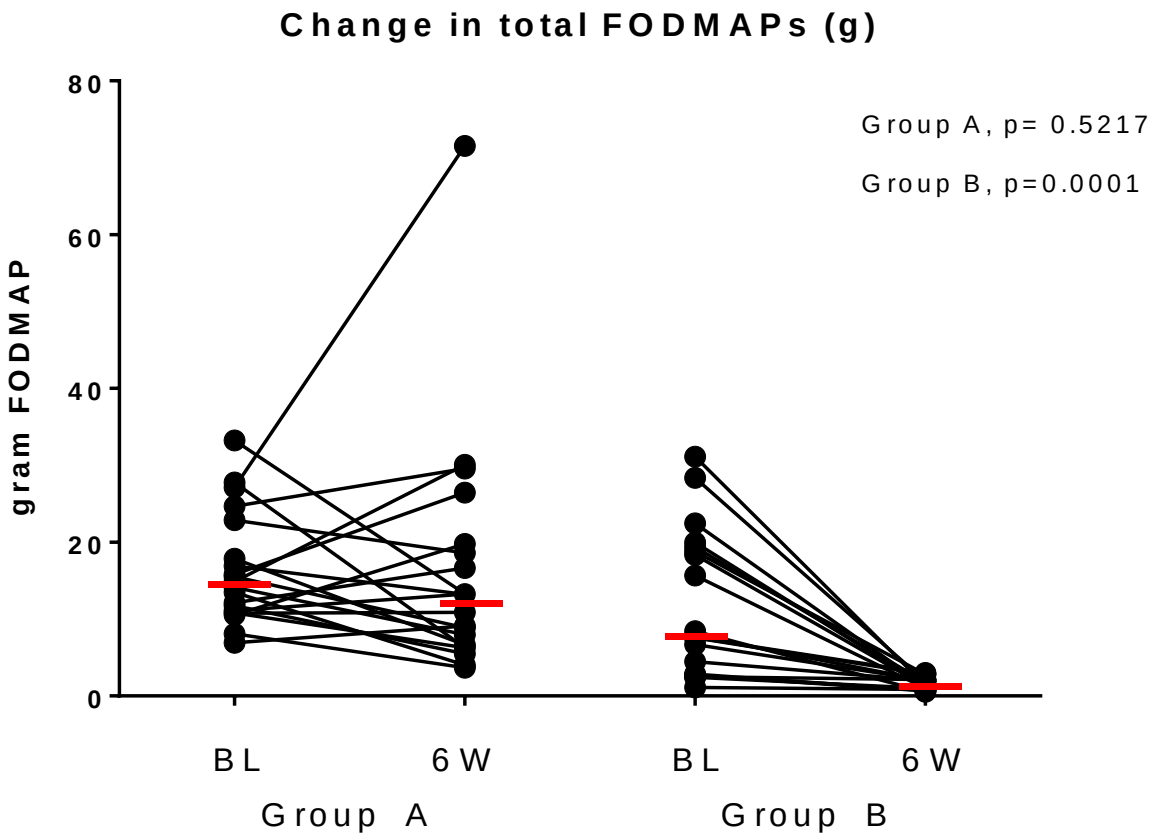


Figure 7: Change in total FODMAP intake for each individual from baseline (BL) to six weeks (6W) in grams

Red line = median.

Wilcoxon matched-pairs signed-rank test in group A and paired T-test in group B.

The majority of the total FODMAP intake in group B was lactose, whilst other FODMAPs accounted for a smaller part.

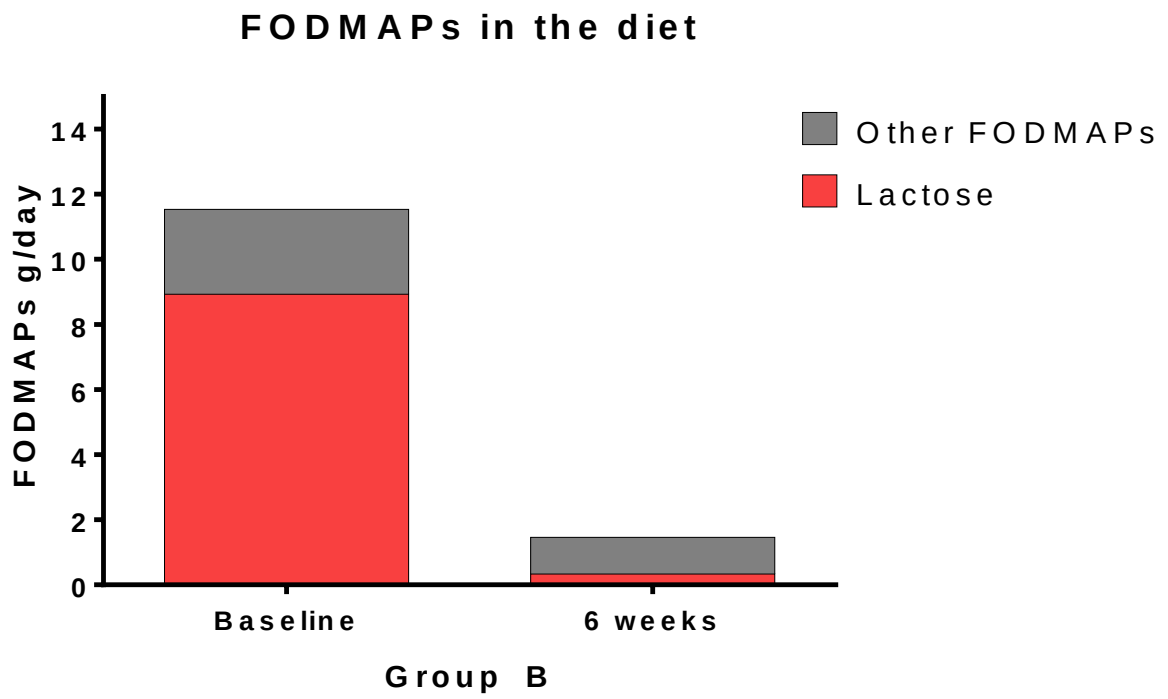


Figure 8: Mean intake of total FODMAPs in grams at baseline and 6 weeks in group B, divided into lactose and other FODMAPs (fructans, galactans, lactose, fructose and polyols)

There was a significant reduction in intake of energy, fat and total FODMAPs (including lactose and other FODMAPs) in group B after six weeks of a low FODMAP diet.

Table 5: Dietary values for group B at baseline and 6 weeks based on 4-day food records. **Data are given in mean daily intake ± SD and median (IQR). P-values and significance levels are shown**

Group B	Baseline	6 weeks	P-value
Energy, kcal	2043 ± 492	1829 ± 446	0.011*
Carbohydrates (incl. fibre), g	227.0 ± 57.3	205.5 ± 72.0	0.063
Dietary fibre, g	20.7 ± 6.4	18.8 ± 8.6	0.101
Fat, g	93 ± 27.1	76.2 ± 20.2	0.012*
Protein, g	89.2 ± 27.3	82.1 ± 20.5	0.213
Calcium, mg	842 ± 377	849 ± 292	0.902
Total FODMAP, g	11.5 ± 9.4	1.6 ± 0.8	0.0001****
Lactose, g	4.7 (1.2-16.4)	0.1 (0.04-0.3)	<0.0001****
Other FODMAPs, g	2.6 ± 1.1	1.1 ± 0.7	<0.0001****

Paired t-test and Wilcoxon signed rank test

There was no significant change in intake of FODMAP in group A. There was a significant difference in fat and protein intake after six weeks of strict gluten free diet.

Table 6: Dietary values for group A at baseline and 6 weeks based on 4-day food records. Data are given in mean daily intake ± SD and median (IQR). P-values and significance levels are shown

Group A	Baseline	6 weeks	P-value
Energy, kcal	2051 ± 609	1790 ± 503	0.119
Carbohydrates (incl. fibre), g	269.4 ± 94.9	230.4 ± 75.7	0.127
Dietary fibre, g	18.8 ± 80.6	16.5 ± 5.1	0.0975
Fat, g	80.6 ± 31.3	64.2 ± 24.4	0.0329*
Protein, g	81.3 ± 30.7	70.67 ± 21.2	0.0483*
Calcium, mg	802 (587-1109)	778 (507-986)	0.231
Total FODMAP, g	14.5 (10.9-21.6)	12.1 (6.4-19.5)	0.522
Lactose, g	11.3 (7.6-17.3)	10.3 (4.8-15.4)	0.784
Other FODMAPs, g	3.1 (1.8-5.5)	3.2 (1.8-8.3)	0.202

Paired t-test and Wilcoxon signed rank test

After six weeks, there was a significant difference between group A and B in amount of FODMAP (p=<0.0001), lactose (p=<0.0001) and other FODMAPs (p=0.0299) in the diet. There were no differences in the daily intake of energy, carbohydrates (incl. fibre), dietary fibre, fat, protein or calcium between group A and B at baseline or after six weeks.

Table 7: P-values for diet comparison in group A and B

Group A vs. Group B	Baseline, p.value	6 weeks, p-value
Energy, kcal	0.960	0.796
Carbohydrates (incl. fibre), g	0.096	0.294
Dietary fibre, g	0.372	0.325
Fat, g	0.188	0.0987
Protein, g	0.394	0.0911
Calcium, mg	0.698	0.369
Total FODMAP, g	0.0786	<0.0001****
Lactose, g	0.150	<0.0001****
Other FODMAPs, g	0.1	0.0299*

Unpaired t-test and Mann-Whitney U test

3.4 Microbiota

3.4.1 Dysbiosis Index

Dysbiosis was defined by a Dysbiosis Index determined by the detection of bacteria and bacterial abundance through 54 probes, where a DI-score >2 equalled dysbiosis. The degree of dysbiosis was compared to a healthy cohort and an IBS cohort [122].

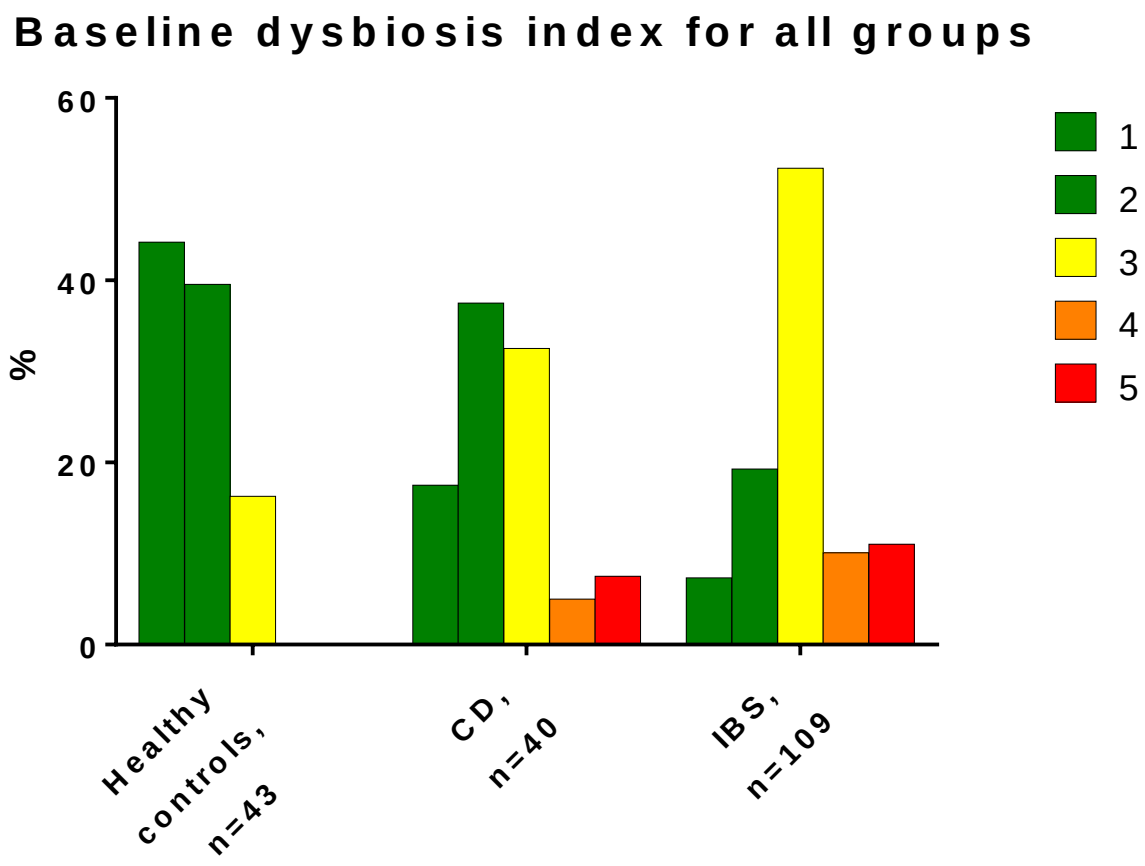


Figure 9: Distribution of DI-score (1-5) in a healthy cohort, study cohort of patients with coeliac disease and an IBS cohort

In our study group, 45% of the patients were dysbiotic at baseline. In comparison, 16% were dysbiotic in the healthy controls and 73% in the IBS cohort. The majority of the CD patients with IBS-like symptoms had mild dysbiosis (DI = 3).

Table 8: Distribution of Dysbiosis Index scores in the study population (CD) compared to healthy controls and an IBS cohort

DI-score	Healthy controls, n	Coeliac disease (CD), n	IBS, n
1	19	7	8
2	17	15	21
3	7	13	57
4	0	2	11
5	0	3	12

Our patient group had significantly different degree of dysbiosis from both the healthy population (p=0.0007) and the IBS-population (p=0.0091).

Table 9: Mean DI scores for all cohorts. P-values are given for the difference between the study cohort versus the other cohorts

Cohort	n	DI, mean	P-value
Healthy	43	1.72	0.0007***
Coeliac disease (CD)	40	2.48	-
IBS	109	2.98	0.0091**

Unpaired t-test (IBS vs. CD) and Mann-Whitney U test (healthy vs. CD)

3.4.2 Dysbiosis and diet intervention

After the diet interventions, the rate of dysbiosis stayed constant in group A, but more patients seemed to have more severe dysbiosis. In group B, more patients seemed to have normobiosis after six weeks on diet.

Table 10: Percentage of individuals in the different DI-groups in group A and B at baseline and after six weeks

	Group A		Group B	
DI group	**Baseline**	**6 weeks**	**Baseline**	**6 weeks**
Normal (1-2)	60 %	60 %	50 %	60 %
Mild (3)	30 %	15 %	35 %	30 %
Severe (4-5)	10 %	25 %	15 %	10 %

Between baseline and six weeks, the mean DI score had increased in group A and decreased in group B. None of the changes in DI score were statistically significant however, with p-values of p=0.852 in group A and p=0.789 in group B. There was not a statistically significant change between the groups after six weeks (p=0.811).

Table 11: Change in DI mean with p-value after six weeks in group A and group B

	Group A			Group B		
	Baseline	**6 weeks**	**p-value**	**Baseline**	**6 weeks**	**p-value**
DI, mean ± SD	2.45 ± 0.94	2.55 ± 1.50	0.852	2.50 ± 1.24	2.45 ± 1.10	0.789

Wilcoxon signed rank test in group A and paired t-test in group B

The individual change in dysbiosis index after six weeks on diet varies a lot after six weeks on diet, as shown in figure 10.

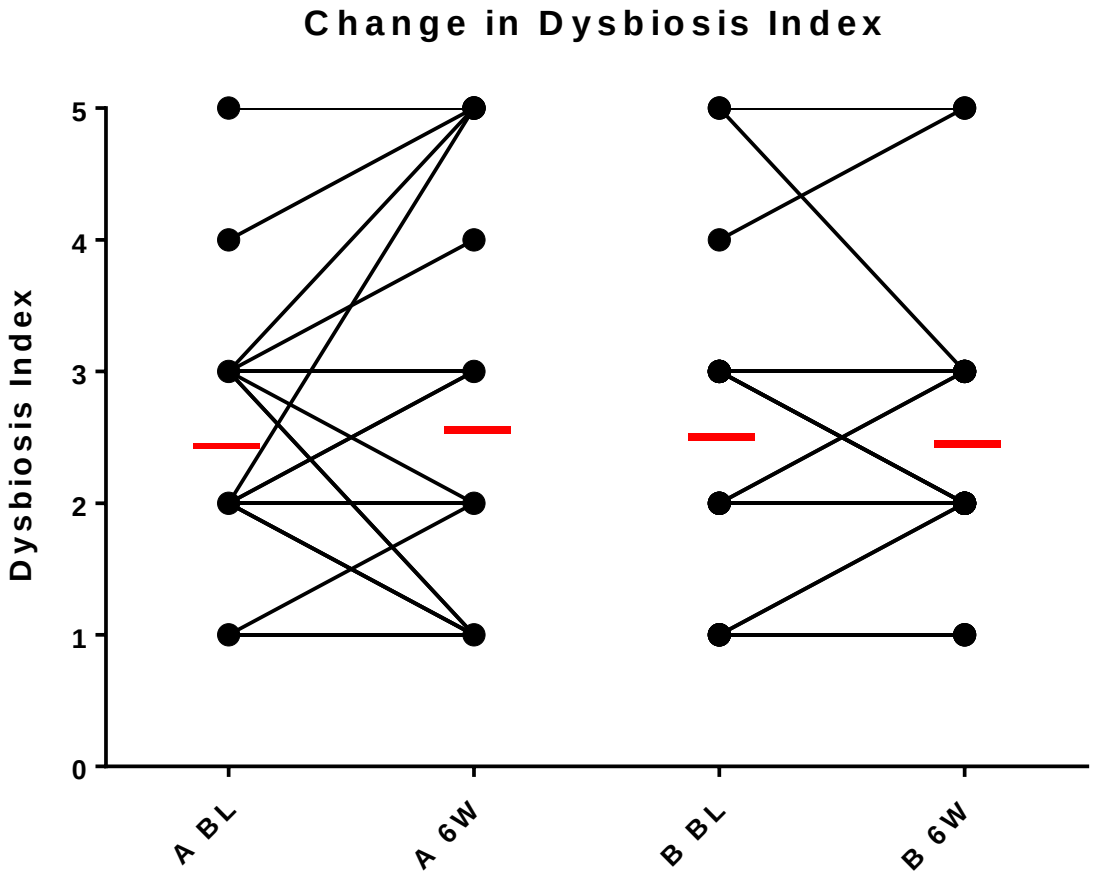


Figure 10: Individual changes in Dysbiosis Index in group A and B from baseline to six weeks

Red line = median

The graph in figure 11 show that group A has a constant rate of dysbiois, but more severe dysbiosis after six weeks of a strict gluten free diet. However, this was not statistically significant.

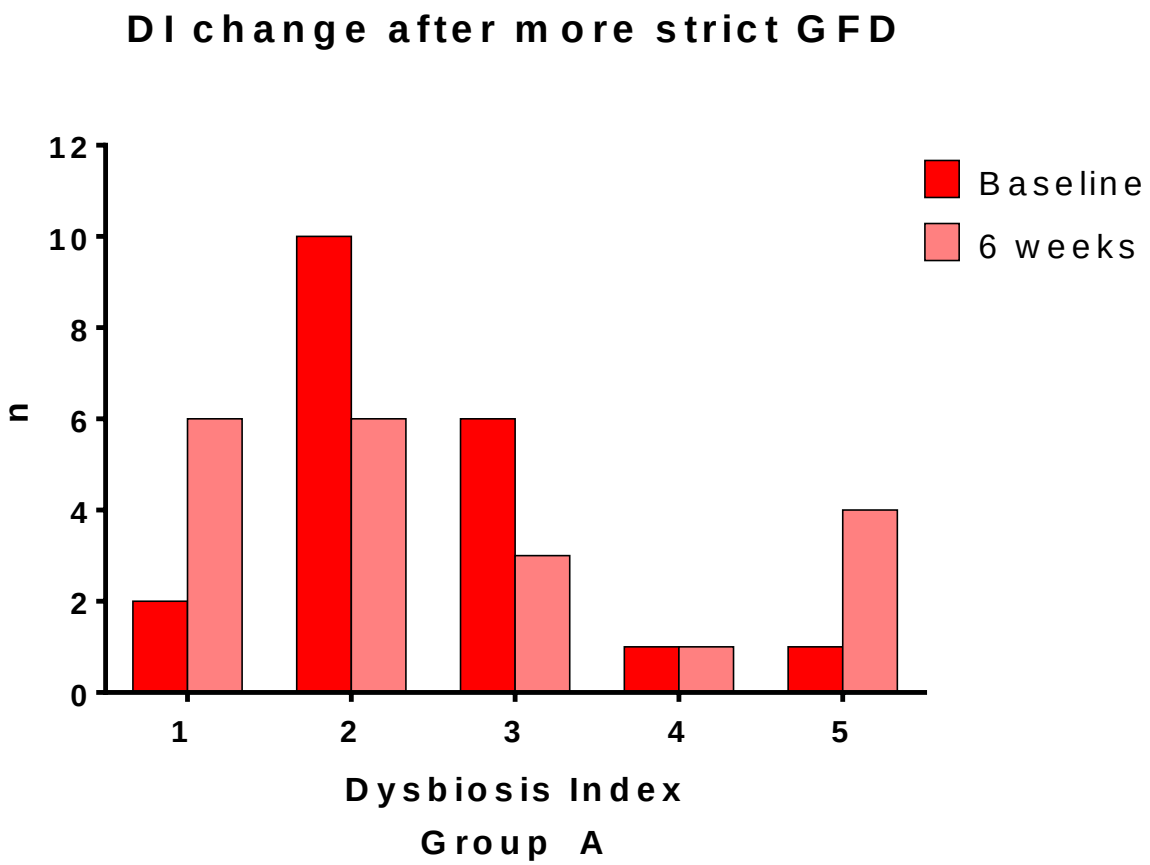


Figure 11: Change in DI-score in group A after six weeks of diet

The graph in figure 12 show that group B moved towards less dysbiosis after six weeks of low FODMAP diet, but it was not a statistically significant change.

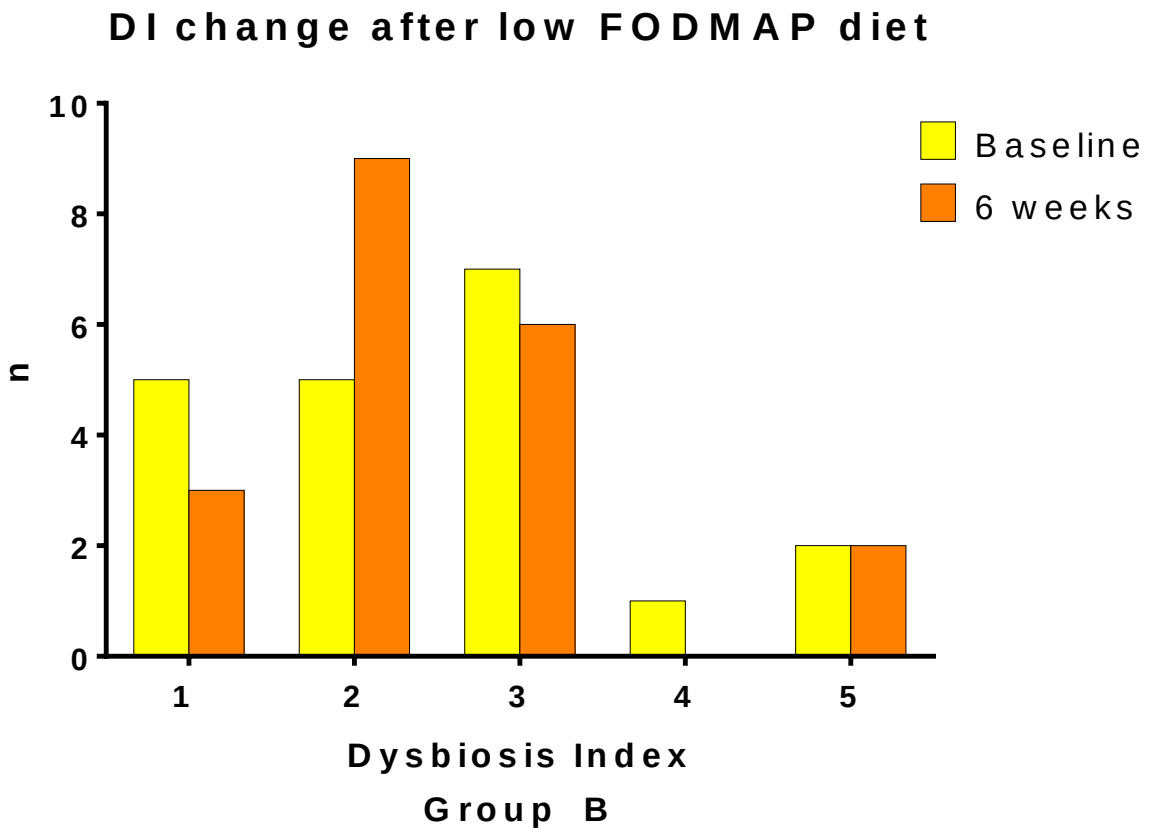


Figure 12: Change in DI score in group B after six weeks of diet

3.4.3 Bacteria at baseline

Several bacterial genera were statistical significantly reduced or increased in the CD patients with IBS-like symptoms compared to the healthy controls, as displayed in the following figures. The figures also display the different diet groups at six weeks, but these figures do not show any change over time and are only used to characterize the baseline microbiota composition. The data are all displayed in figures with mean, median, IQR and confidence intervals. The figures where the 95% confidence interval lie outside the normal mean display statistically significant changes.

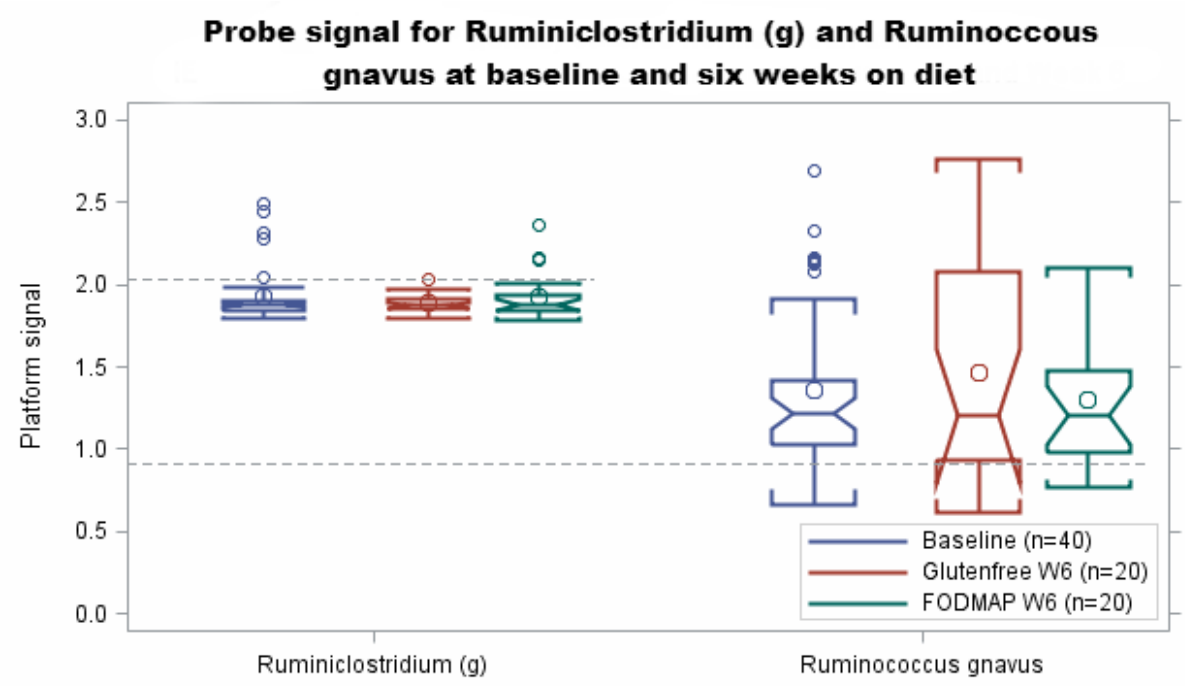


Figure 13: Probe signal for *Ruminiclostridium (genus)* and *Ruminococcus gnavus* at baseline and after six weeks of diets The box displays IQR and the line within the box is the median. The big circle equals mean. The interval shows 95% Confidence Interval. Normal mean (dotted line) equals mean of a healthy cohort. g, genus. The single points outside the 95% confidence intervals are outliers.

The abundance of the genus *Ruminiclostridium* was lower at baseline compared to healthy controls. *Ruminicoccus gnavus* was increased at baseline, but this was not statistically significant as the 95% confidence interval cross the normal mean (dotted line).

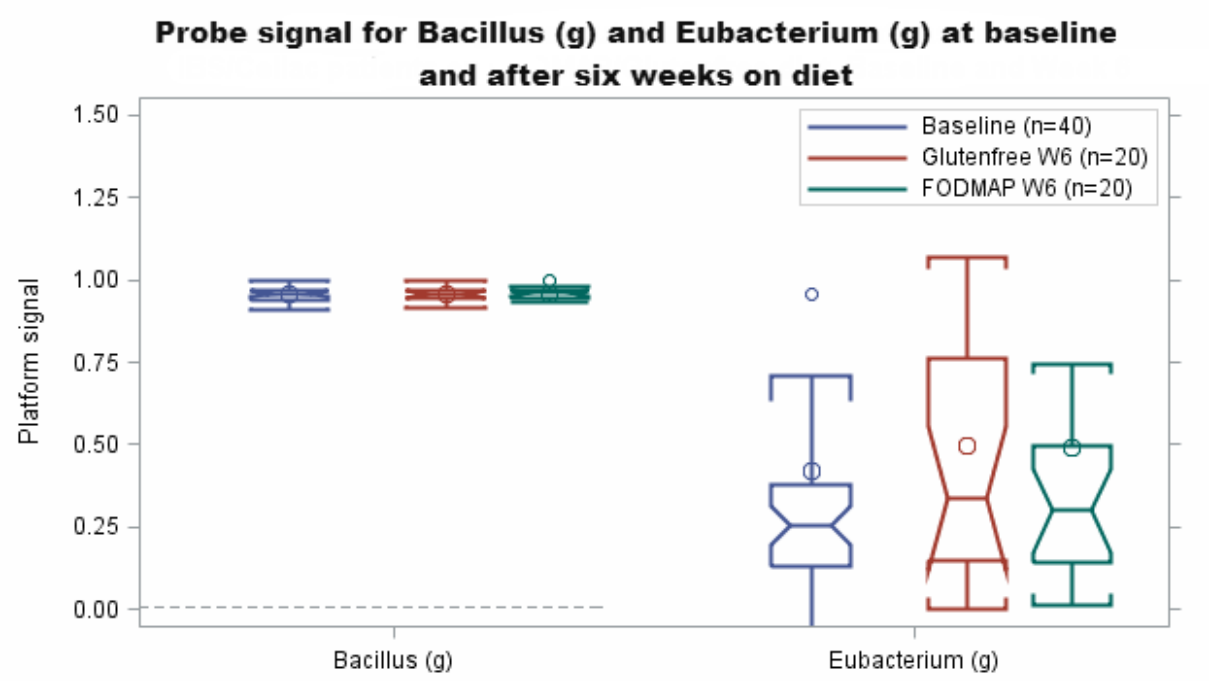


Figure 14: Probe signal for *Bacillus (genus)* and *Eubacterium (genus)* at baseline and after six weeks of diets. The box displays IQR and line within the box is median. The big circle equals mean. The interval shows 95% Confidence Interval. Normal mean (dotted line) equals mean of a healthy cohort. For *Eubacterium (g)*, the normal mean equals 1.6, but this line lies outside the figure. g, genus. The single points outside the 95% confidence intervals are outliers.

Bacillus (genus) had increased values at baseline, whilst *Eubacterium (genus)* was lower than the normal mean. The normal mean for *Eubacterium (genus)* lies outside the figure, but equals 1.6. Both differences were statistically significant.

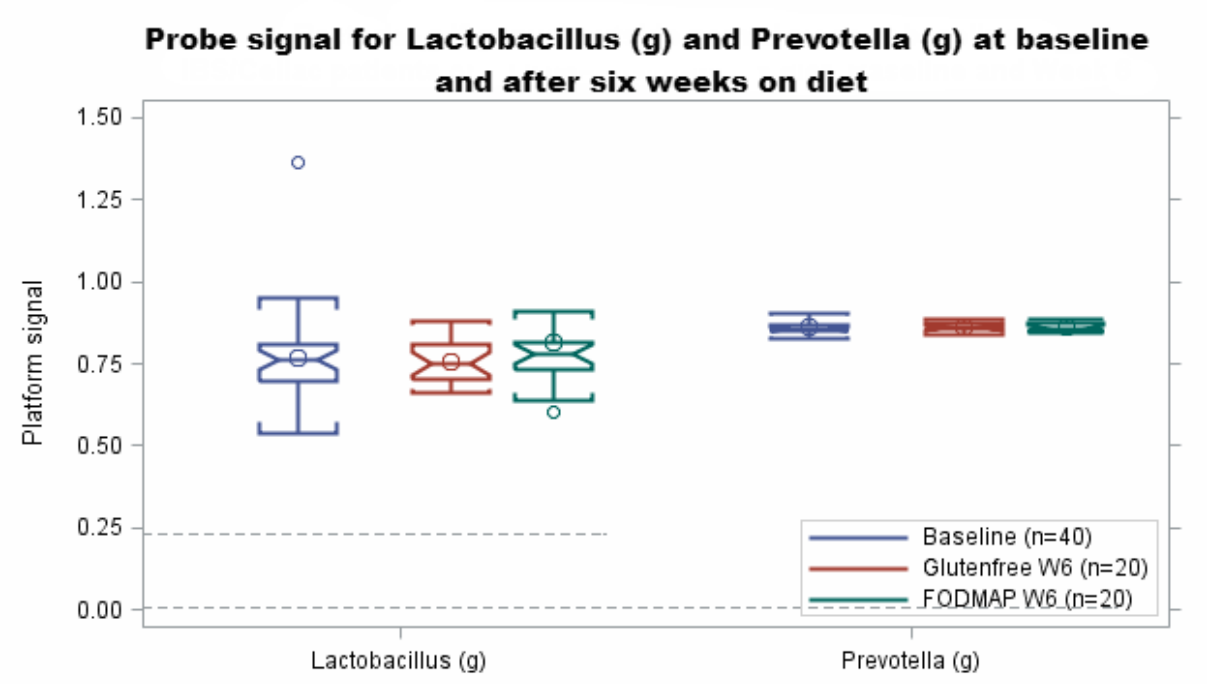


Figure 15: Probe signal for *Lactobacillus (genus)* and *Prevotella (genus)* at baseline and after six weeks of diets. The box displays IQR and the line within the box is the median. The big circle equals mean. The interval shows 95% Confidence Interval. Normal mean (dotted line) equals mean of a healthy cohort. g, genus. The single points outside the 95% confidence intervals are outliers.

There was a higher abundance of both genera *Lactobacillus* and *Prevotella* at baseline compared to a normal mean, as shown in figure 15.

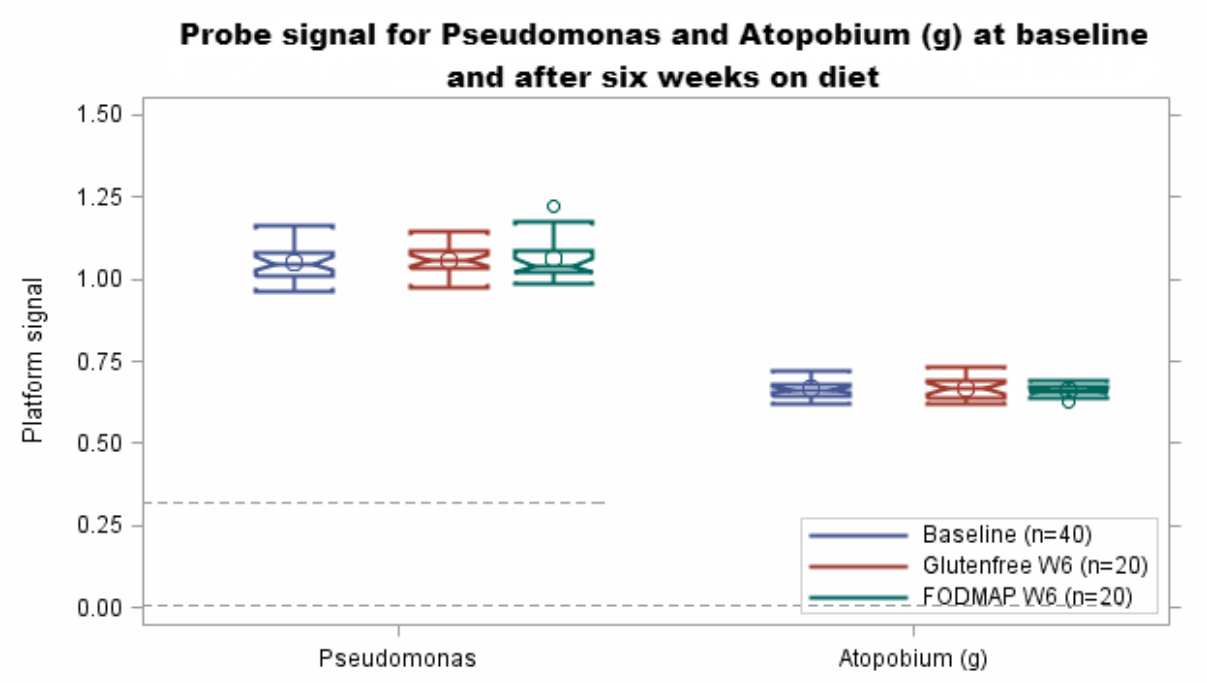


Figure 16: Probe signal for *Pseudomonas* and *Atopobium (genus)* at baseline and after six weeks of diets. The box displays IQR and the line within the box is the median. The big circle equals mean. The interval shows 95% Confidence Interval. Normal mean (dotted line) equals mean of a healthy cohort. g, genus. The single points outside the 95% confidence intervals are outliers.

Pseudonomas, a genus classified under *Proteobacteria*, and the genus *Atopobium*, classified under the phyla *Actinobacteria*, were both increased at baseline.

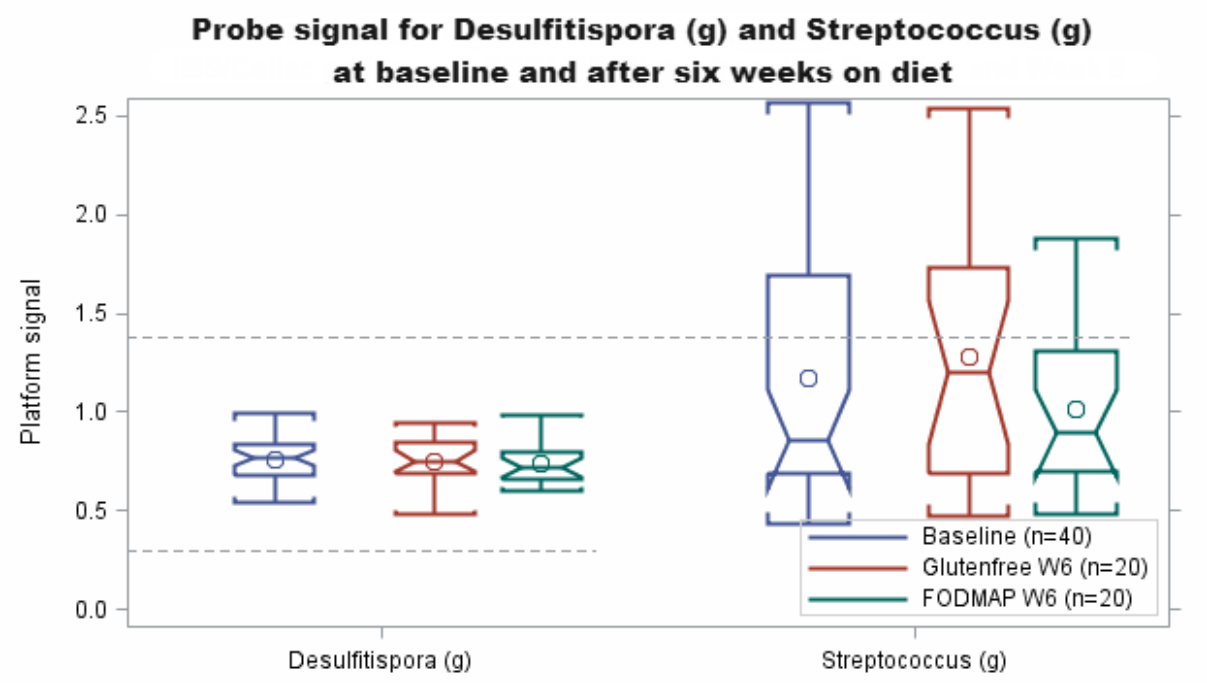


Figure 17: Probe signal for *Desulfitispora (genus)* and *Streptococcus (genus)* at baseline and after six weeks of diets. The box displays IQR and the line within the box is tbe median. The big circle equals mean. The interval shows 95% Confidence Interval. Normal mean (dotted line) equals mean of a healthy cohort. g, genus. The single points outside the 95% confidence intervals are outliers

Desulfitispora (genus) had significantly higher abundance than normal mean at baseline. *Streptococcus (genus)* had lower abundance than normal mean at baseline, but this was not statistically significant as the 95% confidence interval cross the normal mean (dotted line).

For each of the patients' faecal samples (baseline and six weeks), the GA-map Dysbiosis Test™ provided a microbiota profile with selected bacteria on different taxonomic levels. The probes presented are for specific intestinal bacterial genera and species shown to be associated with gastrointestinal disorders. Some genera or species are reported together because their signals were not distinguishable. The level of bacteria are presented in values from -3 to 3, where 0 equals the level in a healthy cohort. The values are an expression for the detected probe signal, and for how far away from a healthy cohort the bacterial level in each patients is. Numbers -3 to -1 refers to a reduced level, where -3 is the farthest away from a healthy cohort, and 1 to 3 equals increased levels where 3 is the farthest away. These deviations cannot be regarded as statistically significant, but are still of significance when looking at dysbiosis.

Table 12: Overview of level of selected bacteria in genera and species for patients with dysbiosis at baseline. The numbers -3 to 3 represent deviations from a healthy cohort which equals 0. Each DI-score represents different patients

	Firmicutes						Verucro-microbia	Bacteriodetes		Proteo-bacteria	Actino-bacteria
DI	Rum.a/ b	Rum.g	F.prau	Lact (g)	Strep. sa & th	D.invi	A.mun	B.frag	Ali (g)	Sh/Es (g)	Bf (g)
5	0	3	-2	0	1	0	0	0	-2	1	0
5	0	0	0	1	0	0	0	0	0	3	0
5	0	0	0	0	2	0	0	3	0	0	-1
4	0	0	0	0	0	0	1	2	0	3	-1
4	2	2	0	0	0	0	0	2	0	0	-1
3	0	1	0	0	1	2	2	0	-2	0	-1
3	0	0	0	0	2	0	0	0	0	0	-1
3	0	2	-1	0	1	0	0	2	-3	1	0
3	0	2	-1	0	2	0	2	0	0	1	0
3	0	0	0	0	0	0	2	1	0	2	-1
3	2	2	0	1	1	0	0	2	0	2	0
3	0	0	-1	0	0	0	0	2	0	0	-1
3	0	2	0	0	1	0	0	0	0	0	-1
3	0	0	0	0	0	0	0	0	1	0	-1
3	0	0	0	0	1	1	1	0	-3	0	0
3	0	0	0	0	0	0	0	0	1	0	-1
3	0	0	0	0	0	0	0	0	0	0	0
3	0	0	-1	0	0	0	0	0	0	0	0

DI, Dysbiosis Index; Rum.a/b, *Ruminococcus albus/bromii*; F.prau, *Faecalibacterium prausnitzii*; Lact, *Lactobacillus*; Strep.sa&th, *Streptococcus sanguinis*&*thermophilus*; D.invi, *Dialister invisus*; A.mun, *Akkermansia munciniphila*; B.frag, *Bacteroides fragilis*; Ali, *Alistipes*; Sh/Es, *Shigella*/*Escherichia*; Bf, *Bifidobacterium.*

The different genus and species presented in table 12 show that there are different bacteria contributing to the dysbiosis in different patients at baseline. The bacteria presented cannot alone account for the dysbiosis in the patients, but are contributors when their abundance deviate from the healthy cohort. There did not seem to be any clear pattern of what bacteria contributed in all patients, but there were however some observations of bacteria that more frequently deviated from a normal level. A reduced level of the genus *Bifidobacterium* (phylum *Actinobacteria)* was seen in 55% of the dysbiotic patients. This genus was reduced also in 55% in all the patients combined (not shown in table). An increased level of *Streptococcus sanguinins* and *Streptococcus thermophilus* was seen in 50% of the dysbiotic patients. The genera *Shigella/Escherichia*, *Bacteroides fragilis* and *Ruminicoccus gnavus* had increased

abundance in about 40% out of the dysbiotic patients. Notably, the level of which the bacteria deviate from a normal level varies between the individual patients.

The genera *Shigella/Eschericia* had increased abundance in three out of five with the highest DI-score (4 and 5), and also at a value of 3 for two of the patients. This observation might indicate that these genera contribute more to a severe dysbiosis.

Table 13: Overview of level of higher taxonomy groups in patients with dysbiosis at baseline. Numbers -3 to 3 represent deviations from a healty cohort which equals 0. Each DI-score represent different patients

DI	Bs / Prev	Firm (B)	Firm (C)	Pb
5	1	0	3	0
5	0	0	0	3
5	1	1	0	0
4	0	0	0	3
4	-1	0	1	0
3	0	1	0	0
3	0	2	0	0
3	0	0	2	0
3	1	1	1	0
3	0	-2	0	2
3	0	1	1	2
3	1	2	0	0
3	1	0	0	0
3	1	0	0	0
3	1	0	0	0
3	0	0	0	0
3	0	0	0	0
3	2	0	0	0

Bs/Prev, *Bacteroides/Prevotella;* Firm (B), *Firmicutes (Bacilli);* Firm (C*), Firmicutes (Clostridia)*; Pb, *Proteobacteria*.

Table 13 shows higher taxonomic groups, which includes many species of bacteria. More patients seem to deviate from the genera *Bacteroides/Prevotella,* followed by *Firmicutes (Bacilli), Firmicutes (Clostridia)* and *Proteobacteria. Bacteroides/Prevotella* were increased in 52.5% of the total study group. These phyla will overlap some with the those presented in table 12 as some are classified under these genera or phyla, however, the probes for the phyla in table 13 detect many more bacteria.

3.4.4 Bacteria and the low FODMAP diet

A statistically significant reduction in the genus *Bacteroides* was seen in patients on the FODMAP diet, but not in patients on a stricter GFD (p=0.024). This was the only bacteria of those analysed that had a statistically significant change in abundance after a low FODMAP diet.

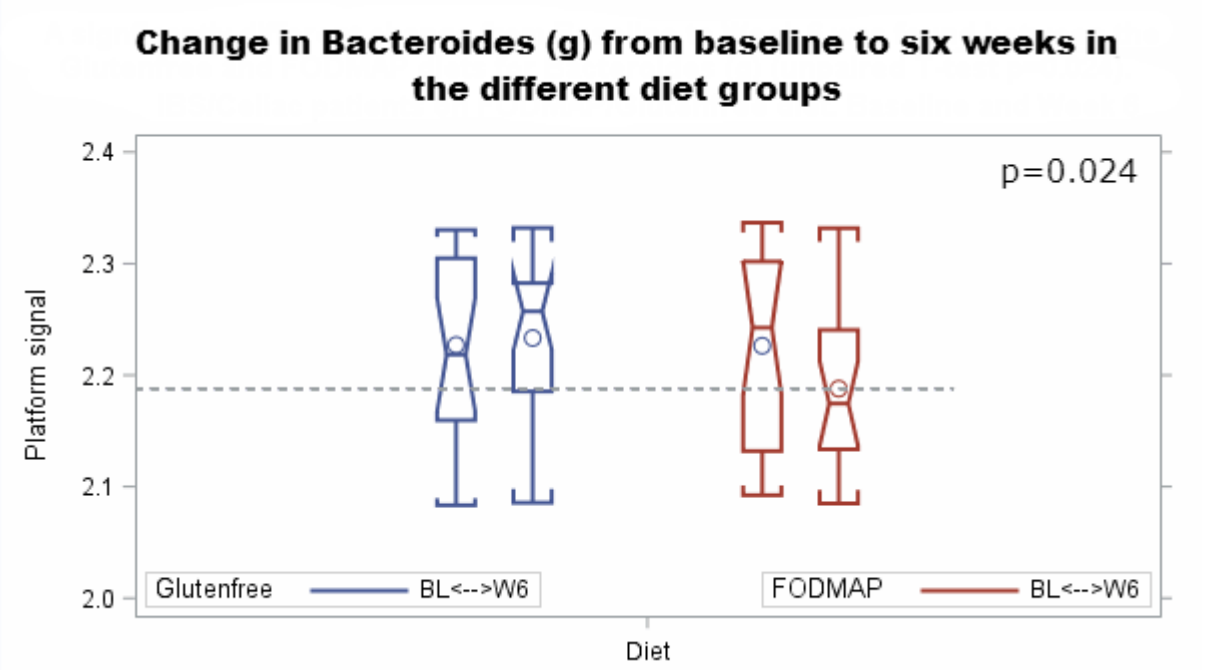


Figure 18: Probe signal for *Bacteroides (genus)* at baseline and after six weeks of diets. These bacteria were statistically significant reduced after a low FODMAP diet, but not after a stricter GFD. The box displays IQR and line within the box is median. The big circle equals mean. The interval shows 95% Confidence Interval. Normal mean (dotted line) equals mean of a healthy cohort. Unpaired t-test was performed. g, genus.

In table 14, the change in specific bacteria and genera are listed for each patient from baseline to six weeks of a low FODMAP diet. There were some individual changes, but no clear pattern shared between the patients. The table also show individual change in DI-score within the group following a LFD.

Table 14: The change in DI-score and bacterial species and genera in patients from baseline to six weeks on a low FODMAP diet. Numbers -3 to 3 represent deviations from a healty cohort which equals 0. Each row represent a patient at baseline or six weeks, where the grey shaded rows equals baseline level

Pat.	DI	Rum. a/b	Rum.g	F.prau	Lact.	Strep.sa & th	D.invi	A.mun	B.frag	Alist.	Sh/Es	Bifido.
1 BL	3	0	0	-1	0	0	0	0	2	0	0	-1
1 6w	3	0	2	0	0	0	0	0	2	0	0	-1
2 BL	3	0	2	0	0	1	0	0	0	0	0	-1
2 6w	2	0	2	0	0	0	0	0	0	0	0	0
3 BL	1	0	0	0	0	1	0	1	0	0	1	0
3 6w	1	0	0	-1	0	0	0	0	1	0	1	-1
4 BL	5	0	0	0	1	0	0	0	0	0	3	0
4 6w	3	0	0	0	2	1	0	0	0	0	0	0
5 BL	1	1	0	0	0	0	0	0	1	0	0	-1
5 6w	1	0	0	0	0	0	0	1	1	0	2	-1
6 BL	2	0	0	0	1	0	0	1	0	0	0	-1
6 6w	2	0	0	0	1	1	0	0	0	0	0	-1
7 BL	4	2	2	0	0	0	0	0	2	0	0	-1
7 6w	5	0	2	0	0	0	1	0	3	0	2	-1
8 BL	2	0	0	0	0	0	0	0	2	0	1	-1
8 6w	2	0	0	0	0	0	0	0	0	-3	1	0
9 BL	1	0	0	0	0	0	0	0	0	-3	0	-1
9 6w	1	0	0	0	0	0	0	0	0	-3	0	-1
10 BL	3	0	0	0	0	0	0	0	0	1	0	-1
10 6w	3	0	2	-1	0	0	0	0	0	0	0	-1
11 BL	1	0	0	0	0	0	1	0	2	-3	0	0
11 6w	2	0	0	0	0	0	1	2	0	-3	0	0
12 BL	3	0	0	0	0	1	1	1	0	-3	0	0
12 6w	3	0	1	0	0	0	0	0	0	-3	1	-1
13 BL	3	0	0	0	0	0	0	0	0	1	0	-1
13 6w	2	0	0	0	0	0	0	0	0	0	0	-1
14 BL	2	1	0	0	0	0	0	1	0	0	0	-1
14 6w	3	0	0	0	0	0	0	0	0	0	2	-1
15 BL	1	0	0	0	0	0	0	0	0	0	1	-1
15 6w	2	0	0	0	0	0	0	1	0	0	0	-1
16 BL	2	0	0	0	0	0	0	2	0	0	0	-1
16 6w	3	1	0	0	0	0	0	2	0	0	0	-1
17 BL	3	0	0	0	0	0	0	0	0	0	0	0
17 6w	2	0	0	0	0	0	0	1	3	0	2	-1
18 BL	3	0	0	-1	0	0	0	0	0	0	0	0
18 6w	2	0	0	-1	0	0	0	1	0	0	0	1
19 BL	2	0	0	-1	0	0	0	0	0	0	-1	-1
19 6w	2	0	0	0	0	0	0	0	0	0	0	-1
20 BL	5	0	0	0	0	2	0	0	3	0	0	-1
20 6w	5	0	0	0	0	0	0	0	3	0	0	-1

BL, baseline; 6w, 6 weeks; Pat, patient; DI, Dysbiosis Index; Rum.a/b, *Ruminococcus albus/bromii*; F.prau, *Faecalibacterium prausnitzii*; Lact, *Lactobacillus*; Strep.sa&th, *Streptococcus sanguinis*&*thermophilus*; D.invi, *Dialister invisus*; A.mun, *Akkermansia munciniphila*; B.frag, *Bacteroides fragilis*; Ali, *Alistipes*; Sh/Es, *Shigella*/*Escherichia*; Bf, *Bifidobacterium.*

Individual changes on higher taxonomy levels are shown in table 15. Also here there will be some overlap with those bacteria presented in table 14. There was no clear pattern in bacteria on higher taxonomy levels amongst the patients.

Table 15: The change in DI-score and bacteria at higher taxonomy levels in patients from baseline to six weeks on a low FODMAP diet. Numbers -3 to 3 represent deviations from a healty cohort which equals 0. Each row represents a patient at baseline or six weeks, where the grey shaded rows equals baseline level

Pat.	DI	Bs / Prev	Firm (B)	Firm (C)	Pb
1 BL	3	1	2	0	0
1 6w	3	1	1	0	0
2 BL	3	1	0	0	0
2 6w	2	0	0	0	0
3 BL	1	0	0	0	0
3 6w	1	1	0	0	0
4 BL	5	0	0	0	3
4 6w	3	0	0	2	0
5 BL	1	0	-1	0	0
5 6w	1	0	0	0	1
6 BL	2	1	0	0	0
6 6w	2	1	0	0	0
7 BL	4	-1	0	1	0
7 6w	5	0	0	1	2
8 BL	2	1	-1	0	0
8 6w	2	-3	0	1	2
9 BL	1	1	0	0	0
9 6w	1	1	0	0	0
10 BL	3	1	0	0	0
10 6w	3	1	0	0	0
11 BL	1	0	0	1	0
11 6w	2	0	0	0	0
12 BL	3	1	0	0	0
12 6w	3	2	0	0	0
13 BL	3	0	0	0	0
13 6w	2	1	0	0	0
14 BL	2	1	0	0	0
14 6w	3	0	0	0	2
15 BL	1	0	0	0	0
15 6w	2	1	0	0	0
16 BL	2	0	0	1	0
16 6w	3	0	-1	2	0
17 BL	3	0	0	0	0
17 6w	2	0	0	0	2
18 BL	3	2	0	0	0
18 6w	2	0	0	0	0
19 BL	2	0	0	0	0
19 6w	2	0	0	0	0
20 BL	5	1	1	0	0
20 6w	5	1	0	0	0

3.5 Breath testing

As shown in figure 19, the hydrogen breath test taken at baseline and after 6 weeks showed no significant change in hydrogen after a low FODMAP diet in group B, median 266 vs. 244 ppm, (p= 0.927). There was no significant change in group A with median 424 vs. 90 ppm, (p= 0.140) after a stricter gluten free diet.

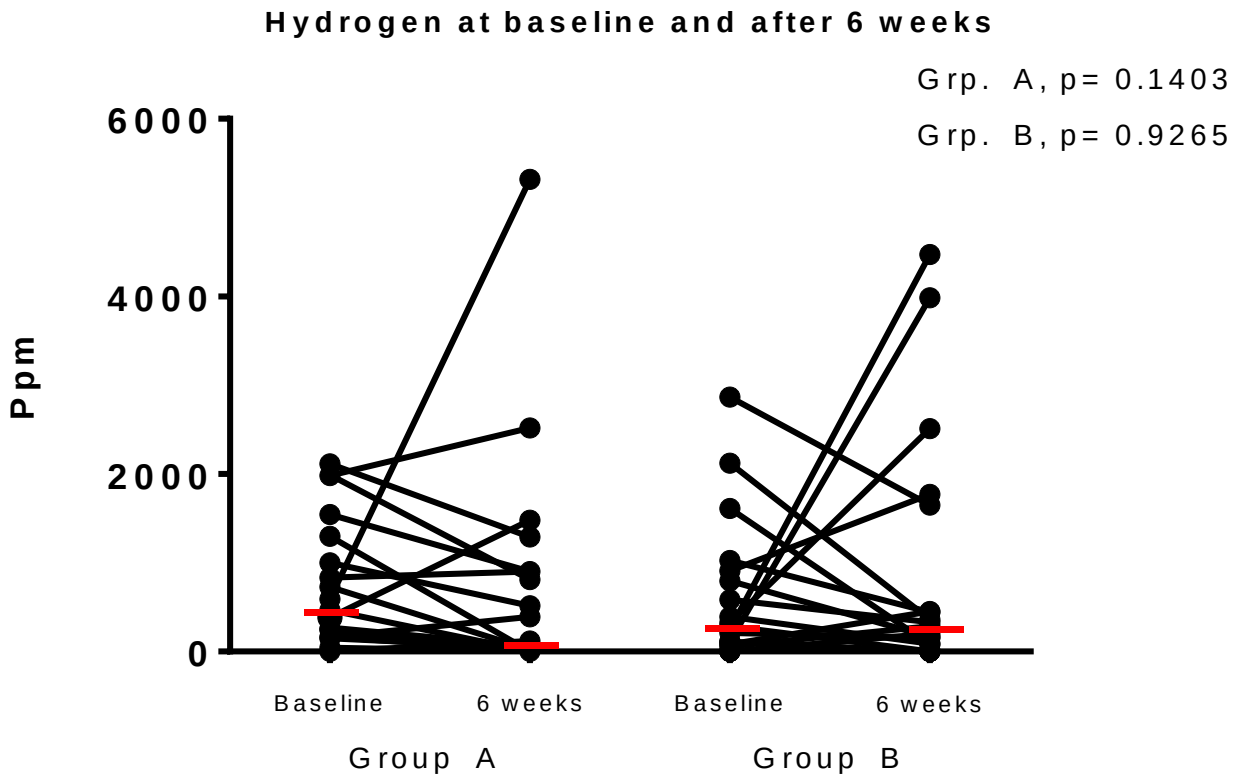


Figure 19: Area under the curve for hydrogen gas at baseline and after six weeks of diet in group A and B

Red line = median. Wilcoxon signed rank test.

There was no significant difference in hydrogen production at baseline or six weeks when comparing responders to non-responders of the LFD, when response was defined as an IBS-SSS reduction of 100 or more. At baseline, median ppm in responders was 266.3 ppm versus 453.8 ppm in non-responders, p=0.985. At six weeks, responders had median ppm of 180 versus median ppm of 322.5 in non-responders, p=0.732.

No significant difference was seen in methane production after 6 weeks of diet in group A (p=0.1563) or group B (p= >0.9999). Seven patients in group A produced methane at baseline, whilst six patients produced methane at six weeks. The methane production by three patients in group B remained stable.

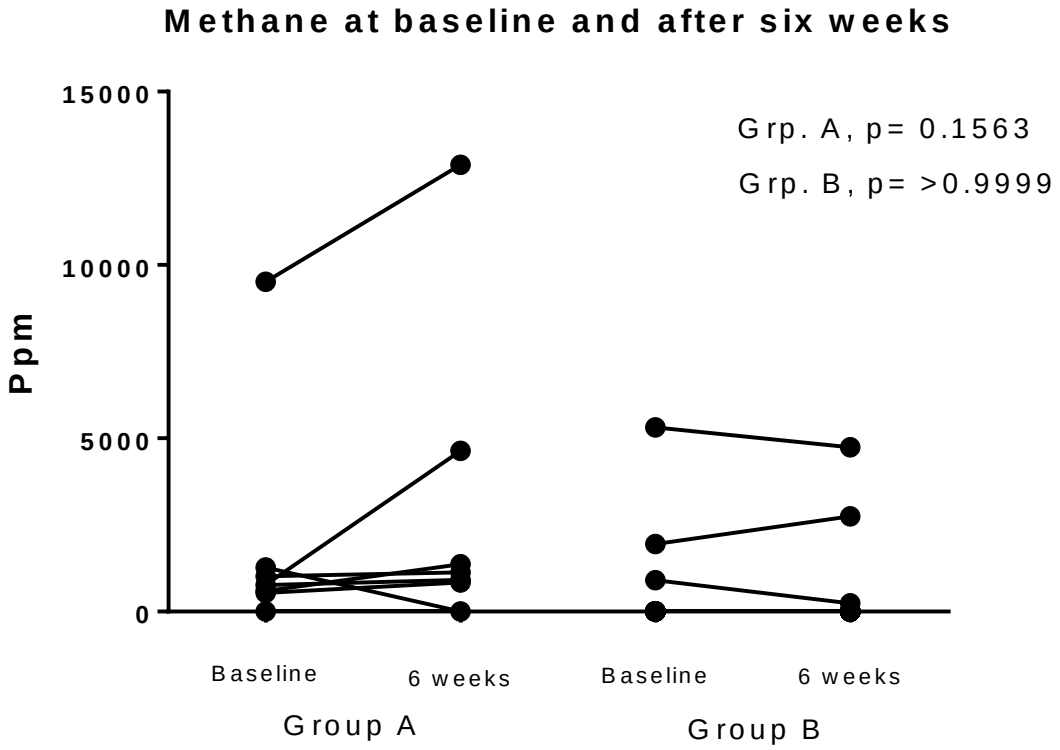


Figure 20: Area under the curve for methane gas at baseline and after six weeks of diet in group A and B

Wilcoxon signed rank test.

3.6 IBS-SSS

3.6.1 IBS-SSS total score

Symptoms were registered at baseline, three weeks and six weeks using IBS-SSS. There was a significant reduction in IBS-SSS in both groups after six weeks of diet. Group A had a reduction in IBS SSS score from mean 259.8 to 203.7 (p=0.0022). In group B, IBS-SSS score was reduced from mean 263.3 to 144.6 (p=<0.0001).

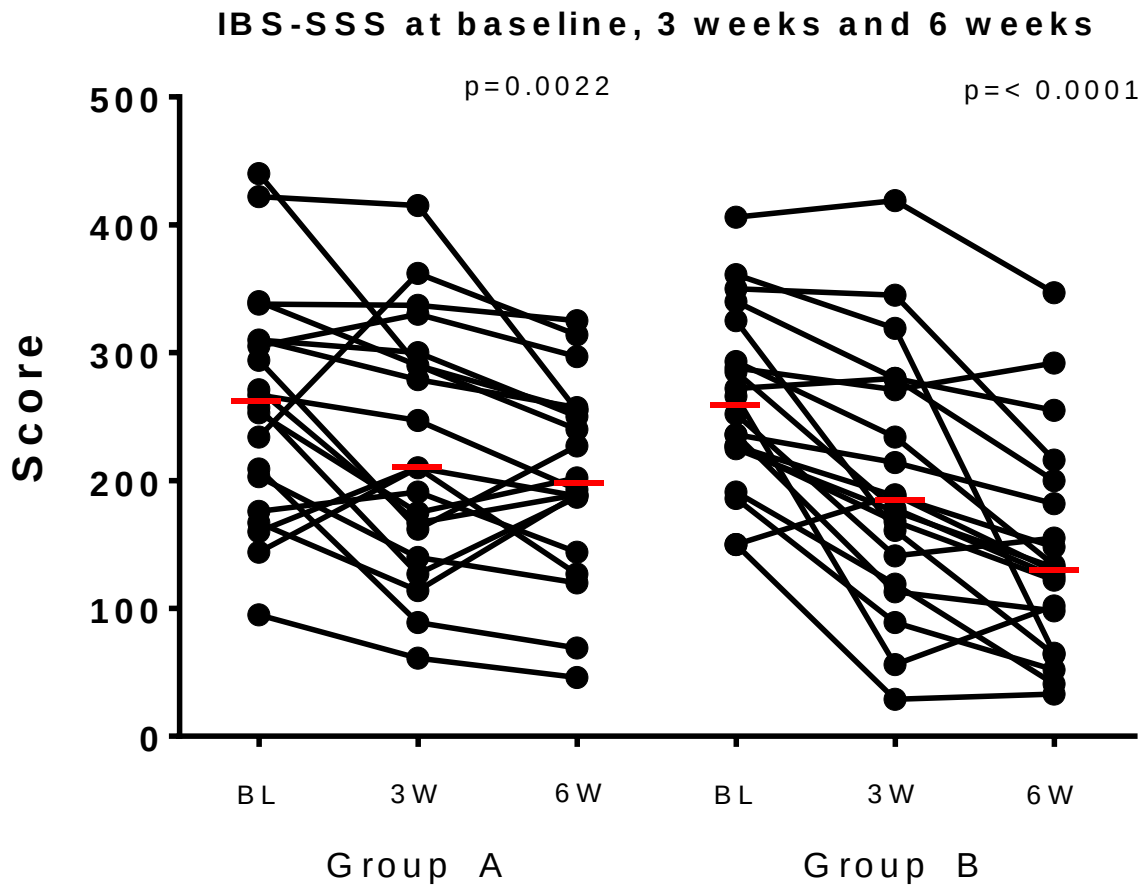


Figure 21: Change in IBS-SSS total score from baseline, after three weeks and six weeks of diet in group A and B

0 = satisfaction, 100 = dissatisfaction. Red line = median.

RM one-way ANOWA and Tukey's multiple comparison test.

Tukey's multiple comparison test show that the biggest change in symptom score in group A was between baseline and three weeks with a mean difference of 35.0, but this was not significant. There was a significant reduction from baseline to six weeks, with a mean total symptom score change of 56.1. In group B, there was a large score reduction both between baseline and three weeks (mean diff. 64.9) and between three weeks and six weeks (mean diff. 53.9), which both were statistically significant changes. The change in total mean score was 118.7. After diet, two patients were in remission (IBS-SSS <75) in group A versus five patients in group B.

3.6.2 IBS-SSS main questions

The IBS-SSS total score is comprised of five main questions which each have a VAS-scale that ranges from 0 to 100. These questions and their scores are listed in table 16 for group A and in table 17 in group B.

In group A there was a statistically significant score reduction in abdominal pain severity (p=0.0015). There was less effect on the other four questions.

Table 16: Mean score for the five different main questions at baseline, three weeks and six weeks in group A Data are given mean ± SD and p-values for change in score from baseline to six weeks

Group A	Baseline	3 weeks	6 weeks	p-value
1. Abdominal pain severity (0-100)	47.8 (±27.1)	33.2 (±29.4)	27.5 (±24.2)	0.0015**
2. Abdominal pain duration (days x 10)	40.3 (±28.6)	42 (±39.3)	33 (±24.5)	0.372
3. Abdominal distension (0-100)	51.3 (±31.3)	43.9 (±26.7)	42.3 (±25.2)	0.243
4. Bowel habit satisfaction (0-100)	67 (±27.4)	56.6 (±26.3)	53.5 (±21.9)	0.0724
5. Life disruption (0-100)	57.3 (±28)	49.3 (±25.3)	47.6 (±22.6)	0.155

Repeated measures one-way ANOVA and Friedman test

There was a statistically significant score reduction on all five questions after a low FODMAP diet in group B. The diet seemed to have the greatest effect on abdominal pain, abdominal distension and on life disruption.

Table 17: Mean score for the five different main questions at baseline, three weeks and six weeks in group B. Data are given mean ± SD or median (IQR). P-values for change in score from baseline to six weeks

Group B	Baseline	3 weeks	6 weeks	p-value
1. Abdominal pain severity (0-100)	39.1 (±20.7)	25.1 (±21.9)	14.5 (0-23.5)	<0.0001****
2. Abdominal pain duration (days x 10)	42.8 (±28.5)	38.8 (±32.3)	20 (0-40)	0.0016**
3. Abdominal distension (0-100)	58.4 (±19.8)	38.8 (±27.5)	19.5 (2-34.5)	<0.0001****
4. Bowel habit satisfaction (0-100)	63.2 (±26.1)	48.8 (±28.7)	44.9 (±23.8)	0.0196*
5. Life disruption (0-100)	61.4 (±24.3)	47.1 (±25.6)	33.7 (±28.9)	<0.0001****

Repeated measures one-way ANOVA and Friedman test

3.6.3 Responders to low FODMAP diet and bacterial pattern

There was detected a distinctive pattern in bacteria for those who responded to a LFD, when response was defined as an IBS-SSS-score reduction of 100. This included less of the genus *Lactobacillus*, less *Firmicutes (Clostridia)* and more of the genus *Apotobium* This pattern was identified through a Fisher's linear discriminant analysis with 80% accuracy.

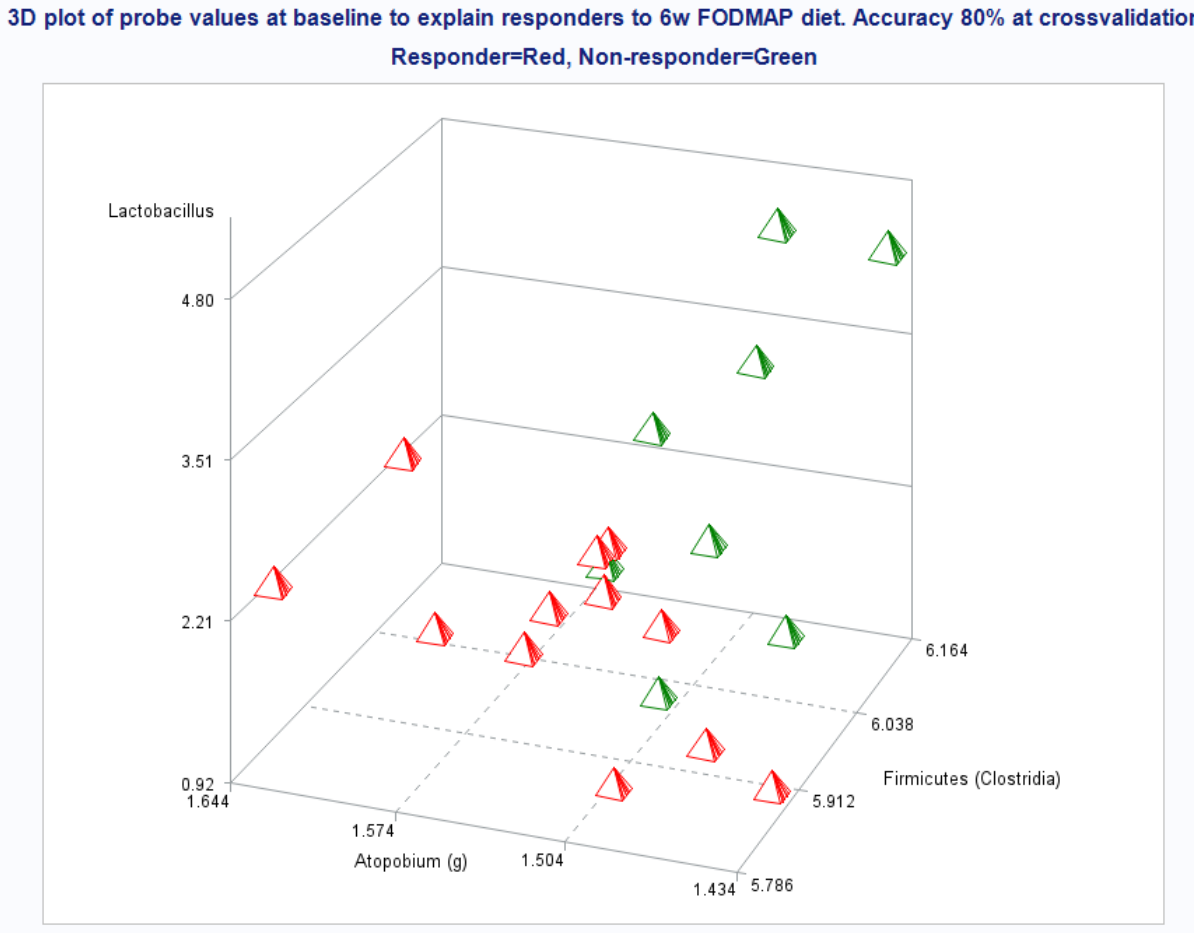


Figure 22: 3D plot of probe signal values in responders to the LFD

Responders to the LFD (red) seem to have less *Lactobacilli (g)*, more *Atopobium (g)* and less *Firmicutes (Clostridia)* than non-responders (green) at baseline. Responders was defined as an IBS-SSS score reduction of 100**.** Fisher's linear discriminant analysis was performed.

3.7 Compliance

3.7.1 Compliance during the intervention period

We used a self-reporting questionnaire in order to evaluate compliance in both groups throughout the intervention period. The form was filled out at six weeks. The table below show the results of the questions that included a VAS-scale (0-100 mm).

Table 18 show that there was a high self-reported degree of adherence to the diet in both groups, with a mean of 93.3% in group B and a median of 97% in group A. It is also clear that the LFD was more difficult to follow compared to the strict gluten free diet. More patients were satisfied with the LFD than the strict gluten free diet for symptom relief.

Table 18: Self-reported compliance during the intervention period in group A and group B

	How satisfied are you with the diet as symptom relief? (0-100, where 100 is very dissatisfied)	How carefully how you followed the diet? (0-100, where 100 is full adherence)	How was it to follow the diet? (0-100, where 100 is very challenging)
Group B, mean ± SD	41.3 ± 30.8	93.3 ± 8.3	64.3 ± 30.1
Group A, mean ± SD and median (IQR)	66.7 ± 31.1	97 (87.5-100)	27.9 ± 32.8

Data are presented in mean ± SD

3.7.2 The low FODMAP diet

In group B, 50% replied that they wanted to continue the diet, 40% replied "maybe" and 10% replied "no". The patients were also asked if they had any deviations from the diet, where 35% answered "none", 55% answered "1-5 times during the six weeks" and 10% answered "1-3 times a week". Of those who had deviations from the diet, six out of 13 answered "only a mouthful", three out of 13 answered "2-5 mouthfuls", and four answered "a whole meal". Fructose- and lactose containing foods were the most common foods in diet deviation.

In response to how satisfied they were with the dietary guidance, nine of the patients answered that they were very satisfied, nine were satisfied, one answered "OK" and one was not satisfied.

3.7.3 The strict gluten free diet

50% answered that they wanted to continue excluding wheat starch and trace amounts of gluten whilst 25% answered "maybe" and 25% "no". In question on any deviations from the diet, 40% answered "none", 50% answered "1-5 times during the six weeks" and 10% answered "1-3 times a week". The most common reason for any deviations was not knowing that the food contained either wheat starch or traces of gluten. Thirteen were very satisfied with the dietary guidance before commencing the diet, whilst six were satisfied and one answered "OK".

3.7.4 Compliance 4 weeks after end of study

Group B also filled out a questionnaire reporting adherence to the diet one month after end of study, which they sent back by mail. All 20 patients answered the questionnaire.

Table 19 show that the patients have followed the diet to a varying degree after end of intervention.

Table 19: Self-reported compliance 4 weeks after end of study in group B

	How well have you maintained your diet 1 month after end of study? (0-100, of which 100 is only eaten low FODMAP)	How did you find the reintroduction of foods? (0-100, of which 100 is very challenging)
Group B, mean ± SD	61.9 ± 36.5	42.6 ± 32.5

Eight of the patients still followed the diet at this stage, seven patients answered "partially", one patient answered "occasionally" and four patients did not follow the diet anymore. In question to why they no longer followed the diet completely, most answered that the effect of the LFD was not big enough to sacrifice their habitual diet, while some answered that there were only a few foods they reacted to or that they missed too many food items. Only one answered that they had no effect of the diet.

It seems the reintroduction was not too difficult, with a mean of 42.6% where 100% is very challenging. Eight answered that they had started reintroducing FODMAPs, while seven had only reintroduced certain food items. What the majority found most difficult with reintroduction, was to identify whether the symptoms came from that exact food item, and also to differ between "normal symptoms" still persisting on a LFD and symptoms that might come from reintroduction of different FODMAPs. Sixteen out of twenty patients suspected that they reacted to two or more FODMAPs.

In response to whether they were going to continue the diet at this stage, four answered "yes" and 12 answered "partially". One said "maybe", while three said "no". The most frequently mentioned FODMAPs the patients suspected they reacted to were fructans and secondly lactose.

3.8 Blood tests

Blood test were taken at baseline and after six weeks of diet. These included serology tests and some other parameters. IgA TTG antibodies were reduced for four out of five patients and reduced in the one patient with elevated antibodies in group B.

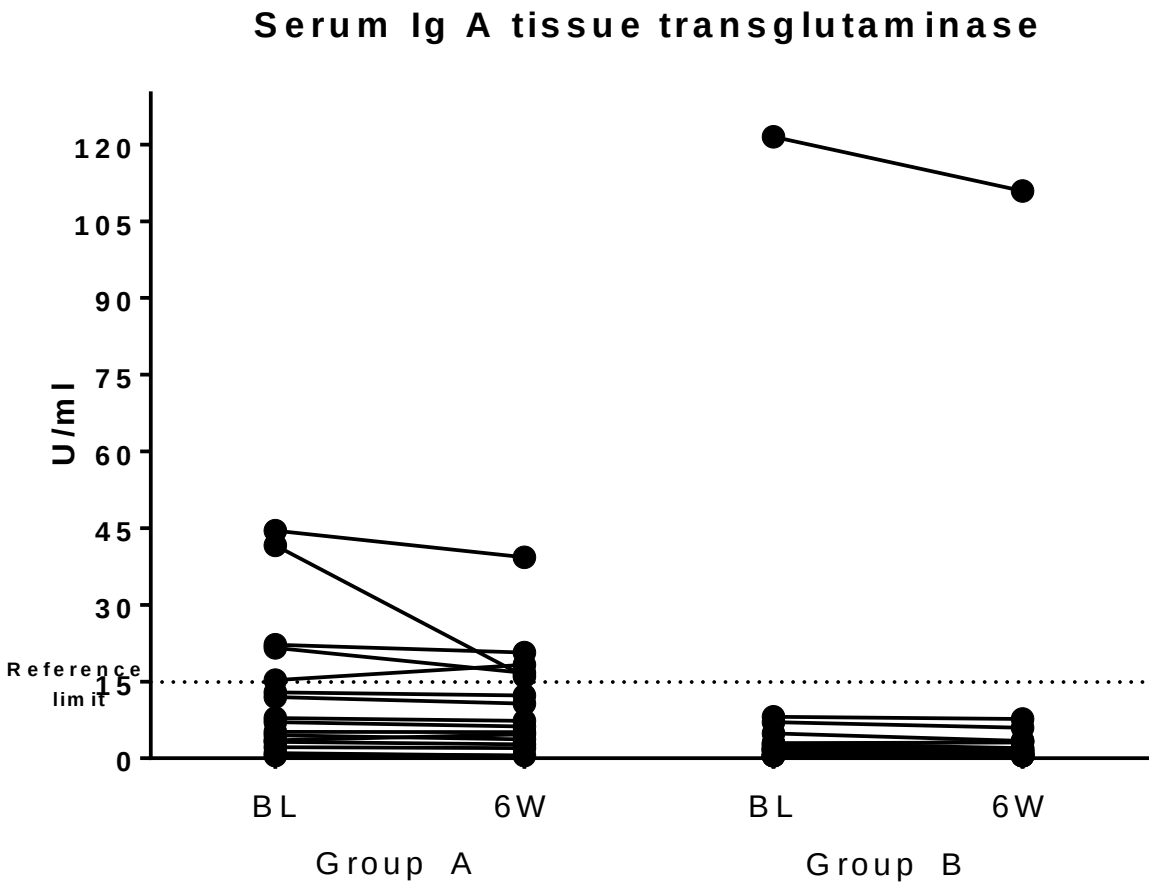


Figure 23: IgA TTG levels at baseline and after six weeks of diet in group A and B
Dotted line equals reference limit 14.9 U/ml. Wilcoxon signed rank test.

The level of IgA TTG and IgG DPG were significantly reduced in both groups. Elevated IgG DPG were however not considered as having elevated antibodies, because we did not have any information on IgA deficiency. Vitamin D was signicantly reduced in group A.

Table 20: Antibodies and Vitamin D levels at baseline and after intervention in both groups

	Group A			**Group B**		
	Baseline	6 weeks	p-value	Baseline	6 weeks	p-value
IgA TTG	5.2	5.1	0.0175*	4.8	4.3	0.0072**
IgG DPG	4.8	4.3	0.0012**	0.9	0.7	0.0039**
Vitamin D	71	63	0.0037**	78	81	0.961

Paired t-test and Wilcoxon signed rank test

4 DISCUSSION

4.1 Main findings

We performed a prospective, randomized and controlled clinical trial to look at the microbiota profile and Dysbiosis Index in coeliac disease patients with IBS-like symptoms, and to see the effect of two different dietary interventions on microbiota and symptoms. One group followed a low FODMAP diet, and the other group followed a stricter GFD excluding wheat starch and trace amounts of gluten from their diet. Both groups followed their assigned diet for six weeks.

There was a successful reduction of total FODMAPs from mean 11.5 to 1.6 grams (p=0.0001), as well as in lactose (p=<0.0001) and other FODMAPs (p=0.0001) in group B. We also found a significant reduction in energy and fat intake in this group. There were no change in FODMAP intake in group A, following a strict GFD. We found a significant change in fat and protein intake in this group.

At baseline, we found that 55% of the patients were normobiotic, whilst 45% had dysbiosis. Of these; 32.5% had a mild dysbiosis (DI=3) and 12.5% had a severe dysbiosis (DI=4-5). Several bacterial genera were statistically significantly different at baseline compared to healthy controls, including *Ruminiclostridium, Bacillus, Eubacterium, Lactobacillus, Prevotella, Pseudomonas, Atopobium* and *Desulfitispora.* There were observations of several bacteria that deviated from the level in healthy controls in the dysbiotic patients, although we cannot determine whether this is statistically significant or not. These bacteria include less *Bifidobacterium,* and more *Streptococus sanguinis, Streptococcus thermophilus, Shigella/Escherichia* and *Bacteroides fragilis.* There was a statistically significant reduction in the genus *Bacteroides* after the LFD compared to the stricter GFD (p=0.024). The degree of dysbiosis tended to decrease on a LFD (p=0.789), and worsen when following a stricter GFD (p=0.852), but these were not statistically significant findings.

IBS-SSS-score was significantly reduced in both groups after six weeks of diet, with p-values of p=0.0022 in group A and p=<0.0001 in group B. Group B had a significant reduction in all five main questions, including abdominal pain and duration, abdominal distension, bowel habit satisfaction and life disruption. Group A had a significant reduction in abdominal pain, but not on the other questions. The responders to a LFD seem to have less *Lactobacillus,*

more *Atopobium (g)* and less *Firmicutes (Clostridia)* at baseline than the non-responders, when response is defined as a score reduction of 100 on IBS-SSS.

There were no significant change in the hydrogen breath test in group A (median 424 vs. 90 ppm, p= 0.140) or group B (median 266 vs. 244 ppm, p= 0.927). Of those producing methane, there were no significant reduction in group A (p=0.1563), nor group B (p= >0.9999). One patient in group A produced methane at baseline, but not after six weeks.

IgA TTG was reduced in four out of five patients in group A and reduced in the one that had elevated levels in group B.

4.2 Discussion of findings

We found that the group of CD patients with IBS-like symptoms had more dysbiosis than a healthy population where 16% suffered from dysbiosis, but less than an IBS population in which 70% had dysbiosis [122]. It was somewhat expected to find some dysbiosis, as an altered microbiota have been detected in untreated and treated CD patients and in IBS patients previously [122-125, 127-129]. What causes our patient group to have an altered bacterial composition is not identified, and there are several potential factors. Firstly, we know that all CD patients have shared HLA DQ2 or DQ8-genes, which might have an impact on the microbiota composition. However, these genes are shared with 30% of the population, and if HLA subtype was important, many more of the general population should be dysbiotic. The tissue antigens make up a very complicated system, however. But also other susceptible genes besides the HLA genes are thought to be involved in CD development, and may affect microbiota. Another shared factor is that all patients follow a GFD, and we know that diet is very potent to affect microbiota. What clearly distinguishes a GFD from a normal diet is the exclusion of gluten, which also involves a reduction in prebiotic carbohydrates such as fructans. Thus, a long term GFD could affect microbiota and either be a possible cause or a factor that maintains dysbiosis. An altered bacterial composition could also have arisen before the CD diagnosis was made, and a GFD might have failed to restore it. Wacklin et al. [147] have suggested that the fact that most patients go a long time before the CD diagnosis is being made, might lead to alterations in the bacterial composition.

It is tempting to assume that dysbiosis in these patients is a contributing factor to the IBS-like symptoms they are experiencing. Although previous studies have detected dysbiosis in both treated and untreated CD patients, we should ideally have studied those groups as well, using the same technique. A DI score for untreated CD patients and treated CD patients without IBS-like symptoms would help determine whether the degree of dysbiosis is distinctive for our patient group or not. Possible mechanisms for why dysbiosis could lead to IBS-like symptoms is the involvement gut microbiota has on maintaining a normal gut barrier and immune response, and a disruption here may lead to such symptoms. It is proposed as a pathogenic factor in IBS that dysbiosis may activate the immune system and as a consequence affect motility and permeability and cause symptoms [52]. If the dysbiosis is in fact causing the IBS-like symptoms, perhaps probiotics could reduce symptoms in these patients. Another important aspect is that our study only looked at faecal microbiota, which does not necessarily have the same composition as mucosal microbiota [148-150]. It is not certain that dysbiosis of the faecal microbiota can explain mechanisms which would mainly involve the mucosal microbiota [151].

Although all included patients met the Rome III-criteria used for IBS diagnosis, we still cannot say whether this particular group of patients actually suffer from IBS in addition to their CD, or if other mechanisms are responsible for their symptoms. Those with elevated antibodies may have IBS-like symptoms due to a gluten contamination in their diet, which causes inflammation and symptoms. Interestingly, the patient with elevated IgA TTG antibodies in group B did experience symptom relief from the FODMAP diet, but it is possible that an ongoing intestinal inflammation will cause reactions to FODMAPs in higher degree than in a healthy intestine. Four out of five with elevated IgA TTG antibodies in group A experienced symptom relief (IBS-SSS reduction >50) and all had reduced antibodies after diet, which indicates that they possibly had gluten contamination in their diet. This is reported to be the most common cause of persisting symptoms in CD [28]. It is also possible that some patients have a concomitant IBS diagnosis, which is proposed in IBD patients in remission with IBS-like symptoms, and has been called IBS-IBD. Considering that as much as 10-20% of the general population suffers from IBS, it is not unlikely that some CD patients have concomitant and unrelated IBS. The prevalence of IBS-like symptoms in CD is reported to be between 15 and 23.3%, which is quite similar to the prevalence of IBS in the general population. However, it is reported even higher in some studies, suggesting that there may be something else than just coexisting IBS, at least for some of the patients. CD involves

inflammation when the intestines are exposed to gluten, which normally resolves on a GFD, but maybe a failure to downregulate this inflammation completely predisposes for IBS-like symptoms, possibly triggered by stress or an infection as seen in IBS. SIBO can also give similar symptoms to those seen in these patients. We did not test for SIBO as it requires an ileo-colonoscopy, and thus we cannot exclude it as a cause. One study did find SIBO to affect CD patients with persistent symptoms [152]. This study did however only use lactulose breath test to test for SIBO and had a small study sample. Maybe this patient group is not a homogeneous group, but rather made up of CD patients with similar symptoms of different origin. Regardless, it is important to address these symptoms as they are known to have great impact on quality of life in IBS patients, and quality of life has also been reported to be lower in CD patients with IBS-like symptoms compared to those without [42, 69].

A low FODMAP diet led to numerically less dysbiosis in these patients, although not a statistically significant finding. This is possibly a random finding, and do not reflect the nature of the diet. The diet involves a temporary elimination of many different foods as well as foods known for their prebiotic effects, and theoretically it does not seem likely that such a diet would improve the bacterial composition. However, we do not know whether the LFD have the same effect on microbiota in these patients as it have in IBS patients. Additionally, the stricter GFD had constant rate of dysbiosis, but led to more patients having severe dysbiosis. Again, this was not a statistically significant finding. This intervention did lead to a reduction in protein and fat intake, but otherwise no change in diet other than the exclusion of wheat starch and products with traces of gluten. It is not evident that this diet intervention would lead to more severe dysbiosis, and might be just random variation over time. If we had a larger study sample, it is possible that we could have seen a more pronounced effect of the diet on dysbiosis. There was also a lot of individual movement in the dysbiosis index of the patients from baseline to six weeks, which indicate that the bacteria that comprise this index is part of a variable or hypervariable part of the microbiota. Thus, we cannot be certain that the changes in DI-score seen in these patients are driven by diet or if these changes would have happened regardless of an intervention.

A paradox of the LFD is that the diet has been shown to have a negative impact on gut microbiota. Interestingly, we did not see the same changes in microbiota after six weeks on diet as seen in IBS populations, such as a reduction in the butyrate-producing bacteria *Faecalibacterium prausnitzii* or more of *Akkermansia muciniphila*, which is a mucus-

associated bacteria. It was not seen any reduction in *Firmcutes (Bacilli)* or *Firmicutes (Clostridia)* either. The phyla *Firmicutes* is known to contain a lot of fermenting bacteria, and it is expected to see less fermenting bacteria after a diet with reduced amount of non-digestible carbohydrates, because these bacteria are being starved on a LFD. An increase in other bacteria might also be expected because when some species are starved and reduced, other bacteria take their place. We did find a statistically significant reduction in the genus *Bacteroides* after the LFD, but not after the stricter GFD. More of the bacteria in this genus is associated with a higher intake of animal products compared to plant polysaccharides [112]. However, the phyla also contains saccharolytic bacteria, which may explain the reduction when FODMAPs are restricted [153]. An explanation to why we did not find the same changes in microbiota as previously seen might lie in the baseline diet of these patients. A study by Halmos et al. [116] looked at the effect of a LFD in patients with inactive Crohn's, and did find some of the expected results on microbiota. However, this change in bacteria was seen between an Australian diet and a LFD, and not between the habitual diet of the patients and a LFD. The authors note that the patients had a low intake of FODMAPs in their habitual diet compared to an Australian diet (23g/day), and that this might have been the reason for lack of response in the microbiota. Even though we have a different patient group, it is possible that an already low FODMAP intake of mean 11.5 g/day in our patient group also before diet intervention led to less response in the microbiota. It must be mentioned that the study by Halmos et al. had a small study sample of eight patients. Previous studies have shown that a diet change over a short period of time does not necessarily affect microbiota very much, especially if the change in diet is not that radical [154, 155]. Or maybe an effect on the microbiota will come apparent in time if the FODMAP diet is continued by the patients. It may also be that we would have seen a greater effect on the microbiota if the patients were to consume a high FODMAP diet more similar to the total FODMAP intake in the general Norwegian population at first, and then switch to a LFD. However, we cannot exclude that that our patient group reacts differently to the diet compared to an IBS patient group when it comes to microbiota and fermentation. Maybe the microbiotic effect of the diet differs amongst different diagnosis, likely due to different bacterial composition at baseline.

At baseline, 55% of the dysbiotic patients had a reduced abundance of the genus *Bifidobacteria*, regardless of the degree of dysbiosis (DI-score 3-5). In fact, 55% of the whole study group had reduced abundance of this genus at baseline. *Bifidobacteria* colonize the intestines at a very early stage and are found in breast-fed infants. They are generally regarded

as beneficial bacteria because of their health benefits, such as inhibiting growth of pathogenic bacteria. These bacteria are also able to ferment non-digestible carbohydrates in the colon [156]. Interestingly, a reduction in *Bifidobacteria* has been detected in IBS patients when following a LFD [114]. Maybe the reduction in *Bifidobacteria* at baseline in our patients can be linked to a reduction in FODMAP intake after commencing a GFD, as this involves less FODMAPs. A reduction of *Bifidobacteria* has been seen in healthy individuals following a GFD [157]. A reduced level of *Bifdobacteria* has also been seen in both treated and untreated children with CD before, as well as in IBS patients [158-160]. Also patients without dysbiosis (DI-score 1-2) had a reduction in *Bifidobactera*, which might indicate that their microbiota composition is driven by some imbalance. This also indicates that *Bifidobacteria* were not the bacteria contributing most to dysbiosis in these patients. However, a reduction in one genus opens for other bacteria to multiply. *Streptococcus sanguinis* and *Streptococcus thermophilus* were both increased in 50% of the dysbiotic patients at baseline. *S. sanguinis* is regareded as a commensal bacteria most known for its presence in the oral cavity [161]. *S. thermophilus* is a bacteria producing lactic acid and used as a probiotic for its health benefits, such as easing digestion of milk and decreasing malabsorption symptoms [162, 163]. It is not clear why these bacteria seem to be increased in some of the dysbiotic patients, but maybe these commensal bacteria become less beneficial for the host in an altered gut environment. *Shigella* and *Escherichia* are two genera possibly contributing to severe dysbiosis in this patient group, and are in general increased in 40% of the dysbiotic CD patients. These genera are closely related to each other, include pathogenic bacteria and are normally just a small part of the human microbiota. *Shigella* is the cause of dysentery, an intestinal inflammation causing bloody diarrhoea [164]. *Shigella/Escherichia* are also found to contribute to dysbiosis in IBS and IBD populations [122, 165]. Although just an observation, is seems reasonable that these pro-inflammatory bacteria could be associated with severe dysbiosis in our patients. *Shigella/Escherichia* are classified under the phyla *Proteobacteria*, and bacteria belonging to this phyla have been reported increased in treated and untreated CD patients in both faecal and duodenal samples [159, 160, 166], and in the mucosal microbiota of CD patients with persistent symptoms [147]. *Ruminococcus gnavus* was also increased in about 40% of the dysbiotic patients, and increased abundance has also been detected in IBD patients and in IBS patients previously [167, 168]. These bacteria have the ability to form toxic products through b-glucuronidase activity, which may cause local colonic inflammation [169]. A higher abundance of *Bacteroides fragilis* has been detected in children with both active and non-active CD in a previous study [170]. It has been linked to altered permeability and production

of proinflammatory cytokines when in contact with gliadin peptides, and therefore associated with CD pathogenesis. Its effect in CD patients on a GFD is not clear, but it may be that a higher abundance compared to normal is a result of the microbiota failing to restore after CD diagnosis was made.

It must be mentioned that although all 54 probes were used to determine the Dysbiosis Index in these patients, only selected probes were used for creating an individual microbiota profile before and after diet due to their association with gastrointestinal diseases. This means that also other bacteria not presented in this study may contribute to dysbiosis, and does not make these findings specific enough to call it a microbiota signature in these patients. We have no information on diversity either. However, these bacteria might still be involved in the persistent symptoms of these patients. If this finding can be reproduced in other studies on CD patients, it could be targeted with probiotics. Probiotics have been tested as a treatment alternative in IBS patients, and although what species of bacteria are most effective remains uncertain, it has proven to have effect on symptoms [171]. There are little data on probiotics in CD patients, and to our knowledge, none are performed on CD patients with persistent symptoms [172].

When looking at the total study population of 40 patients, there was a statistically significant decrease in the genera *Ruminiclostridium* and *Eubacterium,* and an increase in *Bacillus, Lactobacillus, Prevotella, Pseudomonas, Atopobium* and *Desulfitispora* at baseline. There have been done some studies on microbiota composition in patients with active and non-active CD, mostly in children and mostly on duodenal microbiota, but none have found a distinct microbiota profile in CD patients. As far as we know, the composition of microbiota in CD patients with IBS-like symptoms has not been assessed before, and it is too early to tell what an alteration in these bacteria might entail. These results should be reproduced in order to be able to draw firm conclusions from it.

We found a distinctive pattern in baseline bacteria in the responders of the LFD, with less *Lactobacillus*, more *Atopobium (genus)* and less *Firmicutes (Clostridia)* compared to non-responders. If this is a true finding, it can be useful in determining who would benefit from the diet. Why exactly these bacteria might predict response is not so clear. *Clostridia* (phylum *Firmicutes)* is an abundant genus in the gut, important for homeostasis, and metabolic and immune functions [173]. *Atopobium* is a genus under the *Actinobacteria* phylum. An

important feature for bacteria in both these genera is the fermenting capacity of non-digestible carbohydrates and production of SCFA, called saccharolytic bacteria. A pattern with higher abundance of saccharolytic bacteria at baseline was found in responders to a LFD in children with IBS [117]. A possible mechanism for why more of these bacteria could determine diet response is their production of SCFA and gas creating symptoms in IBS patients. It is likely that those with lot of these bacteria would respond symptomatically to a diet where one cuts down on the main substrate for these bacteria, thus starving them. In our patients, there was more of some saccharolytic bacteria and less of others. It is not clear why exactly this would predict a response, but perhaps some saccharolytic bacteria are more important than others when it comes to diet response? Or maybe less *Firmicutes (Clostridia)* is an expression for higher abundance of other bacteria that determine diet response?

An unexpected finding of the breath tests was the lack of reduction in the AUC for hydrogen after a LFD, despite a reduction in FODMAP intake and symptom relief. Reduction in fermentable substrate should in theory lead to less production of hydrogen from colonic bacteria. This has been shown in previous studies in patients with IBS on a LFD [85, 133]. Hydrogen breath test has been used in a LFD study to assess adherence to the diet, where a reduction in hydrogen was associated with a successful reduction in FODMAP intake and fermentation [85]. If we were to transfer this onto our study, our results imply that the patients did not reduce their FODMAP intake during the intervention. This is not very likely, with a self-reported compliance of 93% in group B and a reduction of FODMAPs from a mean of 11.5 to 1.6g confirmed by patients' food records. One can speculate also here, that since the FODMAP intake at baseline was relatively low, a further reduction did not impact the hydrogen production. It is also noteworthy that we did not see a shared decrease in fermenting bacteria after diet amongst the patients following the LFD. However, maybe another method would have been better for assessing the degree of fermentation. We decided to perform the breath test based on the food the patients had eaten the day before, but we had no information on what their last meal before the breath test was, and the patients were given no restrictions other than to follow their assigned diet. This opens for the possibility that some ate slowly digestible foods, which might have increased the amount of hydrogen. There was also seen a bigger reduction in hydrogen in group A, whom did not change their total FODMAP intake. Maybe more patients in this group by random ate easily digestible GFD meals containing low FODMAP the night before the second breath tests were taken? It might have been a better approach to ask the patients to bring their habitual breakfast to eat, and measured excreted

hydrogen based on that meal for three or more hours. This is more similar to previous methods used when assessing the LFD, which have shown reduced hydrogen production when on diet.

Methane was produced in ten individuals at baseline. Six of these had IBS-C, three had IBS-M and one had IBS-D. This is in accordance with previous data, showing that methane production is associated with constipation. One patient in group A did not produce methane after six weeks, which might be due to change in bacterial composition of methanogens. This does not imply that methanogenic bacteria are absent, as they are present in the microbiota of the majority of the population. However, they need to be in a sufficient amount for methane gas to be detected [174].

We detected an effect of the LFD on symptoms and give evidence for the effect of low FODMAP diet in CD patients with IBS-like symptoms, measured by IBS-SSS score reduction. A further improvement in symptoms during the last three weeks of diet shows that an elimination period of six weeks seems reasonable to evaluate effect and exclude a placebo effect. We also detected statistically significant symptom improvement in group A. Although not as effective as the LFD, it seems that some patients also benefit from excluding wheat starch and trace amounts of gluten from their diet. This is supported by serologic tests. It is also possible that some were stricter with their GFD in general during the intervention, and therefore experienced some symptom relief.

Despite being a complex diet, there is reported a high compliance of the LFD in previous studies, and adherence of up to 75% could be expected. In accordance with this, our patients reported an adherence of 93%, which is likely to result from the patients experiencing symptom relief. There was observed a reduction in energy and fat intake in group B, which may be a result of a limited choice of foods. A reduction in fat and protein intake was also seen in group A, which implies that a diet intervention itself may lead to a lower food intake, perhaps due to more awareness of what one eats and/or skipping certain meals/snacks to avoid having to weigh and register. There is reported an underestimation of energy intake when registering food intake, but this should however be similar at baseline and six weeks. In general, the patients seemed to maintain a rather balanced diet throughout the intervention, also in fibre intake and calcium which is one of the concerns when following a LFD. The patients were encouraged to use lactose-free products and many did not need to change their

major carbohydrate sources, such as bread and pasta, due to already being on a GFD. This may have helped maintain an adequate intake. A reduction in vitamin D in group A may be season related, but also associated with the reduction in protein intake which may include less foods rich in vitamin D.

It is fair to say that in general the patients seemed satisfied with the LFD instructions, as nine were very satisfied and nine were satisfied, but one patient answered "OK" and one was not satisfied. This might be an expression for the complexity of the diet, and that although instructed by a dietitian it can be difficult to comprehend the diet. The patients were not supplied with any meals during the intervention, which requires more of the patients in terms of planning and knowledge of the diet. However, the patients were encouraged to make contact if they had questions of any kind. Dietitian delivered diet instructions therefore seems important to ensure comprehension and compliance.

4.3 Study limitations

4.3.1 Recruitment and inclusion criteria

We should have kept record of how many patients we assessed for inclusion, later contacted and from where they came during the recruiting phase of patients. This would have given us a clearer picture of what patient group we actually included in the study, and how representative they were for the CD population.

Perhaps we should have excluded patients who had positive serology, as this finding indicates gluten contamination in the diet. Also, patients with possible gluten contamination in diet can have an ongoing inflammation which can affect microbiota, thus dysbiosis in these patients may be a consequence of gluten in their diet. Although recent abnormal histological findings was an exclusion criterion, we did not collect new duodenal biopsies. Thus, we cannot rule out RCD completely as a cause of persistent symptoms.

Some of the patients were already familiar with the term FODMAP, and some randomized to group B were excited to try the diet, whilst some in group A were disappointed because they wanted to try the FODMAP-diet. This might have created some bias, with a placebo effect in group B due to expectations of effect, and a nocebo effect in group A, where some patients expected the intervention to have little or no effect on their symptoms. The intervention in

group A did not involve any great changes in the patients' diets, which also may have contributed to a nocebo effect. We could have excluded all patients with knowledge of FODMAP in order to reduce the possible placebo/nocebo-effect. However, this would have protracted the recruitment process and made it more difficult.

Ideally, we should have had a larger study sample. This could have given bigger effects of the intervention, as well as given us the opportunity to draw firmer conclusions from the study, rather than observations. Still, the patient number was adequate for assessing symptom relief.

4.3.2 Data collection

We did not weigh the patients, so the weight they reported at baseline is not necessarily as accurate as it could have been. We did not weigh the patients after the diet intervention either, which would have been interesting, as both group consumed less calories at six weeks according to their food records. There was a significant reduction in energy and fat intake in group B, and in fat and protein in group A. There are also studies on the low FODMAP diet that report a lower intake of calories during the diet, which may be an unwanted effect of the diet.

We did not measure total IgA in the patients, so we cannot know whether those with elevated IgG DPG have IgA deficiency or not. Thus, only those with elevated IgA TTG were considered as having elevated antibodies in this study.

4.3.3 Diet instructions

The diet instructions were not given in the same way for all patients, as we were two students who shared this task and took turns on instructing patients in a low FODMAP diet and a strict gluten free diet. We also got more experienced in instructing along the study, which also will lead to some differences in how well the patients were instructed.

4.3.4 Method for microbiota analysis

The GA technology was useful for determining dysbiosis, and provided a microbiota profile report for each patient. This profile included data on bacteria relevant for gastrointestinal disorders, which is very central in this thesis. The test also correlates well with Illumina deep sequencing, as shown in their validation study [122]. However, this method did not provide

information on general bacterial diversity or richness. And although the human gut microbiota is starting to get more defined, the use of probes cannot detect unknown bacteria or low abundance bacteria [111, 122]. PCR amplification may also cause some bias. Due to such limitations, whole genome sequencing is considered to be the ideal analysis which provides the most information on bacterial composition, but this cost much more money and is more time consuming. Another alternative is shotgun metagenomic sequencing where also the functionalities of the bacteria are detected. It has been suggested that the metabolic functionalities of the bacteria are more important than taxonomy [111].

4.3.5 Calculation of FODMAP-content

As described under methods, we used Norwegian, Danish and Australian data to calculate FODMAP-content and intake. Most data except lactose were based on Australian analyses, which may differ in FODMAP-content from Norwegian foods and therefore makes our estimations uncertain. For composite dishes, we used standardized recipes and portion sizes to estimate the FODMAP-content. This is probably the most correct way to find the FODMAP-content (unless the patient had specified the recipe and portion size), but still makes our estimates somewhat imprecise. There was also missing data on FODMAP-content for some foods known to contain FODMAPs, so the estimation of total FODMAPs in this study is probably lower than in real life. Total FODMAP-content was calculated and divided into lactose and non-lactose. The ideal would have been to separate between all the different FODMAPs, because they have different effect on the gastrointestinal physiology [83, 175]. Due to time limitations, we were not able to do that. We still wanted to divide between lactose and other FODMAPs, because lactose accounts for a large part of the total FODMAP-content in the diet, but is not likely the biggest symptom trigger in Norwegians.

4.4 Future aspects

Defining this patient group in terms of identifying the cause for the IBS-like symptoms requires further investigation. Although their symptoms resemble those seen in IBS patients, it is not certain that it is a subgroup of IBS.

Dysbiosis should also be assessed in CD patients without IBS like symptoms and in untreated CD patients to determine whether the degree of dysbiosis detected in this study is distinct for the specific patient group or not. The bacterial composition of the duodenal microbiota in

these patients should also be assessed, which is likely to differ from the luminal microbiota and is more involved with the epithelial cells and the immune system.

This study showed that a larger fraction of these patients had dysbiosis and a reduction of *Bifidobacteria* at baseline, as well as deviations in other bacteria. Based on these findings, it would have been interesting to test probiotics as a mean for symptom relief in this patient group. If dysbiosis in fact contributes to the IBS-like symptoms, it is possible that probiotics can give symptom relief.

5 CONCLUSION

We found in this study that 45% of the CD patients were dysbiotic at baseline, which is more than healthy controls, but less than what is found in IBS patients.

We did not find the same negative effect of the low FODMAP diet on microbiota as seen in previous studies on IBS patients. Whether this is caused by a different bacterial composition at baseline, a less radical intervention or different response to the FODMAP diet than IBS patients is hard to tell. A small study sample does not allow us to draw any firm conclusions from this finding.

Although we cannot determine if these patients suffer from IBS in addition to their CD, if CD has triggered IBS or if other mechanisms cause IBS-like symptoms, these patients do resemble IBS patients symptom-wise. Unless any contraindications, they should be offered the same dietary treatment as IBS patients, namely a low FODMAP diet in addition to their gluten free diet. This study has shown it to be effective for symptom relief.

We also found a pattern of bacteria at baseline predictive of response to a low FODMAP diet. These patients seem to have a microbiota composition characterized by less *Lactobacillus* and *Firmicutes (Clostridia),* and more *Atopobium.*

6 REFERENCES

1. Hahn, M., et al., *Modern diagnosis of celiac disease and relevant differential diagnoses in the case of cereal intolerance.* Allergo J Int, 2014. **23**(2): p. 67-77.
2. Lebwohl, B., J.F. Ludvigsson, and P.H. Green, *Celiac disease and non-celiac gluten sensitivity.* Bmj, 2015. **351**: p. h4347.
3. Dieli-Crimi, R., M.C. Cenit, and C. Nunez, *The genetics of celiac disease: A comprehensive review of clinical implications.* J Autoimmun, 2015. **64**: p. 26-41.
4. Carlsson, A., *Currently diagnosed cases of coeliac disease are just the tip of the iceberg.* Acta Paediatr, 2016. **105**(4): p. 346-8.
5. Wierdsma, N.J., et al., *Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients.* Nutrients, 2013. **5**(10): p. 3975-92.
6. Rashid, M. and J. Lee, *Serologic testing in celiac disease: Practical guide for clinicians.* Can Fam Physician, 2016. **62**(1): p. 38-43.
7. Downey, L., et al., *Recognition, assessment, and management of coeliac disease: summary of updated NICE guidance.* Bmj, 2015. **351**: p. h4513.
8. Fraser-Mayall, H., *Coeliac disease*, in *The Manual of Dietetic Practice*, J. Gandy and T.B.D. Association, Editors. 2014, WILEY Blackwell. p. 426-441.
9. Green, P.H., K. Rostami, and M.N. Marsh, *Diagnosis of coeliac disease.* Best Pract Res Clin Gastroenterol, 2005. **19**(3): p. 389-400.
10. Oberhuber, G., G. Granditsch, and H. Vogelsang, *The histopathology of coeliac disease: time for a standardized report scheme for pathologists.* Eur J Gastroenterol Hepatol, 1999. **11**(10): p. 1185-94.
11. Caja, S., et al., *Antibodies in celiac disease: implications beyond diagnostics.* Cell Mol Immunol, 2011. **8**(2): p. 103-9.
12. Sollid, L.M., et al., *Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer.* J Exp Med, 1989. **169**(1): p. 345-50.
13. Kaukinen, K., et al., *HLA-DQ typing in the diagnosis of celiac disease.* Am J Gastroenterol, 2002. **97**(3): p. 695-9.
14. Sollid, L.M., *Coeliac disease: dissecting a complex inflammatory disorder.* Nat Rev Immunol, 2002. **2**(9): p. 647-55.
15. Barker, J.M. and E. Liu, *Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions.* Adv Pediatr, 2008. **55**: p. 349-65.
16. Kagnoff, M.F., *Celiac disease: pathogenesis of a model immunogenetic disease.* J Clin Invest, 2007. **117**(1): p. 41-9.
17. Mesin, L., L.M. Sollid, and R. Di Niro, *The intestinal B-cell response in celiac disease.* Front Immunol, 2012. **3**: p. 313.
18. Meresse, B., G. Malamut, and N. Cerf-Bensussan, *Celiac disease: an immunological jigsaw.* Immunity, 2012. **36**(6): p. 907-19.
19. Alaedini, A. and P.H. Green, *Autoantibodies in celiac disease.* Autoimmunity, 2008. **41**(1): p. 19-26.
20. Murray, J.A., et al., *Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease.* Am J Clin Nutr, 2004. **79**(4): p. 669-73.
21. Burgin-Wolff, A., et al., *Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease.* Scand J Gastroenterol, 2002. **37**(6): p. 685-91.
22. O'Mahony, S., P.D. Howdle, and M.S. Losowsky, *Review article: management of patients with non-responsive coeliac disease.* Aliment Pharmacol Ther, 1996. **10**(5): p. 671-80.

23. Corrao, G., et al., *Mortality in patients with coeliac disease and their relatives: a cohort study.* Lancet, 2001. **358**(9279): p. 356-61.
24. Boettcher, E. and S.E. Crowe, *Dietary proteins and functional gastrointestinal disorders.* Am J Gastroenterol, 2013. **108**(5): p. 728-36.
25. Stasi, E., et al., *Frequency and Cause of Persistent Symptoms in Celiac Disease Patients on a Long-term Gluten-free Diet.* J Clin Gastroenterol, 2016. **50**(3): p. 239-43.
26. Leffler, D.A., et al., *Etiologies and predictors of diagnosis in nonresponsive celiac disease.* Clin Gastroenterol Hepatol, 2007. **5**(4): p. 445-50.
27. Abdallah, H., et al., *Refractory celiac disease.* Curr Gastroenterol Rep, 2007. **9**(5): p. 401-5.
28. Rubio-Tapia, A., et al., *ACG clinical guidelines: diagnosis and management of celiac disease.* Am J Gastroenterol, 2013. **108**(5): p. 656-76; quiz 677.
29. Mooney, P.D., et al., *Treatment failure in coeliac disease: a practical guide to investigation and treatment of non-responsive and refractory coeliac disease.* J Gastrointestin Liver Dis, 2012. **21**(2): p. 197-203.
30. Ryan, B.M. and D. Kelleher, *Refractory celiac disease.* Gastroenterology, 2000. **119**(1): p. 243-51.
31. Spatola, B.N., et al., *Persistence of elevated deamidated gliadin peptide antibodies on a gluten-free diet indicates nonresponsive coeliac disease.* Aliment Pharmacol Ther, 2014. **39**(4): p. 407-17.
32. Kagnoff, M.F., *Introduction: celiac disease.* Semin Immunopathol, 2012. **34**(4): p. 471-2.
33. Malamut, G. and C. Cellier, *Complications of coeliac disease.* Best Pract Res Clin Gastroenterol, 2015. **29**(3): p. 451-8.
34. Chey, W.D., J. Kurlander, and S. Eswaran, *Irritable bowel syndrome: a clinical review.* Jama, 2015. **313**(9): p. 949-58.
35. Drossman, D.A., et al., *Severity in irritable bowel syndrome: a Rome Foundation Working Team report.* Am J Gastroenterol, 2011. **106**(10): p. 1749-59; quiz 1760.
36. Ostgaard, H., et al., *Diet and effects of diet management on quality of life and symptoms in patients with irritable bowel syndrome.* Mol Med Rep, 2012. **5**(6): p. 1382-90.
37. Lovell, R.M. and A.C. Ford, *Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis.* Clin Gastroenterol Hepatol, 2012. **10**(7): p. 712-721.e4.
38. Vandvik, P.O., S. Lydersen, and P.G. Farup, *Prevalence, comorbidity and impact of irritable bowel syndrome in Norway.* Scand J Gastroenterol, 2006. **41**(6): p. 650-6.
39. Mulak, A. and Y. Tache, *Sex difference in irritable bowel syndrome: do gonadal hormones play a role?* Gastroenterol Pol, 2010. **17**(2): p. 89-97.
40. Longstreth, G.F. and G. Wolde-Tsadik, *Irritable bowel-type symptoms in HMO examinees. Prevalence, demographics, and clinical correlates.* Dig Dis Sci, 1993. **38**(9): p. 1581-9.
41. Heitkemper, M., et al., *Impact of sex and gender on irritable bowel syndrome.* Biol Res Nurs, 2003. **5**(1): p. 56-65.
42. Drossman, D.A., et al., *International survey of patients with IBS: symptom features and their severity, health status, treatments, and risk taking to achieve clinical benefit.* J Clin Gastroenterol, 2009. **43**(6): p. 541-50.

43. Grodzinsky, E., et al., *More negative self-esteem and inferior coping strategies among patients diagnosed with IBS compared with patients without IBS--a case-control study in primary care.* BMC Fam Pract, 2015. **16**: p. 6.
44. Han, C.J. and G.S. Yang, *Fatigue in Irritable Bowel Syndrome: A Systematic Review and Meta-analysis of Pooled Frequency and Severity of Fatigue.* Asian Nurs Res (Korean Soc Nurs Sci), 2016. **10**(1): p. 1-10.
45. Spiller, R., et al., *Guidelines on the irritable bowel syndrome: mechanisms and practical management.* Gut, 2007. **56**(12): p. 1770-98.
46. Bohn, L., et al., *Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial.* Gastroenterology, 2015. **149**(6): p. 1399-1407.e2.
47. El-Salhy, M., *Recent developments in the pathophysiology of irritable bowel syndrome.* World J Gastroenterol, 2015. **21**(25): p. 7621-36.
48. El-Salhy, M., et al., *Is irritable bowel syndrome an organic disorder?* World J Gastroenterol, 2014. **20**(2): p. 384-400.
49. Sinagra, E., et al., *Inflammation in irritable bowel syndrome: Myth or new treatment target?* World J Gastroenterol, 2016. **22**(7): p. 2242-55.
50. Keszthelyi, D., F.J. Troost, and A.A. Masclee, *Irritable Bowel Syndrome: Methods, Mechanisms, and Pathophysiology. Methods to assess visceral hypersensitivity in irritable bowel syndrome.* American Journal of Physiology - Gastrointestinal and Liver Physiology, 2012. **303**(2): p. G141-G154.
51. Agrawal, A. and P.J. Whorwell, *Review article: abdominal bloating and distension in functional gastrointestinal disorders--epidemiology and exploration of possible mechanisms.* Aliment Pharmacol Ther, 2008. **27**(1): p. 2-10.
52. Simren, M., et al., *Intestinal microbiota in functional bowel disorders: a Rome foundation report.* Gut, 2013. **62**(1): p. 159-76.
53. Posserud, I., A. Ersryd, and M. Simren, *Functional findings in irritable bowel syndrome.* World J Gastroenterol, 2006. **12**(18): p. 2830-8.
54. Bures, J., et al., *Small intestinal bacterial overgrowth syndrome.* World J Gastroenterol, 2010. **16**(24): p. 2978-90.
55. Quartero, A.O., et al., *Bulking agents, antispasmodic and antidepressant medication for the treatment of irritable bowel syndrome.* Cochrane Database Syst Rev, 2005(2): p. Cd003460.
56. Sanders, M.E., *Probiotics: definition, sources, selection, and uses.* Clin Infect Dis, 2008. **46 Suppl 2**: p. S58-61; discussion S144-51.
57. Hoveyda, N., et al., *A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome.* BMC Gastroenterol, 2009. **9**: p. 15.
58. Gough, E., H. Shaikh, and A.R. Manges, *Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection.* Clin Infect Dis, 2011. **53**(10): p. 994-1002.
59. Tan, G., D.C. Hammond, and G. Joseph, *Hypnosis and irritable bowel syndrome: a review of efficacy and mechanism of action.* Am J Clin Hypn, 2005. **47**(3): p. 161-78.
60. Drossman, D.A., et al., *Cognitive-behavioral therapy versus education and desipramine versus placebo for moderate to severe functional bowel disorders.* Gastroenterology, 2003. **125**(1): p. 19-31.
61. Bohn, L., et al., *Self-Reported Food-Related Gastrointestinal Symptoms in IBS Are Common and Associated With More Severe Symptoms and Reduced Quality of Life.* Am J Gastroenterol, 2013. **108**(5): p. 634-641.

62. Simren, M., et al., *Food-related gastrointestinal symptoms in the irritable bowel syndrome.* Digestion, 2001. **63**(2): p. 108-15.
63. Biesiekierski, J.R. and J. Iven, *Non-coeliac gluten sensitivity: piecing the puzzle together.* United European Gastroenterol J, 2015. **3**(2): p. 160-5.
64. Biesiekierski, J.R., et al., *No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates.* Gastroenterology, 2013. **145**(2): p. 320-8.e1-3.
65. Ahmad, O.F. and A. Akbar, *Dietary treatment of irritable bowel syndrome.* Br Med Bull, 2015. **113**(1): p. 83-90.
66. Spiegel, B.M., et al., *Testing for celiac sprue in irritable bowel syndrome with predominant diarrhea: a cost-effectiveness analysis.* Gastroenterology, 2004. **126**(7): p. 1721-32.
67. Sanders, D.S., et al., *A primary care cross-sectional study of undiagnosed adult coeliac disease.* Eur J Gastroenterol Hepatol, 2003. **15**(4): p. 407-13.
68. Shahbazkhani, B., et al., *Coeliac disease presenting with symptoms of irritable bowel syndrome.* Aliment Pharmacol Ther, 2003. **18**(2): p. 231-5.
69. O'Leary, C., et al., *Celiac disease and irritable bowel-type symptoms.* Am J Gastroenterol, 2002. **97**(6): p. 1463-7.
70. Hauser, W., et al., *Predictors of irritable bowel-type symptoms and healthcare-seeking behavior among adults with celiac disease.* Psychosom Med, 2007. **69**(4): p. 370-6.
71. Barratt, S.M., et al., *Reflux and irritable bowel syndrome are negative predictors of quality of life in coeliac disease and inflammatory bowel disease.* Eur J Gastroenterol Hepatol, 2011. **23**(2): p. 159-65.
72. Usai, P., et al., *Effect of gluten-free diet and co-morbidity of irritable bowel syndrome-type symptoms on health-related quality of life in adult coeliac patients.* Dig Liver Dis, 2007. **39**(9): p. 824-8.
73. Sainsbury, A., D.S. Sanders, and A.C. Ford, *Prevalence of irritable bowel syndrome-type symptoms in patients with celiac disease: a meta-analysis.* Clin Gastroenterol Hepatol, 2013. **11**(4): p. 359-65.e1.
74. Barbara, G., et al., *The immune system in irritable bowel syndrome.* J Neurogastroenterol Motil, 2011. **17**(4): p. 349-59.
75. Spiller, R. and K. Garsed, *Postinfectious irritable bowel syndrome.* Gastroenterology, 2009. **136**(6): p. 1979-88.
76. Gonsalkorale, W.M., et al., *Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component?* Gut, 2003. **52**(1): p. 91-3.
77. Halpin, S.J. and A.C. Ford, *Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis.* Am J Gastroenterol, 2012. **107**(10): p. 1474-82.
78. Quigley, E.M.M., *Overlapping irritable bowel syndrome and inflammatory bowel disease: less to this than meets the eye?* Therap Adv Gastroenterol, 2016. **9**(2): p. 199-212.
79. Barrett, J.S. and P.R. Gibson, *Clinical ramifications of malabsorption of fructose and other shortchain carbohydrates* Practical Gastroenterology, 2007. **31**: p. 51-65.
80. Khan, M.A., et al., *Low-FODMAP Diet for Irritable Bowel Syndrome: Is It Ready for Prime Time?* Dig Dis Sci, 2015. **60**(5): p. 1169-77.
81. Barrett, J.S., et al., *Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon.* Aliment Pharmacol Ther, 2010. **31**(8): p. 874-82.

82. Marciani, L., et al., *Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome.* Gastroenterology, 2010. **138**(2): p. 469-77, 477.e1.
83. Muir, J.G., et al., *Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC).* J Agric Food Chem, 2009. **57**(2): p. 554-65.
84. Gibson, P.R. and S.J. Shepherd, *Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach.* J Gastroenterol Hepatol, 2010. **25**(2): p. 252-8.
85. Halmos, E.P., et al., *A diet low in FODMAPs reduces symptoms of irritable bowel syndrome.* Gastroenterology, 2014. **146**(1): p. 67-75.e5.
86. McIntosh, K., et al., *FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial.* Gut, 2016.
87. Pedersen, N., et al., *Ehealth monitoring in irritable bowel syndrome patients treated with low fermentable oligo-, di-, mono-saccharides and polyols diet.* World J Gastroenterol, 2014. **20**(21): p. 6680-4.
88. Perez y Lopez, N., E. Torres-Lopez, and F. Zamarripa-Dorsey, *Clinical response in Mexican patients with irritable bowel syndrome treated with a low diet low in fermentable carbohydrates (FODMAP).* Rev Gastroenterol Mex, 2015. **80**(3): p. 180-5.
89. Maagaard, L., et al., *Follow-up of patients with functional bowel symptoms treated with a low FODMAP diet.* World J Gastroenterol, 2016. **22**(15): p. 4009-19.
90. Barrett, J.S., et al., *Comparison of the prevalence of fructose and lactose malabsorption across chronic intestinal disorders.* Aliment Pharmacol Ther, 2009. **30**(2): p. 165-74.
91. Farup, P.G., K.W. Monsbakken, and P.O. Vandvik, *Lactose malabsorption in a population with irritable bowel syndrome: prevalence and symptoms. A case-control study.* Scand J Gastroenterol, 2004. **39**(7): p. 645-9.
92. Turnbull, G.K., *Lactose intolerance and irritable bowel syndrome.* Nutrition, 2000. **16**(7-8): p. 665-6.
93. Vandenplas, Y., *Lactose intolerance.* Asia Pac J Clin Nutr, 2015. **24 Suppl 1**: p. S9-13.
94. Shepherd, S.J., M.C. Lomer, and P.R. Gibson, *Short-chain carbohydrates and functional gastrointestinal disorders.* Am J Gastroenterol, 2013. **108**(5): p. 707-17.
95. Barrett, J.S. and P.R. Gibson, *Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) and nonallergic food intolerance: FODMAPs or food chemicals?* Therap Adv Gastroenterol, 2012. **5**(4): p. 261-8.
96. Lomer, M.C., *Review article: the aetiology, diagnosis, mechanisms and clinical evidence for food intolerance.* Aliment Pharmacol Ther, 2015. **41**(3): p. 262-75.
97. de Roest, R.H., et al., *The low FODMAP diet improves gastrointestinal symptoms in patients with irritable bowel syndrome: a prospective study.* Int J Clin Pract, 2013. **67**(9): p. 895-903.
98. Shepherd, S.J. and P.R. Gibson, *Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management.* J Am Diet Assoc, 2006. **106**(10): p. 1631-9.
99. Gearry, R.B., et al., *Reduction of dietary poorly absorbed short-chain carbohydrates (FODMAPs) improves abdominal symptoms in patients with inflammatory bowel disease-a pilot study.* J Crohns Colitis, 2009. **3**(1): p. 8-14.

100. Bocci, V., *The neglected organ: bacterial flora has a crucial immunostimulatory role.* Perspect Biol Med, 1992. **35**(2): p. 251-60.
101. Carding, S., et al., *Dysbiosis of the gut microbiota in disease.* Microb Ecol Health Dis, 2015. **26**: p. 26191.
102. Aagaard, K., et al., *The placenta harbors a unique microbiome.* Sci Transl Med, 2014. **6**(237): p. 237ra65.
103. Wassenaar, T.M. and P. Panigrahi, *Is a foetus developing in a sterile environment?* Lett Appl Microbiol, 2014. **59**(6): p. 572-9.
104. Moles, L., et al., *Bacterial diversity in meconium of preterm neonates and evolution of their fecal microbiota during the first month of life.* PLoS One, 2013. **8**(6): p. e66986.
105. DiGiulio, D.B., *Diversity of microbes in amniotic fluid.* Semin Fetal Neonatal Med, 2012. **17**(1): p. 2-11.
106. Jimenez, E., et al., *Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section.* Curr Microbiol, 2005. **51**(4): p. 270-4.
107. Canani, R.B., et al., *Epigenetic mechanisms elicited by nutrition in early life.* Nutr Res Rev, 2011. **24**(2): p. 198-205.
108. Kashtanova, D.A., et al., *Association between the gut microbiota and diet: Fetal life, early childhood, and further life.* Nutrition, 2015.
109. Hajela, N., et al., *Gut microbiome, gut function, and probiotics: Implications for health.* Indian J Gastroenterol, 2015. **34**(2): p. 93-107.
110. Arumugam, M., et al., *Enterotypes of the human gut microbiome.* Nature, 2011. **473**(7346): p. 174-80.
111. Salazar, N., et al., *The human intestinal microbiome at extreme ages of life. Dietary intervention as a way to counteract alterations.* Front Genet, 2014. **5**: p. 406.
112. De Filippo, C., et al., *Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa.* Proc Natl Acad Sci U S A, 2010. **107**(33): p. 14691-6.
113. Muir, J.G. and P.R. Gibson, *The Low FODMAP Diet for Treatment of Irritable Bowel Syndrome and Other Gastrointestinal Disorders.* Gastroenterol Hepatol (N Y), 2013. **9**(7): p. 450-2.
114. Staudacher, H.M., et al., *Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome.* J Nutr, 2012. **142**(8): p. 1510-8.
115. Halmos, E.P., et al., *Diets that differ in their FODMAP content alter the colonic luminal microenvironment.* Gut, 2015. **64**(1): p. 93-100.
116. Halmos, E.P., et al., *Consistent Prebiotic Effect on Gut Microbiota With Altered FODMAP Intake in Patients with Crohn's Disease: A Randomised, Controlled Cross-Over Trial of Well-Defined Diets.* Clin Transl Gastroenterol, 2016. **7**: p. e164.
117. Chumpitazi, B.P., et al., *Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome.* Aliment Pharmacol Ther, 2015. **42**(4): p. 418-27.
118. Gong, J. and C. Yang, *Advances in the methods for studying gut microbiota and their relevance to the research of dietary fiber functions.* Food Research International, 2012. **48**(2): p. 916-929.
119. Sekirov, I., et al., *Gut microbiota in health and disease.* Physiol Rev, 2010. **90**(3): p. 859-904.
120. Kircher, M. and J. Kelso, *High-throughput DNA sequencing--concepts and limitations.* Bioessays, 2010. **32**(6): p. 524-36.

121. Petersen, C. and J.L. Round, *Defining dysbiosis and its influence on host immunity and disease.* Cell Microbiol, 2014. **16**(7): p. 1024-33.
122. Casen, C., et al., *Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD.* Aliment Pharmacol Ther, 2015. **42**(1): p. 71-83.
123. Salonen, A., W.M. de Vos, and A. Palva, *Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives.* Microbiology, 2010. **156**(Pt 11): p. 3205-15.
124. Jeffery, I.B., et al., *An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota.* Gut, 2012. **61**(7): p. 997-1006.
125. Kassinen, A., et al., *The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects.* Gastroenterology, 2007. **133**(1): p. 24-33.
126. Rajilic-Stojanovic, M., et al., *Intestinal microbiota and diet in IBS: causes, consequences, or epiphenomena?* Am J Gastroenterol, 2015. **110**(2): p. 278-87.
127. Cenit, M.C., et al., *Intestinal Microbiota and Celiac Disease: Cause, Consequence or Co-Evolution?* Nutrients, 2015. **7**(8): p. 6900-23.
128. McLean, M.H., et al., *Does the microbiota play a role in the pathogenesis of autoimmune diseases?* Gut, 2015. **64**(2): p. 332-41.
129. Marasco, G., et al., *Gut Microbiota and Celiac Disease.* Dig Dis Sci, 2016.
130. Gasbarrini, A., et al., *Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference.* Aliment Pharmacol Ther, 2009. **29 Suppl 1**: p. 1-49.
131. Simrén, M. and P. Stotzer, *Use and abuse of hydrogen breath tests.* Gut, 2006. **55**(3): p. 297-303.
132. Rana, S.V. and A. Malik, *Breath tests and irritable bowel syndrome.* World J Gastroenterol, 2014. **20**(24): p. 7587-601.
133. Ong, D.K., et al., *Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome.* J Gastroenterol Hepatol, 2010. **25**(8): p. 1366-73.
134. Kunkel, D., et al., *Methane on breath testing is associated with constipation: a systematic review and meta-analysis.* Dig Dis Sci, 2011. **56**(6): p. 1612-8.
135. Chatterjee, S., et al., *The degree of breath methane production in IBS correlates with the severity of constipation.* Am J Gastroenterol, 2007. **102**(4): p. 837-41.
136. Pimentel, M., et al., *Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity.* Am J Physiol Gastrointest Liver Physiol, 2006. **290**(6): p. G1089-95.
137. Di Stefano, M., et al., *Breath Methane Excretion Is not An Accurate Marker of Colonic Methane Production in Irritable Bowel Syndrome.* Am J Gastroenterol, 2015. **110**(6): p. 891-8.
138. Lee, K.N., et al., *Association between symptoms of irritable bowel syndrome and methane and hydrogen on lactulose breath test.* J Korean Med Sci, 2013. **28**(6): p. 901-7.
139. Suresh, K., *An overview of randomization techniques: An unbiased assessment of outcome in clinical research.* J Hum Reprod Sci, 2011. **4**(1): p. 8-11.
140. Shih, D.Q. and L.Y. Kwan, *All Roads Lead to Rome: Update on Rome III Criteria and New Treatment Options.* Gastroenterol Rep, 2007. **1**(2): p. 56-65.

141. Francis, C.Y., J. Morris, and P.J. Whorwell, *The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress.* Aliment Pharmacol Ther, 1997. **11**(2): p. 395-402.
142. Farivar, S.S., W.E. Cunningham, and R.D. Hays, *Correlated physical and mental health summary scores for the SF-36 and SF-12 Health Survey, V.I.* Health Qual Life Outcomes, 2007. **5**: p. 54.
143. Optum. *Optum™ SF-36v2® Health Survey*. 2016 [cited 2016 10.04.16]; Available from: https://www.optum.com/optum-outcomes/what-we-do/health-surveys/sf-36v2-health-survey.html.
144. Biesiekierski, J.R., et al., *Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals.* J Hum Nutr Diet, 2011. **24**(2): p. 154-76.
145. Meieriprodukter, O.f. *Laktoseinnhold i meieriproduktene*. 2013 [cited 2016 10.04.2016]; Available from: http://www.melk.no/laktoseintoleranse/laktoseinnhold-i-meieriproduktene/.
146. Muir, J., *FODMAPs across the globe: FODMAP composition of Australian and international foods and challenges of implementing a low FODMAP diet in the USA*, in *Paper presented at the Gastrodiet 2015, Monash University, Prato, Italy.* 2016, November.
147. Wacklin, P., et al., *Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet.* Am J Gastroenterol, 2014. **109**(12): p. 1933-41.
148. Rangel, I., et al., *The relationship between faecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients and healthy subjects.* Aliment Pharmacol Ther, 2015. **42**(10): p. 1211-21.
149. Zoetendal, E.G., et al., *Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces.* Appl Environ Microbiol, 2002. **68**(7): p. 3401-7.
150. Carroll, I.M., et al., *Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome.* Gut Pathog, 2010. **2**(1): p. 19.
151. Tang, M.S., et al., *Inferred metagenomic comparison of mucosal and fecal microbiota from individuals undergoing routine screening colonoscopy reveals similar differences observed during active inflammation.* Gut Microbes, 2015. **6**(1): p. 48-56.
152. Tursi, A., G. Brandimarte, and G. Giorgetti, *High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal.* Am J Gastroenterol, 2003. **98**(4): p. 839-43.
153. Flint, H.J., et al., *Microbial degradation of complex carbohydrates in the gut.* Gut Microbes, 2012. **3**(4): p. 289-306.
154. Lappi, J., et al., *Intake of whole-grain and fiber-rich rye bread versus refined wheat bread does not differentiate intestinal microbiota composition in Finnish adults with metabolic syndrome.* J Nutr, 2013. **143**(5): p. 648-55.
155. Wu, G.D., et al., *Linking long-term dietary patterns with gut microbial enterotypes.* Science, 2011. **334**(6052): p. 105-8.
156. Roberfroid, M., et al., *Prebiotic effects: metabolic and health benefits.* Br J Nutr, 2010. **104 Suppl 2**: p. S1-63.
157. De Palma, G., et al., *Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects.* Br J Nutr, 2009. **102**(8): p. 1154-60.

158. Kerckhoffs Aè, P.M., et al., *Lower Bifidobacteria counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients.* World J Gastroenterol, 2009. **15**(23): p. 2887-92.
159. Collado, M.C., et al., *Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease.* J Clin Pathol, 2009. **62**(3): p. 264-9.
160. Di Cagno, R., et al., *Different Fecal Microbiotas and Volatile Organic Compounds in Treated and Untreated Children with Celiac Disease.* Appl Environ Microbiol, 2009. **75**(12): p. 3963-71.
161. Caufield, P.W., et al., *Natural History of Streptococcus sanguinis in the Oral Cavity of Infants: Evidence for a Discrete Window of Infectivity.* Infect Immun, 2000. **68**(7): p. 4018-23.
162. Correa, N.B., et al., *A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants.* J Clin Gastroenterol, 2005. **39**(5): p. 385-9.
163. Saavedra, J.M., et al., *Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus.* Lancet, 1994. **344**(8929): p. 1046-9.
164. Ud-Din, A. and S. Wahid, *Relationship among Shigella spp. and enteroinvasive Escherichia coli (EIEC) and their differentiation.* Braz J Microbiol, 2014. **45**(4): p. 1131-8.
165. Sokol, H., et al., *Temperature Gradient Gel Electrophoresis of Fecal 16S rRNA Reveals Active Escherichia coli in the Microbiota of Patients with Ulcerative Colitis.* J Clin Microbiol, 2006. **44**(9): p. 3172-7.
166. Wacklin, P., et al., *The duodenal microbiota composition of adult celiac disease patients is associated with the clinical manifestation of the disease.* Inflamm Bowel Dis, 2013. **19**(5): p. 934-41.
167. Joossens, M., et al., *Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives.* Gut, 2011. **60**(5): p. 631-7.
168. Rajilic-Stojanovic, M., et al., *Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome.* Gastroenterology, 2011. **141**(5): p. 1792-801.
169. Beaud, D., P. Tailliez, and J. Anba-Mondoloni, *Genetic characterization of the beta-glucuronidase enzyme from a human intestinal bacterium, Ruminococcus gnavus.* Microbiology, 2005. **151**(Pt 7): p. 2323-30.
170. Sánchez, E., J.M. Laparra, and Y. Sanz, *Discerning the Role of Bacteroides fragilis in Celiac Disease Pathogenesis.* Appl Environ Microbiol, 2012. **78**(18): p. 6507-15.
171. Clarke, G., et al., *Review article: probiotics for the treatment of irritable bowel syndrome--focus on lactic acid bacteria.* Aliment Pharmacol Ther, 2012. **35**(4): p. 403-13.
172. de Sousa Moraes Lí, F., et al., *Intestinal Microbiota and Probiotics in Celiac Disease.* Clin Microbiol Rev, 2014. **27**(3): p. 482-9.
173. Lopetuso, L.R., et al., *Commensal Clostridia: leading players in the maintenance of gut homeostasis.* Gut Pathog, 2013. **5**(1): p. 23.
174. Triantafyllou, K., C. Chang, and M. Pimentel, *Methanogens, methane and gastrointestinal motility.* J Neurogastroenterol Motil, 2014. **20**(1): p. 31-40.
175. Murray, K., et al., *Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI.* Am J Gastroenterol, 2014. **109**(1): p. 110-9.

7 APPENDIX

Appendix 1: Rome III criteria

Appendix 2: IBS-SSS and additional questions

Appendix 3: SF-36

Appendix 4: 4-day food record registration

Appendix 5: Compliance questionnaires for low FODMAP diet (six weeks and one month after end of intervention)

Appendix 6: Compliance questionnaire for a stricter gluten free diet

Appendix 7: Information on gluten free diet

Appendix 8: Information on low FODMAP diet (recipes are excluded)

Appendix 9: Information and consent form

Appendix 10: Research protocol

Appendix 11: REC approval

Appendix 12: Abstract ESPEN

Appendix 13: Abstract UEGW

Appendix 1: Roma III-kriteriene

Diagnostikk av IBS-symptomer etter Roma III

DATO: ……..
NAVN: ……
ALDER: ……..

1. ***IBS-KRITERIER*** (Sett ring rundt svaret)

Spørsmål	Svar	
1.1.Har du vært plaget av smerter eller ubehag i magen i minst 3 dager per måned i løpet av de siste 3 månedene?	Ja	Nei
1.2.Har du hatt disse plagene i 6 måneder eller mer?	Ja	Nei
1.3.Er plagene forbundet med endret hyppighet av avføring?	Ja	Nei
1.4.Er plagene forbundet med endret form eller utseende av avføringen?	Ja	Nei
1.5.Reduseres plagene dersom du får tømt deg skikkelig for avføring?	Ja	Nei

2 Tilleggsspørsmål for å karakterisere plagene (Sett ring rundt svaret)

Spørsmål	Svar	
2.1 Hvis du har diaré, hender det at avføringen er fast inn i mellom?	Ja	Nei
2.2 Hvis du har forstoppelse, hender det at avføringen er løs inn i mellom?	Ja	Nei
2.3 Er ufullstendig tømming av avføring et problem for deg?	Ja	Nei
2.4 Har du avføring om natta?	Ja	Nei
2.5 Hva har du mest av?	Diaré	
	Forstoppelse	
	Om lag likt	

3 Kvantisering av IBS symptomer
Angis på en skala fra 0 til 10, der 0 = ingen symptomer og 10 = alvorlige symptomer

(Angi med tall fra 0 til 10)

Spørsmål Svar	
3.1 Kvalme	
3.2 Oppblåsthet	
3.3 Magesmerter	
3.4 Forstoppelse	
3.5 Diaré	
3.6 Anoreksi (ulyst på mat)	

Appendix 2: IBS-SSS

1. Har hatt tilfredsstillende lindring av dine IBS-smerter/-ubehag de siste 7 dager? Sett en ring rundt svaret ditt. **JA** **NEI**

2. a) Har du magesmerter? Sett en ring rundt svaret ditt. **JA** **NEI**

b) Dersom ja, hvor sterke er magesmertene? (marker på linja)

0 % _____ 100 %

Ingen smerte Veldig mye smerte

c) Oppgi antall dager du har kjent magesmerter i løpet av en 10 dagers periode. Dersom du f.eks. skriver 4 betyr det at du har smerte 4 av 10 dager. Om du har smerte hver dag, skriver du 10.

Antall dager med smerte: _____

3. a) Har du oppblåst og/eller spent mage? Sett en ring rundt svaret ditt. **JA** **NEI**

b) Dersom ja, hvor mye plaget er du? (marker på linja)

0 % _____ 100 %

Ingen plager Veldig mye plager

4. Hvor fornøyd er du med dine avføringsvaner? (marker på linja)

0 % _____ 100 %

Veldig fornøyd Veldig misfornøyd

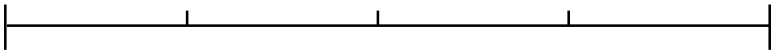
5. Angi med en strek på linja nedenfor hvor mye dine IBS-plager påvirker livet ditt generelt.

0 % _____ 100 %

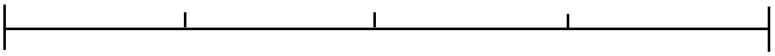
Ingen påvirkning Stor påvirkning

Lider du av følgende:

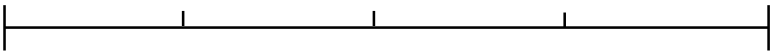
a) Kvalme og/eller oppkast?

0 % aldri  100 % hele tiden

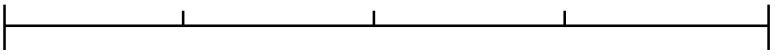
b) Vanskelig for å spise opp alt ved måltidet?

0 % aldri  100 % hele tiden

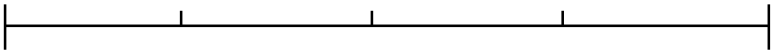
c) Hodepine?

0 % aldri  100 % hele tiden

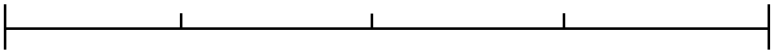
d) Ryggsmerter?

0 % aldri  100 % hele tiden

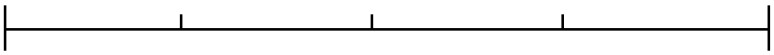
e) Uopplagt eller trøtt?

0 % aldri  100 % hele tiden

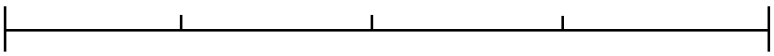
f) Raping og/eller gassavgang?

0 % aldri  100 % hele tiden

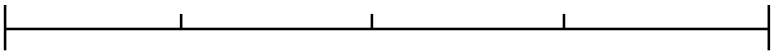
g) Halsbrann?

0 % aldri  100 % hele tiden

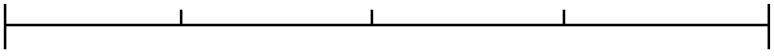
h) Hyppig eller plutselig trang til vannlating?

0 % aldri  100 % hele tiden

i) Smerter i låret?

0 % aldri  100 % hele tiden

j) Smerte i muskler og ledd?

0 % aldri  100 % hele tiden

Appendix 3: SF-36

SF-36 Norsk versjon

Vi spør deg her om hvordan du opplever din egen helse. Vi ønsker å vite hvordan du føler deg og hvordan du mestrer dine vanlige aktiviteter.

Vær snill å svare på alle spørsmål. Noen av spørsmålene ligner på hverandre, men alle er forskjellige. Ta deg tid til å lese spørsmålene nøye og svar med et kryss for det alternativ som du velger!

Takk for at du svarer på disse spørsmålene!

Pasientnummer:
Dato:
Besøksnummer:

---

1. Stort sett, vil du si at din helse er:

Utmerket	**Meget god**	**God**	**Nokså god**	**Dårlig**
○	○	○	○	○

2. **Sammenlignet med for ett år siden**, hvordan vil du si at helsen din stort sett er nå?

Mye bedre nå enn for ett år siden	**Litt bedre nå enn for ett år siden**	**Omtrent den samme som for ett år siden**	**Litt dårligere nå enn for ett år siden**	**Mye dårligere nå enn for ett år siden**
○	○	○	○	○

3. De neste spørsmålene handler om gjøremål som du kanskje utfører i løpet av en vanlig dag. Er **din helse nå slik at den begrenser deg** i utførelsen av disse aktivitetene? Hvis ja, hvor mye?

	Ja, begrenser meg mye	**Ja, begrenser meg litt**	**Nei, begrenser meg ikke i det hele tatt**
Anstrengende aktiviteter, som å løpe, løfte tunge gjenstander, delta i anstrengende idrett	○	○	○
Moderate aktiviteter, som å flytte et bord, støvsuge, gå en tur eller drive med hagearbeid	○	○	○
Løfte eller bære en handlekurv	○	○	○
Gå opp trappen **flere** etasjer	○	○	○
Gå opp trappen **en** etasje	○	○	○
Bøye deg eller sitte på huk	○	○	○
Gå **mer enn to kilometer**	○	○	○
Gå **noen hundre meter**	○	○	○
Gå **hundre meter**	○	○	○
Vaske deg eller kle på deg	○	○	○

4. I løpet av **de siste fire ukene**, har du hatt noen av følgende problemer i ditt arbeid eller i andre av dine daglige gjøremål på grunn av din fysiske helse?

	Hele tiden	**Det meste av tiden**	**En del av tiden**	**Litt av tiden**	**Ikke i det hele tatt**
Har du **redusert tiden** du har brukt på arbeidet ditt eller andre aktiviteter	○	○	○	○	○
Har du **utrettet mindre** enn du hadde ønsket	○	○	○	○	○
Har du vært hindret i visse **typer** arbeid eller andre aktiviteter	○	○	○	○	○
Har du hatt **vansker** med å utføre arbeidet ditt eller andre aktiviteter	○	○	○	○	○

(for eksempel fordi det krevde ekstra anstrengelser)					

5. I løpet av **de siste fire ukene**, har du hatt følelsesmessige problemer som har ført til vanskeligheter i ditt arbeid eller i andre av dine daglige gjøremål (for eksempel fordi du har følt deg deprimert eller engstelig)

	Hele tiden	**Det meste av tiden**	**En del av tiden**	**Litt av tiden**	**Ikke i det hele tatt**
Har du **redusert tiden** du har brukt på arbeidet ditt eller andre aktiviteter	○	○	○	○	○
Har du **utrettet mindre** enn du hadde ønsket	○	○	○	○	○
Har du ikke arbeidet eller utført andre aktiviteter **like nøye** som vanlig	○	○	○	○	○

6. I løpet av de **siste 4 ukene**, i hvilken grad har din fysiske helse eller følelsesmessige problemer hatt innvirkning på din vanlige sosiale omgang med familie, venner, naboer eller foreninger?

Ikke i det hele tatt	**Litt**	**Endel**	**Mye**	**Svært mye**
○	○	○	○	○

7. Hvor sterke **kroppslige** smerter har du hatt i løpet av **de siste 4 ukene?**

Ingen	**Meget svake**	**Svake**	**Moderate**	**Sterke**	**Meget sterke**
○	○	○	○	○	○

8. I løpet av **de siste 4 ukene**, hvor mye har smerter påvirket ditt vanlige arbeid (gjelder både arbeid utenfor hjemmet og husarbeid)?

Ikke i det hele tatt	Litt	En del	Mye	Svært mye
○	○	○	○	○

9. De neste spørsmålene handler om hvordan du har følt deg og hvordan du har hatt det **de siste 4 ukene**. For hvert spørsmål, vennligst velg det svaralternativet som best beskriver hvordan du har hatt det. Hvor ofte i løpet av **de siste 4 ukene** har du …

	Hele tiden	Det meste av tiden	Endel av tiden	Litt av tiden	Ikke i det hele tatt
følt deg full av tiltakslyst?	○	○	○	○	○
følt deg veldig nervøs?	○	○	○	○	○
vært så langt nede at ingenting har kunnet muntre deg opp?	○	○	○	○	○
følt deg rolig og harmonisk?	○	○	○	○	○
hatt mye overskudd?	○	○	○	○	○
følt deg nedfor og trist?	○	○	○	○	○
følt deg sliten?	○	○	○	○	○
følt deg glad?	○	○	○	○	○
følt deg trøtt?	○	○	○	○	○

10. I løpet av de **siste 4 ukene**, hvor mye av tiden har din fysiske helse eller følelsesmessige problemer påvirket din sosiale omgang (som det å besøke venner, slektninger osv.)?

Hele tiden	Nesten hele tiden	En del av tiden	Litt av tiden	Ikke i det hele tatt
○	○	○	○	○

11. Hvor RIKTIG eller GAL er hver av følgende påstander for deg?

	Helt riktig	**Delvis riktig**	**Vet ikke**	**Delvis gal**	**Helt gal**
Det virker som jeg blir litt lettere syk enn andre	○	○	○	○	○
Jeg er like frisk som de fleste jeg kjenner	○	○	○	○	○
Jeg forventer at min helse vil bli dårligere	○	○	○	○	○
Min helse er utmerket	○	○	○	○	○

Appendix 4: 4-day food record registration

HELSE BERGEN - ALT HANDLAR OM MENNESKE
Haukeland universitetssjukehus

KOSTREGISTRERING

NAVN _____

ADRESSE _____

_____

FØDSELSNR _____

HØYDE _____

VEKT _____

KLINISK ERNÆRINGSFYSIOLOG _____

Skjemaet returneres i utfylt stand til:

Helse Bergen HF
Avdeling for klinisk ernæring
Haukeland Universitetssjukehus
Pb 1400
5021 Bergen

Tlf. 55 97 38 32

INNEN: _____

Slik går du frem:

For at vi skal kunne beregne næringsstoffinntaket ditt så nøyaktig som mulig, er det nødvendig at du noterer *alt* du spiser og drikker i løpet av en 4 dagers sammenhengende periode. Perioden onsdag til lørdag (evt. søndag til onsdag) er best, for da får du med én helgedag.

Det er vesentlig at du spiser slik som du pleier i registreringsperioden.

- Angi klokkeslett for hver gang du spiser eller drikker noe.
- Beskriv mat og drikke så nøyaktig som mulig
  - *Brød*: Type, navn, grovhet, tykkelse på skiver, antall skiver. Ev. rundstykke, knekkebrød..
  - *Fett på brødet:* Type, navn, mengde, lett eller vanlig
  - *Pålegg:* Type, mengde, produktnavn, lett eller vanlig
  - *Middag:* Type kjøtt, fisk, kjøttfarse-/fiskeprodukt. Produktnavn. Fettprosent.
  - *Frukt og grønnsaker:* Rå, kokt eller hermetisk.
- Beskriv hvordan maten er tilberedt.
  - Kokt, bakt, stekt, grillet eller varmet i mikrobølgeovn
  - Er maten er renset for skinn og/eller fett?
- Hjemmelagede matretter beskrives i detalj, gjerne ved å skrive ned oppskriften bak på arket.
- Notér alt tilbehør, som saus, pickles, rømme, dressing eller krem, med navn/produsent. Oppgi også om du bruker sukker på gryn, grøt eller i te.
- Få med alle mellommåltider, samt tilfeldig spising og drikke utenom de faste måltidene.
- Kosttilskudd, som tran, vitamintabletter o.l. skal også noteres, med navn, produsent og mengde.
- Mengder kan beskrives på følgende måte:
  - aller helst skal du veie maten og føre mengden opp i gram
  - hvis du ikke kan veie, kan du angi mengder i husholdningsmål, som spiseskje, glass, desiliter eller antall, alt ettersom hva som er hensiktsmessig
  - oppgi størrelse på glassene du bruker i dl

Eksempel:

Kl	*Tirs* **dag** *14 / 1 / 11*	**Produktnavn/Produsent**	**Vekt**
0730	*1 butikkskåret skive kneip*	*Bakers*	*30g*
	m/ skrapet lag margarin	*Soft Soya*	
	3 høvelskiver hvitost, 16% fett	*Norvegia, Tine*	
	1 stor grapefrukt		*200g*
	1 stort glass lettmelk (Stort glass = 2 dl)	*Tine*	
1100	*1 beger fruktyoghurt*	*Yoplait Dobbel 0%, mango*	*125g*
	1 melkesjokolade	*Freia*	*100g*
	1 kopp svart kaffe		*150g*
1500	*kokt torsk*		*140g*
	3 små potete, kokt		*150g*
	3 toppede ss revet gulrot		
	1 ss remulade	*Idun*	
	2 store glass saft	*Lerum uten tilsatt sukker*	

Kl	..... dag ... / ... / ...	**Produktnavn/Produsent**	Vekt

Kl	..... dag ... / ... / ...	**Produktnavn/Produsent**	Vekt

Kl	..... dag ... / ... / ...	**Produktnavn/Produsent**	Vekt

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Kl	..... dag ... / ... / ...	**Produktnavn/Produsent**	Vekt

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Appendix 5: Compliance questionnaires for the low FODMAP diet

Overholdelse av lav-FODMAP dietten gjennom 6 uker

Hvor fornøyd er du med lav-FODMAP dietten som symptomlindring?

Svært fornøyd Svært misfornøyd

0% _____ 100%

Kan du tenke deg å fortsette på dietten:

- O Ja
- O Kanskje
- O Nei
- O Kun dersom jeg får videre veiledning

Hvis nei, hvorfor:

- O For tidkrevende
- O Savner for mange matvarer
- O Ble ikke bedre
- O For dyrt

Hvor nøye har du fulgt lav-FODMAP dietten gjennom de 6 ukene?

Ikke fulgt den i det hele tatt Kun spist lav-FODMAP mat

0% _____ 100%

Hvor ofte hadde du avvik fra dietten løpet av de 6 ukene:

- O Ingen ganger
- O 1-5 ganger i løpet av de 6 ukene
- O 1-3 ganger i uken
- O 4-6 ganger i uken

Hvor store mengder FODMAPs inntok du ved avvik fra dietten?

- O En munnfull
- O 2-5 munnfull
- O Et helt måltid
- O Alle måltidene i løpet av dagen

Hvor lenge gikk du på dietten før du spiste matvarer med FODMAPs :

- O Ingen dager
- O 1-3 dager
- O 4-7 dager
- O 2-3 uker
- O 3-5 uker

Hvilken matvarer inneholdt avvik fra dietten:

- O **Fruktose**holdige matvarer som eple, pære, honning, juice, tørket frukt (rosiner, svisker, aprikos), asparges
- O **Laktose**holdige matvarer som melk/fløte/yoghurt og matvarer med laktose (vafler, boller, kaker, is etc.), melkesjokolade.
- O **Fruktan**holdige matvarer som inneholder hvete, rug og bygg som for eksempel brød, boller, vafler, kjeks, middagsmat med hvetemel.
- O **Fruktan**holdige matvarer som inneholder løk eller hvitløk, f.eks middagsmat, krydder, ferdigretter.
- O **Galaktan**holdige matvarer som bønner, linser, kikerter eller pistasjnøtter.
- O **Polyoler** som man finner i sukkerfrie pastiller eller tyggis.
- O **Polyoler** som man finner i avokado, aprikos, blomkål, plomme, sopp, vannmelon.

Hvordan synes du det var å følge dietten:

Kjempelett Veldig utfordrende

0% 100%

Hvorfor spiste du matvarer som inneholdt FODMAPs:

- O Spiste kun lav-FODMAP mat
- O Ikke tilgang på lav-FODMAP mat på restaurant/gatekjøkken
- O For tidkrevende å lage lav-FODMAP mat
- O Hadde lyst på mat med FODMAP
- O Lav-FODMAP mat var for dyr
- O Visste ikke at matvaren inneholdt FODMAPs

Hvor fornøyd er du med informasjonen du fikk om dietten:

- O Meget fornøyd
- O Fornøyd
- O Ok
- O Misfornøyd
- O Meget misfornøyd

Overholdelse av lav-FODMAP dietten én måned etter diettslutt

Har du fulgt dietten de siste 4 ukene?

- O Ja
- O Litt
- O Innimellom
- O Nei

Hvor godt har du oppretthold lav-FODMAP dietten etter 1 mnd?

Gått tilbake til
mitt normale kosthold — Kun spist lav-FODMAP

0% ——— 100%

Hva er grunnen til at du ikke spiser 100 % lav-FODMAP lenger?

- O Ikke aktuelt, følger fortsatt dietten for fullt
- O Merket ikke noe effekt av dietten
- O Merket ikke god nok effekt til å ofre mitt vanlige kosthold
- O Det er kun noen matvarer jeg reagerer på
- O Savnet for mange matvarer

Dersom du har fulgt dietten, har du reintrodusert noen FODMAPs?

- O Ja
- O Nei
- O Kun noen matvarer
- O Prøvd, men ble dårlig av alt

Hvordan synes du det var å reintrodusere matvarer til dietten?

Kjempelett — Meget vanskelig

0% ——— 100%

Hva var utfordrende med reintrodusering av matvarer:

- O Visste ikke hvordan jeg skulle gjøre det
- O At jeg mest sannsynligvis kom til å få symptomer av den matvaren
- O Vanskelig å skille «normale symptomer» med strikt diett (jeg ble ikke helt frisk med dietten) og symptomer jeg evt får når jeg innfører ulike FODMAPs igjen
- O Vanskelig å vite om jeg fikk symptomer fra akkurat den matvaren
- O Hadde ikke problemer med re-introdusering
- O Ville ikke reintrodusere noen matvarer

Hva var det du prøvde å reintrodusere først?

- O **Fruktose**holdige matvarer som eple, pære, honning, juice, tørket frukt (rosiner, svisker, aprikos), asparges
- O **Laktose**holdige matvarer som melk/fløte/yoghurt og matvarer med laktose (vafler, boller, kaker, is etc.), melkesjokolade.
- O **Fruktan**holdige matvarer som inneholder hvete, rug og bygg som for eksempel brød, boller, vafler, kjeks, middagsmat med hvetemel.
- O **Fruktan**holdige matvarer som inneholder løk eller hvitløk, f.eks middagsmat, krydder, ferdigretter.
- O **Galaktan**holdige matvarer som bønner, linser, kikerter eller pistasjnøtter.
- O **Polyoler** som man finner i sukkerfrie pastiller, tyggis, avokado, aprikos, blomkål, plomme, sopp og vannmelon.

Kommer du til å fortsette på lav- FODMAP dietten fremover?

- O Ja, 100 %
- O Delvis
- O Nei
- O Kanskje

Hvilken type FODMAP tror du at du ikke tåler? Flere kan krysses av.

- O Tåler alle
- O Tåler ingen
- O **Fruktose**holdige matvarer som eple, pære, honning, juice, tørket frukt (rosiner, svisker, aprikos), asparges
- O **Laktose**holdige matvarer som melk/fløte/yoghurt og matvarer med laktose (vafler, boller, kaker, is etc.), melkesjokolade.
- O **Fruktan**holdige matvarer som inneholder hvete, rug og bygg som for eksempel brød, boller, vafler, kjeks, middagsmat med hvetemel.
- O **Fruktan**holdige matvarer som inneholder løk eller hvitløk, f.eks middagsmat, krydder, ferdigretter.
- O **Galaktan**holdige matvarer som bønner, linser, kikerter eller pistasjnøtter.
- O **Polyoler** som man finner i sukkerfrie pastiller, tyggis, avokado, aprikos, blomkål, plomme, sopp og vannmelon.

Appendix 6: Compliance questionnaire for a stricter gluten free diet

Overholdelse av strikt glutenfri kost gjennom 6 uker

Hvor fornøyd er du med strikt glutenfri kost som symptomlindring?

Svært fornøyd Svært misfornøyd

0% _____ 100%

Kan du tenke deg å fortsette på dietten:

- O Ja
- O Kanskje
- O Nei
- O Kun dersom jeg får videre veiledning

Hvis nei, hvorfor:

- O For tidkrevende
- O Savner for mange matvarer
- O Ble ikke bedre
- O For dyrt

Hvor nøye har du fulgt strikt glutenfri kost gjennom de 6 ukene?

Ikke fulgt den i det hele tatt Kun spist strikt glutenfri kost

0% _____ 100%

Hvor ofte hadde du avvik fra dietten i løpet av de 6 ukene:

- O Ingen ganger
- O 1-5 ganger i løpet av de 6 ukene
- O 1-3 ganger i uken
- O 4-6 ganger i uken

Hvor store mengder inntok du ved avvik fra dietten?

- O En munnfull
- O 2-5 munnfull
- O Et helt måltid
- O Alle måltidene i løpet av dagen

Hvor lenge gikk du på dietten før du spiste matvarer med hvetestivelse/spor av gluten:

- O Ingen dager
- O 1-3 dager
- O 4-7 dager
- O 2-3 uker
- O 3-5 uker

Hvordan synes du det var å følge dietten:

Kjempelett Veldig utfordrende

0% 100%

Hvorfor spiste du matvarer som inneholdt hvetestivelse/spor av gluten:

- O Spiste kun strikt glutenfri kost
- O Ikke tilgang på mat uten hvetestivelse/spor av gluten på restaurant/gatekjøkken
- O For tidkrevende
- O Hadde lyst på mat med hvetestivelse/spor av gluten
- O Mat uten hvetestivelse/spor av gluten var for dyr
- O Visste ikke at matvaren inneholdt hvetestivelse/spor av gluten

Hvor fornøyd er du med informasjonen du fikk om dietten:

- O Meget fornøyd
- O Fornøyd
- O Ok
- O Misfornøyd
- O Meget misfornøyd

Appendix 7: Information on gluten free diet

HELSE BERGEN
Haukeland universitetssjukehus
Avdeling for klinisk ernæring

Kostråd til deg som skal spise

Glutenfritt

August 2011

Hva er gluten?

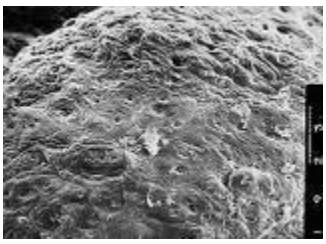
Gluten er en fellesbenevnelse på tre forskjellige, men likevel ganske like, proteiner som finnes i hvete, rug og bygg. Vanlig havre kan også inneholde gluten på grunn av forurensing fra andre kornslag. Kornslagene spelt, dinkel, kamut og rughvete inneholder også gluten.

Hvem trenger glutenfri kost?

Cøliaki

Ved cøliaki skades tarmen ved inntak av gluten, og evnen til å fordøye maten avtar. Dette medfører at en redusert mengde av matens næringsstoffer tas opp som igjen kan føre til magesmerter og underernæring
Hos barn er et vanlig symptom på cøliaki at vekstkurven flater ut.
Hos voksne er de klassiske symptomene på ubehandlet cøliaki jernmangel, diaré og avmagring, mens noen opplever trøtthet og dårlig matlyst. Det er heller ikke uvanlig å ikke ha symptomer i det hele tatt.

Frisk tarm



Skadet tarm

Når de som har fått stilt diagnosen cøliaki konsekvent holder en glutenfri kost, vil tarmen leges. Fordøyelsesproblemene vil avta og kroppen vil kunne utnytte maten på vanlig måte.

Tarmslimhinnen vil alltid reagere på gluten og selv små mengder kan gi tilbakefall, med eller uten symptomer. Derfor må et glutenfritt kosthold følges strengt og vare livet ut.

Det er viktig at ingen starter på glutenfri diett før sikker diagnose er stilt ved tynntarmsbiopsi.

Dermatitis herpetiformis (DH)

Dette er en relativt sjelden hudsykdom med kløende utslett og væskefylte blemmer. Tarmslimhinnen er også angrepet på lignende måte som ved cøliaki, men vanligvis i noe mindre grad. Dette fører i mange tilfeller til underernæring, men sjelden til større problemer med fordøyelsen. Glutenfri kost er en viktig del av behandlingen av DH.

Tarmslimhinnen blir normal på glutenfri kost og hudsymptomene vil ofte bedres, men det kan ta opptil et par år.

Enkelt kan også ha nytte av jodredusert kost, og vil kunne få veiledning i forhold til dette.

Hveteallergi / hveteintoleranse

Ved hveteproteinallergi og hveteintoleranse vil det i tillegg til reaksjon på hvete ofte forekomme kryssreaksjoner på rug og bygg. Derfor brukes ofte glutenfri kost. Samtidig intoleranse overfor havre er mindre vanlig, men *vanlig* havre kan være forurenset med små mengder hvete som gir reaksjon. Velg derfor havre som er merket ren eller glutenfri. (se s. 7).

Grant Cochrane/FreeDigitalPhotos.net

Innholdsdeklarasjon og merking av glutenfrie produkter

Alle ferdigpakkede matvarer skal være merket med en innholdsdeklarasjon, som skal være skrevet på norsk, svensk, dansk eller engelsk. Innholdsdeklarasjonen er det beste hjelpemiddel man har til å vurdere om et sammensatt produkt er glutenholdig.

Merkeforskriften setter særlige krav til merking av allergener. Dette innebærer at en rekke matallergener inkludert gluten/glutenholdige ingredienser alltid skal deklareres i ingredienslisten, selv om de er tilsatt i ørsmå mengder. Det er en rekke

ingredienser som inneholder gluten (se side 10). Matvarer som er merket med svært lavt gluteninnhold eller glutenfritt kan brukes uten at man må sjekke ingredienslisten.

Dessverre er ikke alle matvarer merket med innholdsdeklarasjon. Dette gjelder bl.a. ferskvarer og andre produkter som selges i løs vekt. I slike tilfeller må man forhøre seg hos butikkbetjeningen eller produsenten. **Ikke bruk matvarer med ukjent sammensetning!**

Det forekommer en gang i blant at produsenten endrer oppskrifter slik at tidligere glutenfrie produkter får ny, glutenholdig, ingrediens, sjekk derfor deklarasjonen på produkter du kjenner fra tid til annen.

Et nytt, strengere, regelverk for merking av glutenfrie produkter trådde i kraft i 2012. Det nye regelverket innebær at den øvre grensen for innhold av gluten senkes fra 200 mg gluten/kg til 100 mg gluten/kg.

Svært lavt gluteninnhold: Produkter merket med ”svært lavt gluteninnhold” kan ikke ha et gluteninnhold som overstiger 100 mg/kg i produktet som selges til forbrukeren. Gluteninnholdet i disse matvarene er så lave at de kan trygt brukes av de aller fleste med cøliaki.
Glutenfri:
Produkter merket med ”glutenfri” skal inneholde mindre enn 20 mg gluten/kg ferdig produkt. Produkter som tidligere ble merket ”naturlig glutenfri” skal i henhold til den nye forskriften merkes ”glutenfri”.

Ubearbeidet frukt, bær, grønnsaker, kjøtt og fisk vil ikke bli merket som glutenfrie selv om de er naturlig glutenfrie men kan trygt benyttes, se også Kostsirkelen side 6.

Image: m_bartosh / FreeDigitalPhotos.net

"Kan inneholde spor av..."

Mange matvarer er merket med "spor av" hvete, gluten eller andre glutenholdige ingredienser. Denne merkingen innebærer ikke at hvete eller andre glutenholdige ingredienser inngår som en del av produktet, men at produktet er laget i omgivelser der det også lages glutenholdige varer. Produksjonsomgivelsene innebærer altså en risiko for at det merkede produktet kan være kontaminert. Erfaring tilsier at disse produktene **kan brukes** av de aller fleste med cøliaki fordi spormengdene er svært små og langt under grenseverdiene som gjelder.

Image: dan / FreeDigitalPhotos.net

Hva innebærer et kosthold ved cøliaki?

Kilde Helsedirektoratet.no

Rene råvarer fra matvaregruppene melk, kjøtt, fisk, egg, ris, poteter, grønnsaker, frukt og bær er naturlig frie for gluten. Disse matvarene er en viktig del av et næringsrikt kosthold, og bør inngå i glutenfritt kosthold.

Spis normale mengder kjøtt, fisk, egg og meieriprodukter, som gir protein, vitaminer og mineraler. Grønnsaker, poteter, frukt og bær gir i tillegg karbohydrater og fiber, og bør brukes i større mengder hver dag. Som i sunn kost ellers bør man begrense inntaket av produkter rike på fett og sukker, som "fast food", kaker, kjeks, godteri og søt drikke.

Havre ved cøliaki

Glutenfri havre / Ren havre
Glutenfri havre tåles av de fleste med cøliaki. Vanlig havre kan være forurenset med gluten fra industriell produksjon og bearbeiding. Det må derfor kun brukes havre og havreprodukter som er merket "ren" eller "glutenfri".
Det anbefales at havre introduseres etter at glutenfri diett er veletablert og pasienten er symptomfri. Eventuelle reaksjoner på havre kan da lettere oppdages og følges opp.
Fordi noen kan få mage-tarmsymptomer på grunn av det høye fiberinnholdet i havre, anbefales gradvis innføring av havre i kostholdet. Bruk av havre kan på en positiv måte bidra til økt fiberinntak i glutenfri kost og kan gi større variasjon i kosten.
For barn anbefaler fagrådet i Norsk cøliakiforening at man venter med å introdusere ren/glutenfri havre til barnet har spist glutenfritt i ca 3-6 mnd.

www.axa.no

Laktoseredusert kost

Fordøyelsen av melkesukker (laktose) er ofte nedsatt når tarmen er skadet. Vanlig melk, brunost og iskrem medfører ofte mageknip og mye luft i tarmene.
Laktosefrie melkeprodukter og alle hvitoster inneholder ingen laktose og tåles godt.
Syrnede melkeprodukter, som kulturmelk og yoghurt i begrensede mengder, samt laktoseredusert lettmelk, tåles også av de fleste. Etter ca 6 uker tåler de fleste noe laktose, og melk og brunost kan gradvis introduseres i kosten igjen.
Dersom du ikke har hatt mageplager, trenger du neppe å ta hensyn til laktose i kosten.

www.tine.no

Oversikt over matvarer som er glutenfrie, samt matvarer som inneholder glutenholdige ingredienser

Matvarer	Glutenfritt/svært lavt gluteninnhold	Glutenholdig, kan ikke brukes
Melk og ost	Alle sorter melk og andre meieriprodukter som Yoghurt, skyr Ost Rømme, kesam, creme fraiche,	 Yoghurt m/müsli Søst, gomme lagd med glutenholdige matvarer
Kjøtt, fisk og egg	Alle sorter rent kjøtt, innmat, farsevarer uten gluten, Alle sorter ren fisk, lever, rogn, farsevarer uten gluten. Alle sorter skalldyr. Eggeretter uten gluten.	Pølser, panert kjøtt og kjøttfarser tilsatt glutenholdige ingredienser. Leverpostei kan inneholde hvetemel. Panert fisk og fiskefarser tilsatt glutenholdige ingredienser. Fiskekaker o.l. kan være tilsatt glutenholdige ingredienser som kavring/hvetemel Griljert og fritert mat kan inneholde mel og kavring.
Gryn, mel og brød	Flak, gryn og mel av amarant, bokhvete, hirse, mais, ris, soya, tapioka, quinoa og teff. Ren havre (merket glutenfri) Polentagryn, potetmel, potetfiber, sagogryn, kastanjemel, ,arrowrot, sesamfrø, solsikkefrø, linfrø, valmuefrø, kokosmasse, sukkerroefiber og psylliumfrøskall. Glutenfrie melblandinger, Glutenfrie varianter av brød, knekkebrød, flatbrød, kjeks, kaker, pølsebrød, rundstykker, kavring og lomper.	Flak, gryn og mel av hvete, rug, bygg og vanlig havre Rughvete, spelt Vanlig havre. Semulegryn, hvetekli, hvetekim Cous-cous, bulgur Vanlig brød, knekkebrød, kjeks, flatbrød og andre bakverk Puffet hvete, puffet havre.

Gryn, mel og brød	Puffet ris, cornflakes uten maltekstrakt, Glutenfri pasta, ris. Barnegrøt: ris- og maisgrøt og andre glutenfrie grøter. Sinlac grøt. Sausejevning av mais.	Vanlig pasta, hveteris, byggris Barnegrøter basert på glutenholdige kornvarer.
Poteter, grønnsaker, frukt og bær	Alle rene varer.	Stuinger o.l. tilsatt glutenholdige ingredienser. Sprøstekt løk kan inneholde hvetemel.
Fett, olje	Olje, margarin, smør, majones, remulade og salatdressing uten gluten.	
Sukker, søtsaker	Søtt pålegg, honning, drops, sjokolade uten glutenholdig kjeks/crisp, ekte marsipan, lakris, lakrisprodukter uten gluten og karamell uten gluten.	Sjokolade/sjokoladepålegg med kjeks/crisp, mandelmasse tilsatt gluten, enkelte lakris- og karamellprodukter, maltekstrakt og konfekt med glutenholdig fyll.
Drikke	Kaffe, te, melk, juice, saft, iste, kakao og smoothie (uten glutenholdig gryn/korn) Øl laget på malt fra glutenfrie kornsorter som hirse, bokhvete, sorghum eller teff.	Smoothie (med glutenholdig gryn/korn) Øl
Diverse	Sauser, gryteretter, supper o.l. uten gluten. Soyasaus uten gluten, buljong uten gluten, krydder og ølgjær uten gluten.	De fleste sauser, gryteretter og supper. Enkelte chipstyper og nøtteblandinger kan være tilsatt kavring/brødsmuler. Maltekstrakt, soyasaus med hvete, buljong med gluten, krydderblandinger tilsatt gluten og ølgjær.

Ingredienser

Produsenter er forpliktet til å oppgi om et sammensatt produkt inneholder glutenholdige ingredienser men det krever at man selv vet hvilke ingredienser det gjelder.

	Ingredienser* som inneholder gluten:	Ingredienser* som er glutenfrie
A		Amarant, Arrowrot,
B	Bygg, Brødsmuler	Bakepulver, Bokhvetemel
C	Cous-cous	Carob,
D	Dinkelhvete, Durumhvete	
E		E-nr. (alle er glutenfrie), Emulgeringsmel,
F	Fullkornsmel	Fruktkjernemel, Fortykningsmiddel,
G	Grynmel, Grahamsmel	Glukosesirup av hvete, Glutamat, Glutaminsyre, Glutinous (ris), Glukosesirup (alle typer), Glyserol, Guarkjernemel, Guargum, Glutenfri havre
H	Havre (vanlig), Havrekli, Havrekorn, Havremel, Hvete, Hvetekim, Hveteprotein, Hvetestivelse**	Havre (glutenfri), Hirse, Humle, Hvetestivelse**, Husk (psylliumfrøskall),
J		Johannesbrødkjernemel,
K	Kamut (egyptisk hvete), Kavring, Kim, Kli, Korn, Kruskablanding	Kastanjemel, Kikertmel (rent), Klumpforebyggende middel, Kostfiber (produsenten opplyser dersom kostfiber er fra glutenholdige råvarer),
		Linfrø,
M	Malt, Maltekstrakt, Maltsirup	Maltodextrin (fra hvete), Maltarom, Maltose, Maltsukker, Mandelmel (rent), Modifisert stivelse (dekstrin fra hvete), MSGsmaksforsterkere E621 og E637,
N	Nudler	
P	Puffet havre, Puffet hvete	Polentagryn, Potetfiber, Potetmel, Prekokt rismel, Psylliumfrø, Psylliumfrøskall (husk)
Q		Quinoamel,
R	Rug, Rugmel, Rugmalt	Ris/villris, Risbakemel, Rismel,
S	Semulegryn, Spelt, Strøkavring, Strømel	Soyagryn, Soyamel, Sorghummel, Sesamfrø,
T	Tritikale	Tapioka, Tarakjernemel, Teff,
V		Valmuefrø
Ø	Ølgjær.	

* Listen er ikke komplett. Se www.ncf.no for bredere oversikt.

**Hvetestivelse kan være glutenfri og glutenholdig, avhengig av hvor godt renset hveten er. Dette opplyses det oftest om på varedeklarasjonen. Ved usikkerhet kontakt produsenten. Hvetestivelse som inngår i glutenfrie produkter er glutenfri.

Stivelse og maltsukker

Både maltsukker, maltodekstrin og maltose er glutenfrie karbohydrater som ikke inneholder gluten. Stivelse kan være utvunnet av hvete, potet, ris eller annet. Dersom det er hvetestivelse skal dette være angitt på pakken. Hvetestivelse er renset for gluten, slik at den kommer under definisjonen "glutenfri", og produkter med hvetestivelse inngår derfor i den glutenfrie kosten. Noen få cøliakere med veldig sensitiv tarm kan likevel reagere på hvetestivelse og må utelate dette fra kosten.

Vegetabilsk protein

Hydrolyserte og modifiserte vegetabilske proteiner, som for eksempel er i buljongpulver og buljongterning, har gjennomgått så store forandringer at de ikke gjenkjennes som gluten og gir dermed ikke tarmskade, selv om det skulle være proteiner fra hvete, rug eller bygg.

Tilsetningsstoffer (E-nummer)

Alle tilsetningsstoffer (E-numre) som er godkjente for bruk i Norge er glutenfrie og hvetefrie. Dette gjelder også alle typer glutamat, glutaminsyre og alle stivelsessorter/fortykningsmidler.

Norsk cøliakiforening (NCF) gir ut et Ingrediensleksikon som kan bestilles fra deres nettsider (www.ncf.no)

Glutenfrie spesialprodukter

Det finnes en rekke glutenfrie varianter av brød, melblandinger, pølsebrød, kjeks, kaker, knekkebrød osv. Slike glutenfrie spesialprodukter bør erstatte de matvarer som må fjernes fra kosten. Produsenter av glutenfrie produkter er (listen er ikke fullstendig).

- Toro
- Finax
- Semper
- Hammermühle
- Schär
- Holmen Crisp
- Drei Pauly

Forhandlere av glutenfrie produkter

De fleste matvarebutikker og helsekostforretninger har glutenfrie produkter i varierende utvalg. Det finnes også flere nettbutikker som har et omfattende sortiment av glutenfrie varer og som leveres per post, f. eks. www.allergikost.no eller www.allergimat.no. For flere nettsteder, se www.ncf.no og klikk på Linker

Mange bakerier produserer glutenfritt brød som kan fås ferskt på bestilling og ellers frossent. Flere spisesteder, også pizza- og hamburgerkjeder, har glutenfrie alternativ.

Gode råd for glutenfri baking og matlaging

Her følger noen tips fra erfarne cøliakere som det kan være verdt å ta med seg:

Det viktigste bakerådet: Bruk fiberhusk i all gjærbakst!
Fiberhusk løses i væske i et par minutter og tilsettes melet. Du får en mye bedre deig å arbeide med, og du får et saftigere bakverk som holder seg lenger uten å smule. Bruk 1 ss per ½-1 kg mel. Fiberhusk er psylliumfrøskall som virker ved å øke innholdet av geldannende fibre i brødet.

- Til brødbaking kan du gjerne velge grov brødmix og tilsette linfrø, sesamfrø, hirse, solsikkefrø og/eller bokhvete for å øke fibermengden i brødet. NB! Mengden bør ikke være for stor, for da holder ikke brødet ikke sammen. Opptil 1 dl pr brød går bra, prøv deg frem. For dem som ikke liker "klumper" i brødet kan man male nøttene før man tilsetter det til brød.
- Frys brødet ferskt hvis du baker mer enn det du trenger for en dag. Del det gjerne opp slik at du kan ta frem mindre mengder om gangen.
- Litt olje i brødet forbedrer holdbarhet og konsistens.
- Bokhvete, hirse, mais, soyabønner og ris finnes som mel, flokker og gryn/bønner, og kan brukes til baking og i matlaging. Det vil øke matens fiberinnhold, næringsinnhold og ikke minst gi variasjon. Ferdig kokte linser kan også tilsettes baksten.
- Pannekaker, vafler, kjeks og kaker kan lages av glutenfri brødmix, gjerne tilsatt fiberhusk. Varier gjerne ved å tilsette soyamel, bokhvetemel og/eller andre typer glutenfrie gryn eller mel.
- Supper, sauser og stuinger kan jevnes med lys melblanding, f.eks Toro's fin kakemix, eller maisenna.
- Glutenfri panering og strøbrød kan lages av glutenfritt brød som tørkes og males, eller det kan kjøpes ferdig i helsekostbutikken.
- De fleste vanlige oppskrifter på kokosboller, kokosmakroner, kransekake og marengs er glutenfrie.
- Dekk brødformen med smurte strimler av bakepapir så slipper brødet.
- Mange av dine «gamle» oppskrifter kan brukes. Erstatt hvetemel med glutenfritt mel, doble mengden hevemiddel, og tilsett et egg for å få det til å henge bedre sammen. I stedet for egg kan du øke mengden glutenfritt mel i forhold til mengden hvetemel.
- Bruk kjøkkenmaskin til gjærbakst, lag deigen løs og la maskinen arbeide deigen godt. Deigen kan være klisset, og i stedet for å bake brødene kan de helles rett i brødformer. Ved utbaking av boller og rundstykker kan det hjelpe å fukte hendene med olje eller lunkent vann og hjelpe til med en skje for å få form. Kjevle og bakeunderlag i plast egner seg ekstra godt til glutenfri baking.
- Tørt bakverk kan fuktes litt og varmes i f. eks brødrister. Mange synes glutenfritt brød smaker bedre når det er ristet.
- Mange foretrekker å kjøpe en brødbakemaskin som baker brød på natten. Da kan du få et ferskt brød hver morgen.

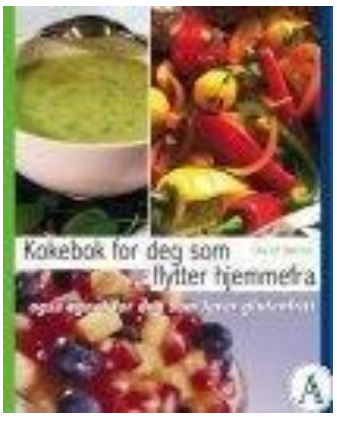
Oppskrifter

Oppskrifter på glutenfritt bakverk og matretter er det ikke blitt plass til i dette heftet. Det finnes en rekke kokebøker med glutenfrie oppskrifter som du kan kjøpe i bokhandelen. Det finnes også flere nettsteder og blogger med oppskrifter og råd om glutenfri matlaging.

Norsk cøliakiforening arrangerer forskjellige matlagings- og bakekurs.

Et utvalg av glutenfrie kokebøker:

- *Glutenfrie tradisjoner fra hele verden.* Henriette Pecühle (2011) Kolofon.
- *Glutenfri mat godt og sunt. Gjærbakst og kaker.* Else Lill Münter Rolfsen, Ruth Solheim Aag (2007) Kolibri forlag.
- *Glutenfri mat - godt og sunt. Småretter og middager*. Else Lill Bjønnes og Bjørn Wiborg (2010) Kolibri forlag.
- *Kokebok for deg som flytter hjemmefra.* Else Lill Bjønnes (2006) Kolibri forlag.



Litt mer informasjon

- om cøliakiforeningen
Norsk Cøliakiforening (NCF) er en interesseorganisasjon/pasientforening for mennesker med cøliaki eller dermatitis herpetiformis (DH). NCF ble opprettet i 1974. De har utarbeidet flere informasjonsbrosjyrer om cøliaki og glutenfritt kosthold og utgir medlemsbladet Cøliaki-nytt fire ganger årlig. De arbeider kontinuerlig for å ivareta medlemmenes interesser gjennom informasjon, opplysning og bidrag til forskning. De arbeider for å bedre tilgangen til glutenfrie produkter og bevisstgjøring av produsenter på betydningen av korrekt deklarering av alle matvarer.

NCF har en informativ hjemmeside på internett som oppdateres jevnlig og har lenker til andre nyttige hjemmesider: www.ncf.no

Du kan også kontakte foreningen per brev eller telefon:
Norsk Cøliakiforening, Pb 351 – Sentrum, 0101 Oslo.
Telefon: 22 40 39 00

- om lokallaget
NCF har et aktivt fylkeslag i Hordaland og Sogn og Fjordane, som arrangerer bl.a. møter og bakekurs. De har opprettet kontaktpersoner som bistår med hjelp til nydiagnostiserte cøliakere.

- om cøliaki-poliklinikken
Du kan også ringe cøliakipoliklinikken på Haukeland universitetssykehus: 55 97 29 40, tirsdager klokken 16-18. Telefonen bemannes av frivillige fra cøliakiforeningen.

- om grunnstønad:
Diagnosen cøliaki gir automatisk rett til grunnstønad for å dekke merutgifter til matvarer. For tiden (juni 2011) gjelder sats 2: 948 kr/mnd, for barn opp til 3 år og sats 4: 1833 kr/mnd, for alle andre.

Første uken på glutenfritt

Kjøp ferdigbakt glutenfritt brød til hele første uken, skjær i skiver og frys ned. Glutenfri baking krever trening. Du kan ikke regne med å få godt bakeresultat de første gangene du prøver, så sørg for å ha nok brød, knekkebrød og kjeks den første tiden. Mesteparten av maten kan du kjøpe i dagligvarebutikken.

Handleliste med glutenfrie matvarer

- Potet og ris
- Glutenfri pasta og glutenfritt brød/knekkebrød
- Rene kjøttprodukter (inkl. karbonadedeig og kjøttdeig)
- Kjøtt (rent) fra kylling, kalkun og andre fugler
- Kjøtt (rent) fra fisk og skalldyr
- Egg
- Hvitost, brunost og de fleste smøreoster
- Syltetøy
- Melk og melkeprodukter (yoghurt, rømme, fløte etc, ev laktosefrie alternativ)
- Frukt og bær
- Grønnsaker og rotfrukter
- Margarin, lettmargarin, smør, flytende margarin og olje
- Salt, pepper og alle rene urtekrydder
- **For jevning**: Maisenna eller potetmel

Husk å alltid sjekke innholdsdeklarasjonen!

- bibliotek, bokhandel eller internett

En glutenfri kokebok er god å ha. Foruten oppskrifter finner du informasjon om andre korn- og melsorter som kan erstatte hvetemel, fibertilskudd og bakeråd. Mye av denne informasjonen finner du også på internett. Start på hjemmesiden til cøliakiforeningen: www.ncf.no og benytt lenkene derfra.

- cøliakiforeningen

Kontaktpersonene i cøliakiforeningen vet at det kan være tøft å få diagnosen cøliaki, spesielt hvis baksten slår feil og maten smaker annerledes. Kontakt de dersom du har spørsmål om glutenfri matlaging eller om du bare vil prate litt.

- trygdekontoret

Kontakt trygdekontoret for å få sendt inn søknad om grunnstønad så fort som mulig, slik at du raskt får utbetaling av merkostnader i forbindelse med dietten. Diagnosetidspunktet regnes som diettstart og du får betalt fra denne dagen. Det er også mulig å søke trygdekontoret om refusjon av utgifter til tannskader som har sammenheng med cøliaki.

-"glutenfri sone"

For å unngå at smuler fra vanlig brød kommer i kontakt med glutenfritt brød, kan det være lurt med egen brødboks til glutenfritt brød, egen skjærefjøl, egen brødkniv og kanskje en egen brødkurv. Dersom det brukes mye, kan det være praktisk med en egen brødrister til glutenfritt brød. Eventuelt kan man bruke toastposer utenpå brødskivene i brødristeren. Toastposer er også praktisk å ha med seg på reise (kan kjøpes blant annet på allergimat.no og allergikost.no).

Appendix 8: Information on the low FODMAP diet

HELSE BERGEN
Haukeland universitetssjukehus
Avdeling for klinisk ernæring

Kostråd ved irritabel tarm
FODMAP-redusert kost

I denne brosjyren finner du informasjon om FODMAP og tips til hvordan en FODMAP-redusert kost kan settes sammen.

Hva er irritabel tarm?
Irritabel tarm, eller irritable bowel syndrome (IBS), er en tilstand som kan medføre ulike plager i mage og tarm. Typiske symptomer er kvalme, mageknip, magesmerter, oppblåsthet, utspilt mage og luft/gass og rumling i tarmen. Man kan også oppleve avføringsforstyrrelser som diaré, forstoppelse eller vekslende løs og hard avføring. Symptomene kan komme og gå i perioder. Stress og bekymring kan forverre symptomene, og for noen kan plagene utløses eller forverres av mat eller drikke. Irritabel tarm kan være plagsom, men ikke farlig. Det er ikke kjent hva som er årsakene til irritabel tarm. Tilstanden er utbredt, og oppleves hos 15-20 % av befolkningen.

Generelle råd ved irritabel tarm

Mange opplever bedring når de følger disse rådene:

- Regelmessige måltider med sunn og variert kost
- Flere små måltider er bedre enn få store
  - 4-6 måltider daglig, med ca. 3-4 timer mellom hvert måltid
- Ro rundt måltidet
- Regelmessig liv med god balanse mellom aktivitet og hvile
- Tilskudd av geldannende fiber, som ViSiblin, FiberHusk, Psyllium og Benefiber. NB! Viktig med rikelig væskeinntak i tillegg.
- Mat som ***kan*** gi problemer:
  - Fet mat
  - Stekt mat
  - Røkt og sterkt saltet mat
  - Sterkt krydret mat
  - Mye kostfiber
  - Mat med mye tungtfordøyelige karbohydrater (FODMAPs)
  - Kaffe og annen koffeinholdig drikke (te, cola og energidrikker)

FODMAP-redusert kost

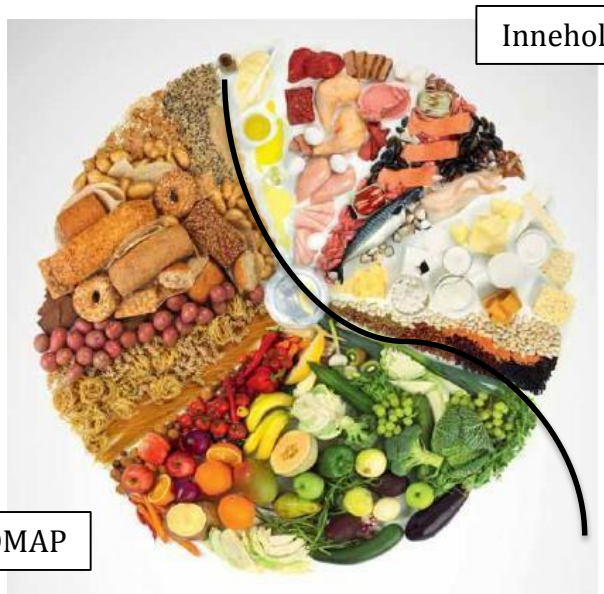
Australske forskere (Peter Gibson og Sue Shepherd) har utviklet en kost, **«FODMAP-redusert kost»**, som har vist seg å redusere plagene hos mange som sliter med irritabel tarm.

Hva er FODMAP?
FODMAP er en forkortelse for **f**ermenterbare **o**ligo-, **d**i- og **m**onosakkarider og (**a**nd) **p**olyoler. Dette er karbohydrater som tynntarmen kan ha vanskeligheter med å bryte ned og absorbere. Når de ufordøyde karbohydratene kommer til tykktarmen, vil de trekke til seg væske og forårsake gjæring. Dette kan gi problemer med gassdannelse, magesmerter, oppblåsthet, diaré og/eller forstoppelse.

Slik går du fram
En rekke studier har vist at flesteparten av alle som lider av irritabel tarm har god effekt av FODMAP-redusert kost. Dersom du vil teste om denne dietten kan ha effekt hos deg, anbefales det å unngå/begrense matvarer med høyt FODMAP-innhold i 4-8 uker.
Se tabelloversikt fra neste siden, hvor matvarene er kategorisert i rød, oransje og grønn kolonne. Matvarer i grønn kolonne har lavt innhold av FODMAP og kan brukes. Motsatt har matvarer i rød kolonne høyt innhold av FODMAP, og bør unngås/begrenses. Matvarer i oransje kolonne kan brukes i moderate mengder. For å få ideer om hvordan kosten kan settes sammen i denne perioden, -se siste del av brosjyren, side 11-19. Der finner du menyforslag og oppskrifter.

Hvis du ikke merker noen bedring/effekt innen 4-8 uker, har det ingen hensikt å fortsette med dietten. Blir du bra bør du på en systematisk måte forsøke og reintrodusere de matvarene du har fjernet fra kosten, slik at du til slutt får et «skreddersydd» kosthold som holder magen i orden, og ikke er mer begrenset enn det behøver å være. Se side 9-10, som handler om reintroduksjon av FODMAPene.

Kilder til FODMAPene finnes i matvaregruppene som er avbildet på venstre side av blå linje.



Bilde: Redigert kostholdssirkel Helsedirektoratet.
FODMAP-redusert kost er ikke det samme som lavkarbo eller glutenfri kost!

Tabelloversikten viser matvarer med høyt, middels og lavt FODMAP-innhold.

Matvaregruppe	Høy FODMAP Unngås/brukes i svært små mengder	I mindre mengder	Kan brukes i moderate mengder
Frukt og bær	Aprikos Eple Fersken Fiken Kirsebær Lychee Mango Nektarin Persimon (sharon) Plommer/ svisker Pære Vannmelon Bær: Bjørnebær Boysenbær Uansett frukttype: Fruktjuice Større porsjoner frisk frukt og smoothie. Hermetisk frukt i egen juice Tørket frukt/bær (se unntak i gul kolonne)	Grapefrukt Granateple Rambutan Tørket frukt/bær: Banan Rosiner Tranebær Kokos	Ananas Appelsin Banan Cantaloupe Dragefrukt Druer Durian Honningmelon Kaktusfiken Kiwi Klementin Papaya Pasjonsfrukt Rabarbra Sitronsaft Stjernefrukt Bær: Blåbær Bringebær Jordbær
Grønnsaker	Artisjokk Asparges Blomkål Hvitløk Løk, hvit og rød Purre (hvit del) Sopp Sukkererter Stangselleri Vårløk (hvit del) Tørkede belgfrukter: - erter - bønner - linser	Aubergine Artisjokk (hermetisk) Avokado Brokkoli Erter Gresskar Butternut Mangold (sølvbete) Rødbete Savoykål	Agurk Alfalfa spirer Aspargesbønner Bønnespirer Fennikel Gulrot Gresskar Hokkaido Kålrot Mais Noriark Okra Oliven (sorte og grønne) Paprika (Grønn) Paprika * (Rød) Pastinakk Potet Purre (grønn del) Rosenkål Reddik

Urter/krydder	Hvitløk Krydderblandinger og marinader med hvitløk og/eller løkpulver.		Basilikum Chili* Estragon Gressløk Ingefær Koriander Persille Rosmarin Timian

Matvaregruppe	**Høy FODMAP** **Unngås/brukes i svært små mengder**	**Lav FODMAP** **Egnede alternativer**
Melkeprodukter/ melkeerstattere	Melk (fra alle pattedyr) Yoghurt Fløte Rømme Brunost og prim Ferske og myke hvite oster (og kremoster) Soyamelk av hele soyabønner Iskrem	Laktosefri melk Laktosefri yoghurt Laktosefri fløte Laktosefri rømme Laktosefri kesam Faste hvite oster (norvegia o.l.) Lagrede hvite oster (brie, camenbert, roquefort etc.) Cottage cheese 4 ss Mozzarella Chevre Feta Kokosmelk Soyamelk av soyaprotein Rismelk Iskrem basert på laktosefri melk/fløte/ rismelk, eller sorbet og saftis.

Sukker/søtstoff	Fruktose (fruktsukker) Høy fruktose maissirup Honning Isomalt (E953) Laktitol (E966) Maltitol (E965) Mannitol (E421) Sorbitol (E420) Xylitol (E967)	Sukker (sukrose) Glukose (druesukker) Sirup, lønnesirup, ris malt (Kunstige) søtstoff som ikke ender på –ol Stevia Aspartam
Kornprodukter	Større mengder: Hvete Rug Byggryn (bygg) *(som hovedingrediens i brød, knekkebrød, bakverk, pasta, kornblandinger)*	Glutenfri havre < 25 g per porsjon Bokhvete < 25 g per porsjon Mais/ polenta Ris Quinoa Brød, pasta og kornvarer som er glutenfrie og/eller basert på ovenfor nevnte kornsorter.

FODMAPs er en gruppe karbohydrater.
Rent kjøtt, fjærkre, fisk, egg og fett/olje inneholder ikke FODMAP.

Mer om ulike typer FODMAP

Hva er forskjellen mellom FODMAP og andre karbohydrater?

Karbohydrater er energirike molekyler som fungerer som energikilde og energilager hos alle planter og dyr. De er bygget opp av én eller flere sukkerenheter som er bundet sammen i korte eller lange kjeder. Under fordøyelsen må karbohydratene i kosten brytes ned til enkle sukkermolekyler før de kan absorberes i tynntarmen og nyttiggjøres av kroppen. Det som kjennetegner FODMAP-karbohydratene, er at de er små (består av én eller noen få sukkerenheter), absorberes dårlig og gjæres lett.

Karbohydrater som vanligvis ikke gir besvær:

- **Glukose** (druesukker) er ett enkelt sukkermolekyl som lett absorberes i tynntarmen. Tåles godt ved IBS. Finnes i frukt og bær.
- **Sukrose** (vanlig sukker) består av et fruktose- og et glukosemolekyl som er bundet sammen. Fordøyes og absorberes lett, men inntaket bør begrenses av hensyn til den generelle helsen.
- **Stivelse** er lange kjeder av glukose. Disse brytes raskt ned og absorberes fullstendig i tynntarmen og er derfor uproblematisk ved IBS. Finnes i kornvarer, pasta, rotgrønnsaker og poteter.
- **Kostfiber** er langkjedede karbohydrater som ikke brytes ned i tynntarmen. Finnes i grove kornvarer, grønnsaker og frukt. Kostfiber er viktig for tarmfunksjonen og bidrar til å gi avføringen riktig konsistens. Vi skiller mellom vannløselige og ikke-vannløselige fibertyper. Vannløselig fiber er mest gunstig ved IBS.

FODMAP:

- **Fruktose** (fruktsukker) er et **monosakkarid**, det vil si at det består av bare ett sukkermolekyl. Finnes i frukt, bær, fruktjuice og honning, ofte sammen med glukose (Tabell 1). Fruktose absorberes godt sammen med like store mengder glukose, og 30-40 % av befolkningen (både friske og personer med IBS) absorberer ikke overskudd av fruktose. Inntak av mat som inneholder mer fruktose enn glukose, kan skape problemer hos dem som lider av irritabel tarm.

- **Laktose** (melkesukker) finnes i melk og melkeprodukter (Tabell 2). Laktose er et **disakkarid** og består av to sukkermolekyler (glukose og galaktose) som er bundet sammen. Under fordøyelsen spaltes de to sukkerenhetene fra hverandre ved hjelp av enzymet laktase, som produseres i tarmslimhinnen. Mangel på enzymet fører til laktosemalabsorpsjon. Genetisk betinget laktasemangel er svært utbredt på verdensbasis, og forekommer ofte hos innvandrere, men sjelden blant etnisk norske. Tarminfeksjoner og skader på tarmen kan også føre til laktasemangel, som regel av forbigående type.

- **Sorbitol** og andre søtstoff som ender på –ol, som mannitol, maltitol og xylitol, er **polyoler** (også kalt sukkeralkoholer). Disse absorberes ikke fullstendig i tynntarmen og større inntak kan forårsake diare og luftplager hos alle. Ved irritabel tarm kan også mindre inntak gi symptomer. Sukkeralkoholer forekommer naturlig i visse typer frukt og grønnsaker og brukes i sukkerfri tyggegummi, drops og pastiller (Tabell 3).

- **Fruktaner** er korte kjeder av fruktose og tilhører gruppen **oligosakkarider**. Finnes i løk, hvete og rug. **Galaktaner** er også **oligosakkarider** og finnes i belgfrukter. Disse stoffene brytes ikke ned av enzymene i tynntarmen, men blir i stedet mat for tykktarmsbakteriene, som produserer gass (Tabell 4).

Tabell 1: Mat som inneholder overskudd av FRUKTOSE	Tabell 3: Mat som inneholder SORBITOL og/eller andre POLYOLER
Frukt og bær Boysenbær Epler Fiken Kirsebær Mango Pærer Vannmelon	**Frukt og bær** Aprikoser Bjørnebær Epler Fersken Kirsebær Moreller Nektariner Plommer Pærer Svisker Vannmelon
Grønnsaker Asparges Sukkererter	**Grønnsaker** Avokado Blomkål Stangselleri Sopp Sukkererter
Søtstoff Fruktose Honning Høy fruktose maissirup	
Gir et høyt totalinntak av fruktose	
Store porsjoner av Frukt Tørket frukt Fruktjuice	**Søtstoff** Isomalt (E953) Laktitol (E966) Maltitol (E965) Mannitol (E421) Sorbitol (E420) Xylitol (E967)
Inneholder ofte fruktose	
Matvarer merket ”Naturlig lett” ”Naturlig søtet”	

Tabell 2: Mat som inneholder LAKTOSE Begrenses ved laktosemalabsorpsjon
Melk og melkeprodukter Melk (fra alle pattedyr) Yoghurt Fløte Rømme Brunost Prim Ferske og myke hvite oster Iskrem

Tabell 4: Mat som inneholder FRUKTANER og/eller GALAKTANER
Frukt Nektarin Persimmon (kaki/sharon) Plommer Rambutan Vannmelon
Grønnsaker Artisjokk Bønner (tørkede) Erter Fennikel Hvitløk Kikerter Kål Linser Løk Purre Sjalottløk
Korn (som hovedingrediens i brød/bakverk, pasta, grøt, müsli) Spelt Hvete Rug
Tilsetningsstoffer Frukto-oligosakkarider (FOS) Galakto-oligosakkarider (GOS) Oligogalaktose Oligofruktose Inulin

Hvorfor tåler noen FODMAP dårligere enn andre?

Sorbitol, fruktaner og galaktaner absorberes dårlig av alle mennesker, og fruktosemalabsorpsjon er like vanlig hos friske personer som hos individer med IBS. Folk flest tåler likevel FODMAP godt.
Grunnen til at personer med irritabel tarm får plager av FODMAP, kan være følgende:

Tarmoverfølsomhet for gassproduksjon

Ved irritabel tarm er tarmen mer følsom for gassen som blir produsert, og den trykkøkningen i tarmen som gassen forårsaker, oppleves mer smertefull og ubehagelig.

Bakteriell overvekst i tynntarmen

Noen av de bakteriene som normalt er lokalisert i tykktarmen, kan bevege seg over i tynntarmen. Dette kalles bakteriell overvekst i tynntarm og forekommer hos opptil 50 % av de som har irritabel tarm. Når FODMAP gjæres av bakterier i tynntarmen, vil gassen som produseres, øke trykket i et smalt parti av tarmen og derfor forårsake mer ubehag og smerte.

Reintroduksjon av FODMAP-grupper

Da man ikke nødvendigvis reagerer på alle FODMAP-gruppene, anbefales det å teste toleransen for hver enkelt gruppe. Før du gjør dette er det anbefalt å følge FODMAP-redusert kost i 4-8 uker, til du er symptomfri, for så å innføre én og én FODMAP-gruppe.
Det er viktig å reintrodusere gruppene enkeltvis på en systematisk måte for å finne din egen toleransegrense og hvilke typer FODMAP du reagerer på.

Forslag til reintroduksjon

Det er opp til den enkelte hva man ønsker å gjeninnføre først. Det kan være en idé å starte med det man har savnet aller mest. Det anbefales å starte med en liten mengde av en matvare som kun inneholder en type FODMAP om gangen.
Se forslag til gode testmatvarer i tabelloversikt under, og ellers på side 7-8.

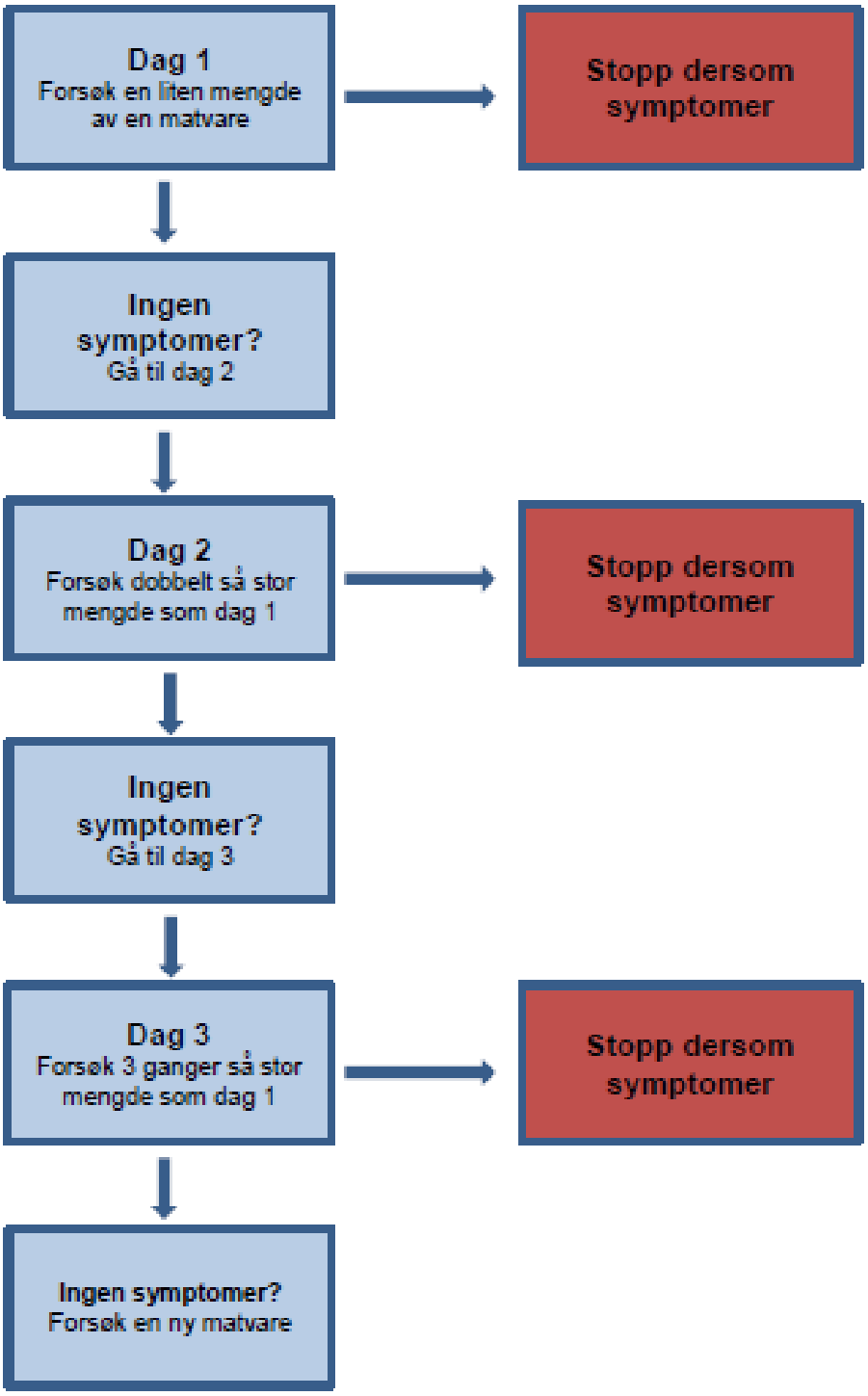
Følgende matvarer egner seg godt til testing fordi de inneholder mye av én FODMAP-type og lite eller ingenting av de øvrige. Start forsiktig og øk etter hvert til normale porsjonsstørrelser:

FODMAP-gruppe	Testmatvare
Fruktose **(tabell 1):**	¼ Mango eller 1 ts honning
Laktose **(tabell 2):**	125 ml melk (søtmelk) eller 1 skive brunost (15g)
Polyoler **(tabell 3):**	2 tørket aprikoser, 1 stk sukkerfri tyggegummi eller et par sukkerfrie pastiller (med sorbitol)
Fruktaner **(tabell 4):**	1 ss tilberedt løk, purre, eller 1 fedd hvitløk.
Galaktaner **(tabell 4):**	2 ss bønner eller linser
Fruktose og polyoler	En kombinasjon av fruktose og sorbitol kan tolereres dårligere enn gruppene enkeltvis. For å teste toleransen for en kombinasjon, kan pære benyttes

Ved laktoseintoleranse

Dersom du gjennom utprøvingen finner ut at du tolererer laktose dårlig, kan du ha nytte av preparater med laktaseenzym. Disse selges reseptfritt på apotek, og finnes i flere varianter. *Kerutabs* tabletter og *Lactrase* kapsler virker slik at man tar 1-3 tabletter/kapsler i forbindelse med måltid som inneholder laktose. Med disse vil du kunne nyte et måltid på restaurant, i selskap, ved festlige anledninger eller på ferie uten å bli dårlig på grunn av laktosen.

Følgende modell kan brukes når du tester en ny matvare:



Gjennomfører du tre hele dager med en ny matvare uten å få symptomer, kan matvaren på sikt kunne inngå i kosten igjen. Test kun nye matvarer når du ikke har symptomer. Når du skal teste neste matvare anbefales det å ta ut den matvaren du har testet av kosten igjen. Dette er viktig fordi summen av de to matvarene med mye FODMAP kan overstige din toleransegrense for total mengde FODMAPs. Dette kan illustreres som et beger som flyter over. Det kan være at man tåler små mengder fruktaner og fruktose, eller fruktaner enkeltvis, men når alle kombineres kan den totale mengden få begeret til å flyte over, og man får symptomer.

Praktiske råd ved FODMAP-redusert kost

Når du reduserer inntaket av FODMAP, er det fortsatt mulig å ha et sunt og variert kosthold. Dette er en veileder til hvordan du selv kan sette sammen måltider som har et lavt innhold av FODMAP.

Frokost/Lunsj/Kvelds

- 2 skiver glutenfritt brød/knekkebrød/rundstykker med pålegg.
- Glutenfri havregrøt med vann/laktosefri melk (og eventuelt bringebær/ jordbær/ blåbær/banan)
- Glutenfri havregryn eller glutenfri cornflakes med laktosefri melk/ yoghurt/ Biola
- Omelett (med skinke, kokt potet, og FODMAP-reduserte grønnsaker som paprika, tomat, vårløk (grønn del), squash, oliven)
- Salat
    - FODMAP-reduserte grønnsaker og frukt, eks salat, tomat, agurk, gulrot, vårløk (grønn del), oliven, appelsin, druer og honningmelon
    - Glutenfri pasta
    - Kylling/kjøtt/egg/fisk/sjømat
    - 1 ss gresskarkjerner

Middag

- Rene produkter av hvitt og rødt kjøtt, egg, fisk og sjømat
    - Les innholdsliste på blandingsprodukter- begrens ingredienser med mye FODMAP
- Poteter, ris, glutenfri pasta, risnudler, quinoa
- Pai/pizzabunn av glutenfritt mel.
- Eggeretter
- Pannekaker lagd med glutenfritt mel og laktosefri melk
- Hjemmelaget suppe av grønnsaker, kjøtt mm.
- Stekte grønnsaker/salat (se lunder oppskrifter)

Tilsett smak til maten:

- Oljer til steking/marinade
- Sitronsaft
- Laktosefri rømme/kesam – med og uten urter
- Friske urter, som for eksempel: basilikum, koriander, persille, rosmarin, timian
- Chili, Ingefær, salt og pepper
- Lønnesirup
- Vårløk (den grønne delen)

* Olje med hvitløksmak (legg store biter hvitløk i olivenolje, la den trekke en ukes tid. Ta vekk hvitløksbitene før du bruker oljen i matlaging)

- Alternativt; -stek hvitløken i oljen, og ta ut hvitløken før du tilsetter resten av ingrediensene.

Pålegg

- Kjøttpålegg: kokt skinke, skinkestek, spekeskinke, kalkun- og kyllingskinke naturell
- Egg
- Reker og annen sjømat
- Rene fiskepålegg som røkelaks, tunfisk, påleggslaks og sardiner
- Kaviar
- Majones
- Ost: Hvitost, brie, cottage cheese , cheddar, edamer, mozzarella, camembert og fetaost.
- Syltetøy av bringebær, jordbær og blåbær.
- Lønnesirup
- Peanøttsmør
- Banan
- Agurk, tomat, paprika, salat

Nøtter/frø

- Hasselnøtter, 10 stk
- Mandler, 10 stk
- Macadamia, 20 stk
- Peanøtter, 28 g
- Pekan, 10 stk
- Valnøtter, 10 halve
- Pinjekjerner, 1 ss
- Chiafrø, 2 ss
- Gresskarkjerner, 2 ss
- Solsikkefrø, 1 ts
- Sesamfrø, 1ss

Snacks

- Sorbetis laget av lav-FODMAP frukt.
- 1 glass smoothie av lav-FODMAP frukt som banan og bær, og laktosefri yoghurt og eventuelt glutenfrie havregryn
- Tortillachips, potetchips med salt/pepper.
- Glutenfrie kjeks og kaker
- Fruktsalat med laktosefri yoghurt og lønnesirup
- Riskaker med peanøttsmør og banan
- Mørk sjokolade
- Pannekaker og vafler med glutenfritt mel og laktosefri melk
- Glutenfri havrekjeks med nøtter og mørk sjokolade
- Haribo Eldorado vingummi.
- Mentos mint, Polo peppermyntepastiller, Wrigleys tyggegummi med sukker.

Mengde frukt: Det anbefaler maks 2-3 porsjoner à 100g med lav FODMAP frukt daglig, da fruktose i større mengder kan gi symptomer

Frukt og bær, lav fodmap	Opptil i en porsjon	Grønnsaker, lav fodmap	Opptil i en porsjon
Ananas	140 g	Agurk	65 g
Appelsin	130 g	Alfalfa spirer	20 g
Banan	100 g	Artisjokk(hermetisk)	30 g
Dragefrukt	330 g	Aspargesbønner	120 g
Druer	150 g	Aubergine	40 g
Durian	100 g	Avokado	20 g
Grapefrukt	80 g	Brokkoli	50 g
Granateple	40 g	Bønnespirer	50 g
Honningmelon	135 g	Fennikel	100 g
Cantaloupe	160 g	Gulrot	60 g
Kaktusfiken	166 g	Gresskar, Butternut	30 g
Kiwi	150 g	Gresskar, Hokkaido	60 g
Klementin	85 g	Kålrot	100 g
Pasjonsfrukt	100 g	Mais	40 g
Papaya	140 g	Mangold (sølvbete)	115 g
Rabarbra	130 g	Noriark	2 stk
Rambutan	30 g	Okra	100 g
Sitronsaft	1 ss	Oliven, sorte og grønne	60 g
Stjernefrukt	94 g	Paprika (Grønn)	100 g
Bær:		Paprika (Rød)	50 g
Blåbær	60 g	Pastinakk	60 g
Bringebær	90 g	Potet	120 g
Jordbær	140 g	Purre (grønn del)	100 g
		Rosenkål	115 g
		Reddik	40 g
		Rødbete	20 g
		Savoykål/ Savoy cabbage	35 g
		Salater	
		Bok Choy/ Pak Choy	160 g
		Eskarollsalat/ Endive	40 g
		Ruccola	35 g
		Ekebladsalat	35 g
		Spinat	150 g
		Sellerirot	35 g
		Squash	100 g
		Søtpotet	70 g
		Tomat	120 g
		Vannkastanjer	55 g
		Vårløk (grønn del)	1 bunt

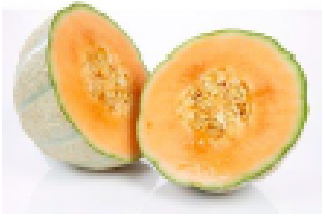


Foto: www.frukt.no

Vær oppmerksom på

- Ikke alle glutenfrie produkter er lav-FODMAP. Veldig ofte tilføres eple eller annet som er høy-FODMAP for bedre smak osv. Sjekk ingredienslisten.
- Konsentrert fruktjuice, f.eks. fra eple og pære, brukes iblant som søtstoff.
- Dipper og dressinger inneholder ofte løk og hvitløk.
- Smakstilsatt vann kan inneholde fruktose - les innholdsfortegnelsen.
- Yoghurt kan være tilsatt fruktose.
- Inulin er en fruktantype og brukes iblant som fiber eller prebiotikum i yoghurt, brød eller müsli.
- Dersom du reagerer på mye kostfiber, begrens matvarer med mye fiber, og velg heller fine produkter enn grove. En gradvis økning av fiber kan bedre toleransen.

På **varedeklarasjonen** skal alle ingrediensene oppgis i rekkefølge etter vekt. Den ingrediensen det er mest av nevnes først, og den det er minst av til sist. Bruk denne kunnskapen når du skal vurdere om en matvare kan inngå i kostholdet ditt. Små mengder FODMAP går som regel bra. Står for eksempel hvete listet opp sent i ingredienslisten inneholder matvaren så lite at de aller fleste tåler det.

For mer inspirasjon, informasjon, matvarelister og oppskrifter:

Helse Bergen: Nasjonal Kompetansetjeneste for Funksjonelle Mage-tarm sykdommer. www.helse-bergen.no/nkfm

Monach University: http://www.med.monash.edu/cecs/gastro/fodmap/
Til mobil: **LowFODMAPdiet App** fra Monach University.

Bøker på norsk: *Stine Junge Albrechtsen, Mette Borre, Lisbeth Jensen, Marianna Lundsteen Jacobsen & Cæcilie Gamsgaard Seidel:*
For deg med irritabel tarm.LowFODMAP-dietten gir ro i magen. Iris forlag 2014

Cecilie Hauge Ågotnes:
LavFODMAP. God mat for sensitive mager. Aschehoug 2015

Julianne Lyngstad:
LavFODMAP. En komplett håndbok for deg med sensitiv mage. Frisk forlag 2014

Appendix 9: Information and consent form

Forespørsel om deltakelse i forskningsprosjektet:

Effekt av FODMAP-reduksjon i tillegg til glutenfri kost ved cøliaki

Bakgrunn og hensikt
Det er kjent at mange pasienter med diagnostisert sikker cøliaki opplever begrenset eller ingen symptomlindring av glutenfri kost, tross normalisering av blodprøver og evt. tynntarmsbiopsi. Dette er derfor et spørsmål til deg om å delta i en forskningsstudie hvor vi vil sammenligne de to behandlingsalternativene som finnes for dette. Den ene gruppen vil få en grundig veiledning i strikt glutenfri kost, mens den andre vil få en FODMAP-redusert kost. Vi vil se på tilleggseffekten av en lav-FODMAP- diett hos personer med cøliaki og mageplager i forhold til en streng glutenfri diett.

FODMAP er en forkortelse for fermenterbare oligo-, di-, og monosakkarider og polyoler. Dette er karbohydrater som gir næring til bakterier i tarmen og som hos enkelte kan forårsake mageplager som diaré, forstoppelse, magesmerter og oppblåsthet. Matvarer som inneholder FODMAP er blant annet hvete, rug, visse melkeprodukter, løk, bønner, søtstoffer, epler, mango, brokkoli og plommer. Lav-FODMAP-diett går ut på å unngå å spise matvarer med høyt innhold av FODMAP.

Du er valgt ut til å få tilbud om å delta i studien fordi du er i alderen 18-60 år, har cøliaki og har vært på cøliakikurs. Du har spist glutenfri kost i minst seks måneder, men har likevel ubehag og mageplager. I tillegg har du scoret 75 eller mer på spørreskjemaet IBS-SSS og scoret på Roma III-kriteriene for IBS, og har dermed symptomer på «irritabel tarm». Det finnes forskning som viser at lav-FODMAP diett kan gi symptomlindring ved irritabel tarm. Det er imidlertid ikke forsket på om dietten kan være nyttig for cøliakere med irritabel tarm, og derfor spør vi om du vil være med på denne studien som kan vise oss om FODMAP-restriksjon i tillegg til glutenfri kost gir mer effektiv symptomlindring enn innskjerpet glutenfri kost alene.

Studien er en åpen, kontrollert studie utført av to masterstudenter i klinisk ernæringsfysiologi, veiledet av overlege/professor ved Universitetet i Bergen og Haukeland Universitetssykehus og klinisk ernæringsfysiolog ved Haukeland Universitetssykehus, som er ansvarlige for prosjektet.

Hva innebærer studien?
Studien innebærer at du over en periode på seks uker enten spiser en strikt glutenfri kost (gruppe A) eller at du spiser en spesiell kost som inneholder lite FODMAP i tillegg til strikt glutenfri kost (gruppe B). Det vil være tilfeldig om du havner i gruppe A eller gruppe B. En lav FODMAP-kost betyr at du må kutte ut ulike typer matvarer. Havner du i gruppe B, vil du senere få en detaljert oversikt over matvarer du ikke kan spise, og en liste med alternativer til de matvarene du må kutte ut. Dersom du velger å delta i studien, vil du bli invitert til et møte der du får utdypende forklaring om hva som skal skje i studien. Dersom du havner i gruppe A

vil du også få mer informasjon om glutenfri kost, men dersom du havner i gruppe B vil du få mer informasjon om lav-FODMAP-dietten. Du skal møte opp på Haukeland Universitetssykehus tre ganger for å ta blodprøver og pusteprøver og for samtaler med studentene i klinisk ernæringsfysiologi. Du skal også avgi avføringsprøver, fylle ut noen spørreskjemaer og fylle ut en kostdagbok for 4 dager. Dette gjøres før oppstart av studien, etter 3 uker og etter 6 uker, og i tillegg skal noen skjemaer fylles ut 4 uker etter avsluttet studie. Dette kan gjøres hjemme og deretter sendes i posten.

Mulige fordeler
Fordelen ved å delta er en mulig bedring i symptomene. Det betyr mulig mindre diaré, mindre forstoppelse, mindre magesmerter og/eller mindre oppblåsthet. En bedring av symptomer fra tarmen vil ofte også medføre en bedring i livskvalitet.

Mulige ulemper
Det er usannsynlig at studien kan medføre bivirkninger eller ubehag. Det er mulig at du ikke får noen bedring av dietten. Utover dette er det ingen risiko forbundet med studien. Du må møte opp på Haukeland Universitetssykehus tre ganger, noe som kan oppleves som belastende og/eller tidskrevende for enkelte. Du må også ta tre avføringsprøver, blodprøver og tre pusteprøver. Dietten du skal følge fører også til at du sannsynligvis må kutte ut en del matvarer som du vanligvis spiser, noe som kan oppleves som vanskelig for noen. Det kan også være en utfordring å gå på diett i sosiale sammenhenger.

Hva skjer med prøvene og informasjonen om deg?
Avføringsprøvene, blodprøvene og pusteprøvene som er tatt av deg, og informasjonen som registreres om deg, skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysninger og prøver vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det betyr at opplysningene er avidentifiserte. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Avføringsprøvene og blodprøvene vil destrueres etter at nødvendige analyser er tatt. Ved publisering av resultatene vil identiteten din ikke komme fram.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst, uten å oppgi noen grunn, trekke ditt samtykke til å delta i studien. Dette vil ikke få noen konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side av dette skrivet. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte studentene i klinisk ernæringsfysiologi Ida Serine M. Strindmo (Telefon: 900 31 585, e-post: ist104@student.uib.no) og/eller Kamilla Nuland (Telefon: 988 45 679, e-post: knu030@student.uib.no). Eventuelt kan ansvarlig lege Jan Gunnar Hatlebakk kontaktes på telefonnummer 977 07 817 eller på e-post jan.hatlebakk@helse-bergen.no.

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*
Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Kriterier for deltakelse
Du er valgt ut til å forespørres til å delta i studien fordi du er i alderen 18-60 år, har cøliaki, har vært på cøliakikurs, gått på glutenfri kost, men har allikevel ubehag og symptomer etter seks måneder. I tillegg har du scoret 75 eller mer på spørreskjemaet IBS-SSS og scoret på Roma III-kriteriene for IBS, og har dermed symptomer på «irritabel tarm».

Bakgrunnsinformasjon om studien
Ikke alle med en sikker cøliakidiagnose blir symptomfrie på glutenfri kost, på tross av at antistoffer i blodprøver og eventuelt tynntarmsbiopsier er normaliserte. Det er kjent at personer med cøliaki har en overhyppighet av irritabelt tarm syndrom (IBS). Cøliakere med IBS kan tenkes å ha en positiv effekt av FODMAP-redusert kosthold, men dette er ikke undersøkt tidligere, og det er derfor nyttig å kartlegge. Kan FODMAP-restriksjon gi symptomlindring hos cøliakipasienter som ikke har tilfredsstillende effekt av bare glutenfri kost?

Matvarer som inneholder mye FODMAPs (fermenterbare oligo-, di-, og monosakkarider og polyoler) kan gi plager fra mage- tarm området, særlig hos de med irritabel tarm. Mat som inneholder FODMAP blir fermentert i tykktarmen. Det betyr at bakterier i tykktarmen omdanner ufordøyd mat til gass og til energi (korte fettsyrer). Dette er en normal og viktig prosess, og det er blant annet essensielt for tarmcellenes helse. Fermentering er noe som i ulik grad skjer hos alle mennesker, men de med irritabel tarm får antageligvis mer plager av dette enn friske.

Ved irritabel tarm skjer det en unormal respons i mage- tarm kanalen som kan skyldes overfølsomhet i tarmen. Det kan også skyldes en unormal respons fra nervesystemet i tarmen, en forstyrrelse i bakteriefloraen, motilitetsforstyrrelse (unormal bevegelse av tarminnholdet) eller smerter på grunn av gassdannelse fordi det blir en utvidelse av tarmen. Dette kan gi de typiske symptomene på irritabel tarm, som oppblåsthet, magesmerter, gassdannelse, diaré og/eller forstoppelse. Tanken ved lav-FODMAP diett er å redusere inntak av mat som kan fermenteres av bakterier slik at det blir mindre fermentering i tarmen, og dermed mindre plager.

Blodprøver, avføringsprøver og pusteprøver
Før oppstart av studien skal du ta en blodprøve for å sjekke betennelsesmarkører. Blodprøven tas på sykehuset i forbindelse med gruppemøtet før oppstart av selve dietten. Du skal også avgi en avføringsprøve som skal analyseres for å studere bakteriesammensetningen. Avføringsprøven kan tas hjemme og sendes i posten i en spesial-emballasje. Pusteprøver vil bli gjort på sykehuset før oppstart av studien for å se på grad av fermentering av bakteriene i tykktarmen. Du vil bli tildelt time for dette og undersøkelsen tar ca. 60 minutter. Blod-, avførings- og pusteprøver vil bli repetert etter tre og seks uker.

Spørreskjemaer og kostregistrering
Du skal svare på fire spørreskjemaer før oppstart av studien, etter tre uker og etter seks uker. Disse skjemaene er Rome III (kriterier for irritabel tarm), IBS-SSS (symptomer på irritabel tarm), SF-36 (livskvalitet) og compliance (overholdelse av FODMAP-dietten). I tillegg skal du utføre en 4-dagers kostregistrering på de samme tidspunktene.

Tidsskjema – hva skjer og når skjer det?
Du har blitt kontaktet og blitt forespurt om å delta i studien. Dersom du er villig til å være med i studien, signerer du samtykkeskjemaet bakerst i dette skrivet. Du skal deretter møte opp på tre møter, som alle vil finne sted på Haukeland sykehus på dagtid så langt det lar seg gjøre.

Du vil bli invitert til et gruppemøte med 4-6 andre deltakere. Avhengig om du er havnet i gruppe A eller gruppe B vil studentene i klinisk ernæringsfysiologi der gi grundig informasjon om strikt glutenfri kost (gruppe A) eller lav-FODMAP-dietten (gruppe B) og hva som skal skje fremover. Du skal også ta en blodprøve og en pusteprøve, før eller etter dette møtet. Dette gjøres også på Haukeland Universitetssykehus og pusteprøven tar ca. 60 minutter, hvor du puster inn i et rør hvert 15.minutt. Litt ventetid før og etter må beregnes. Disse kan gi nyttig informasjon som kan hjelpe deg til å minske IBS-symptomene.

Du vil også få utdelt skriftlig informasjon. Skjemaene SF-36, IBS-SSS og Rome III angir hvor plaget du er av irritabel tarm og hvordan det påvirker din livskvalitet. Disse skal fylles ut og leveres en av studentene før eller etter møtet. Du skal også gjøre en 4 dagers prospektiv kostregistrering der du noterer ned alt du spiser. Etter at du har registrert kosten din i 4 dager, sender du registreringen så fort som mulig i posten til postadresse:

Medisinsk avdeling
Haukeland universitetssjukehus
5021 Bergen

Du skal også avgi en avføringsprøve som du kan levere før eller etter møtet, eller som kan tas hjemme og sendes til adressen ovenfor. Dersom du er havnet i gruppe B, får du også med deg et compliance-skjema som skal fylles ut etter tre uker og tas med neste gang.

Andre møte blir tre uker etter oppstart av studien, altså etter at du har gått på den strikte glutenfrie dietten i tre uker dersom du havnet i gruppe A, eller etter at du har gått på lav FODMAP-diett i tillegg til glutenfri diett i tre uker. Du vil få detaljert informasjon på hva som ikke kan spises og eventuelle alternativer til den matvaren i forkant. Dette møtet blir også et møte med 4-6 deltakere der eventuelle spørsmål og problemer diskuteres. I forkant av dette møtet skal du på ny registrere alt du spiser i løpet av 4 dager ved hjelp av en kostdagbok og ta dette med til møtet. I tillegg skal du på forhånd (samme dag eller dagen før) ha fylt ut skjemaene SF-36 om livskvalitet, IBS-SSS om IBS-symptomer og Rome III om IBS-kriterier som du gjorde ved oppstart av studien, og ta disse med på møtet. Dersom du havnet i gruppe B som går på FODMAP-redusert kost i tillegg til glutenfri kost, skal du også ha med deg et ferdig utfylt compliance-skjema som går på overholdelse av dietten etter tre uker. Før eller etter møtet vil du på ny ta samme pusteprøve som du gjorde første gang og du skal ta med deg en ny avføringsprøve.

Tredje og siste møte blir så når studien er avsluttet, altså etter seks uker. Det betyr at du skal ha gått på dietten i seks uker. Dette er også et møte med 4-6 deltakere. Eventuelle problemer og spørsmål kan tas opp. Du har også mulighet til å kontakte studentene på telefon eller e-post (se kontaktinformasjon på andre side) hvis det oppstår problemer underveis. Du kan eventuelt også ringe den ansvarlige legen. Dersom du er i gruppe A, vil du på dette møte få informasjon om FODMAP-dietten. Dersom du er i gruppe B, vil du på dette møte få informasjon om gradvis reintroduksjon av FODMAPS. Igjen tar du med fire ferdig utfylte skjemaer (SF-36, IBS-SSS, Rome III, compliance) i tillegg til 4 dagers prospektiv kostregistrering. Også i

forkant eller etterkant av dette møtet skal du ta pusteprøve og blodprøve, og du skal også ta med deg en avføringsprøve.

Én måned etter at du har fullført dietten skal du sende inn/levere compliance-skjemaet til oss.

Alternative prosedyrer dersom du velger å ikke delta i studien:

Dersom du ikke ønsker å delta i studien vil det ikke få noen konsekvenser for din videre behandling. Dersom du underveis i studien ønsker å trekke deg kan du ta kontakt når som helst. Da vil du bli invitert til en samtale, og eventuelle problemer vil bli diskutert. Du har selvfølgelig fortsatt rett til å slutte i studien når som helst uten å oppgi grunn. Dersom du ønsker å slutte med dietten, så vil du få tilbud om å få andre generelle råd for hva du kan gjøre for å lindre symptomene.

Studiedeltakerens ansvar:
Som deltaker i denne studien ber vi om at du setter deg inn i informasjonen og følger diettene så godt som mulig. Tid og dato for møtene, pusteprøvene, blodprøvene og avføringsprøvene (skal gjøres samme dag) skal avtales slik at det passer for begge parter. Med tanke på at det vil være 4-6 deltakere på hvert møte i tillegg til to mastergradsstudenter i klinisk ernæringsfysiologi, ber vi om at du er fleksibel på tid og dato for møtene og prøvetakningene. Du må også møte opp til avtalt tid, eller eventuelt ringe i god tid hvis timen ikke passer. Du har også ansvar for å fylle ut skjemaene som avtalt, ta dem med på møtene og sende de i posten før avtalt frist. På det første møtet med studentene vil du få mer nøyaktig informasjon enn det som står i dette skrivet.

Endringer i planen:
Dersom det skjer en endring i planen, eller en tidligere avslutning av dietten, vil du bli informert så raskt som mulig. Du vil også bli orientert dersom ny informasjon blir tilgjengelig som kan føre til at du ikke lenger vil delta i studien. Dersom det oppstår en uforutsett hendelse som gjør at studien må avsluttes vil du bli kontaktet snarest mulig.

Utgifter
Du vil få kompensasjon for reiseutgiftene til og fra Haukeland Universitetssykehus. Du får ikke kompensasjon for eventuell tapt arbeidstid, godtgjørelse for deltakelse eller tilskudd til diett.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som registreres om deg er informasjon om symptomer og livskvalitet fra forskjellige skjemaer. Resultater fra blodprøver, pusteprøver og avføringsprøver vil også bli registrert, men selve prøvene vil bli destruert etter at analysene er gjort. Kontaktinformasjon (navn og telefonnummer) om deg vil bli lagret. Det er kun vi som holder på med studien som har tilgang til opplysninger om deg, og de vil bli lagret innelåst i et skap på studieleders kontor.

Biobank
Avføringsprøver og blodprøver kastes etter analyse, det vil si at det ikke opprettes en biobank.

Utlevering av materiale og opplysninger til andre
Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger kun brukes til denne studien ved Haukeland Universitetssykehus i Bergen. Avidentifiserte opplysninger skal ikke sendes til andre foretak eller foretak i andre land.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi og Haukeland Universitetssykehus' rolle
Studien er finansiert gjennom forskningsmidler fra gastroenterologisk seksjon ved Klinisk Institutt 1 ved Universitetet i Bergen. De vil bidra med personell til analyser av blodprøver. Firmaet Genetic Analysis i Oslo vil analysere avføringsprøvene. Det er ingen mulige interessekonflikter.

Forsikring
Forsikringsordningen som gjelder er pasientskadeerstatning, idet du som deltaker er under behandling ved Haukeland Universitetssykehus.

Informasjon om utfallet av studien
Du har som deltaker i studien rett til å få informasjon om utfallet av studien når dette er klart. Det vil mest sannsynligvis bli våren 2016.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix 10: Research protocol

The effect of FODMAP-reduction in addition to gluten free diet in celiac disease

Background

Celiac disease is a common autoimmune disease, where ingestion of gluten will cause an immune reaction in predisposed individuals. The immune reaction can cause intestinal damage such as villous atrophy, crypt hyperplasia and chronic inflammation. The only treatment of celiac disease is a lifelong gluten-free diet, and strict adherence to such a diet will lead to full mucosal healing and symptom relief in the majority of patients. However, a part of celiac disease patients also have IBS-symptoms in addition to their gluten intolerance, thus not responding fully to a gluten free diet. The inflammation of the mucosa seen in celiac disease is thought to predispose for functional bowel disorders such as IBS. (1) These patients will possibly benefit from a FODMAP-reduced diet, as the diet has been shown to give significant symptom relief and increased quality of life in many IBS-patients. (2, 3)

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder, affecting 10-20% of the general population, and is characterized by altered bowel habits and abdominal discomfort. IBS can be diagnosed using the Rome III-criteria. (4) IBS is a diagnosis of exclusion, which means that the ruling out of other possible diseases is essential before diagnosis is set. The cause behind IBS is not fully understood yet; but several hypotheses have been proposed. Visceral hypersensitivity, gut dysmotilty and disturbance in the brain-gut-axis are assumed to be of importance in its pathophysiology. The best treatment available for IBS-patients per se is diet. The FODMAP-diet was developed by the dietician Susan Sheperd and her research group in1999, when they started to exclude several foods IBS-patients reported as symptom triggering. FODMAP is short for fermentable oligosaccharides, disaccharides, monosaccharaides and polyols, and is a grouping of carbohydrates that are inadequately absorbed in the small intestine and instead fermented by intestinal bacteria.

There are several hypotheses on why FODMAPs trigger symptoms in IBS-patients, some being thought to have more significance than others. FODMAPs are thought to have an osmotic effect on the gut, and also the fermentation by gut bacteria resulting in production of gas and small chain fatty acids are thought to cause pain and discomfort in IBS-patients (5). However, the FODMAPs cannot be considered as the cause of IBS, but the diet represent a

treatment alternative that can offer symptom relief for these patients.

Purpose and objectives

The purpose of the study is to investigate whether celiac disease patients with IBS-symptoms can have a symptomatic and a quality of life benefit from FODMAP-reduction in addition to their gluten free diet. Another objective is to investigate whether a FODMAP-reduced diet will have any effect on the gut microbiota or influence the degree of fermentation by gut bacteria.

H_{a1}: A FODMAP-reduction in addition to a gluten free diet will give symptom relief and increased quality of life in celiac disease patients with IBS-symptoms.
H_{a2}: A FODMAP-reduction will affect the microbiota and the degree of fermentation

Separately from the study of FODMAP-reduction, we will also follow a group of newly diagnosed celiac disease patients for 6 weeks. The objective here is to investigate whether there will be a change in the degree of fermentation and in the microbiota after 6 weeks of gluten free diet.

Design and method

The study is an open, prospective, randomised, and controlled study consisting of an intervention group and a control group, where ideally, each group will consist of approximately 20 subjects. The participants will be recruited between June and December 2015, mainly from the Norwegian Celiac Society (NCF) and Læring- og mestringssenteret (LMS). Some may also be recruited from the polyclinic for celiac disease at Haukeland Universty Hospital.

The intervention group will follow a low FODMAP diet in addition to their gluten free diet for 6 weeks, whilst the control group will follow their regular gluten free diet. The intervention group will receive dietary counselling on how to follow a low FODMAP diet, and the control group will receive additional dietary counselling on the gluten free diet. After end of study, the control group will be offered counselling on the FODMAP-diet.

Inclusion criteria:

- Confirmed celiac disease diagnosis for at least 6 months
- IBS-symptoms confirmed by the Rome III-criterion
- Score > 75 on the IBS-Symptom Severity Score (IBS-SSS)
- Subjects between 18 - 60 years of age

Exclusion criteria:

- Subjects with therapy-resistant celiac disease
- Recent biopsy with abnormal findings

The group of newly diagnosed celiac disease patients will consist of approximately 20 subjects, and will be recruited in the same time period from Læring- og mestringssenteret. The subjects included in this group will be those with a new diagnosis who is about to commence on a gluten free diet.

Variables

Variables included in the study will be the following:

Symptoms

Quality of life

Microbiota

Hydrogen breath test

Blood tests

Collection of data

Data will be collected through questionnaires, biological material and breath tests.

In order to measure symptoms we will use the standardized and validated IBS-Symptom Severity Score (IBS-SSS). This scheme includes five different questions with a score from 0-100, and offers a classification of symptom severity. We will also use the Rome III-criteria to confirm IBS-symptoms. To measure quality of life, we will use the questionnaire Short Form Survey (SF-36). This scheme includes questions regarding physical and mental health. A compliance scheme will be used in the intervention group in order to assess adherence to the diet.

There will be taken serological test for celiac disease at baseline off all participants. These will be included in the study as a possible explanatory variable.
All participants will do a 4-day prospective food dairy at baseline and after the intervention.

At baseline, both groups will fill out these questionnaires regarding their symptoms and quality of life. They will be asked to fill out the same questionnaires after 3 weeks and at the end of study (at 6 weeks). The subjects in the intervention group will also fill out the compliance scheme at 3 weeks and 6 weeks of diet, and also at 10 weeks (4 weeks after end of study). The questionnaires will be filled out when the participants are present at Haukeland University Hospital for collection of data at baseline and after 6 weeks. Ideally, the questionnaires at 3 weeks will be filled out during follow-up at Haukeland University Hospital. For those who are not able to attend this follow-up, the questionnaires will be mailed.

We will perform a breath test in both the intervention group, the control group and in the group of newly diagnosed celiac disease patients. The breath test will be performed using a "Model SC MicroLyzer".
The principle behind the breath test is to measure the amount of hydrogen breathed out, which correlates to the production of hydrogen by the bacteria in the intestines. We will perform a 60-minute breath test, measuring breath at 0, 15, 30, 45 and 60 minutes. The breath test will be done on the basis of their diet, comparing degree of fermentation on their normal diet at baseline, after 3 weeks and after 6 weeks of diet.

The stool samples will be collected at baseline, after 3 weeks and after 6 weeks of diet in the intervention group, control group and in the group of newly diagnosed celiac disease patients. The stool samples will be sent to a laboratory in Oslo; Genetic Analysis (GA), who will perform the microbiota analysis. The stools samples will be tested utilizing DNA sequences within the 16S rRNA gene of the bacteria in order to identify any bacterial imbalance in the microbiota. We want to investigate whether the microbiota changes based on what the participants eat.

Analysis

The data will be summarized in figures and/or tables. We will use STATA or SPSS to perform statistical analysis.

Economy

Funding: Kamilla Nuland, Ida Serine Melhus Strindmo, the main supervisor and co-supervisors will not receive any form of remuneration.

Time schedule

February – April 2015	Writing of protocol and applying to REC
June – December 2015	Recruiting of patients and performance of study
February – March 2016	Data analysis
April – May 2016	Writing of master thesis

Ethics

There is no risk of harm in this study. The intervention and the data collection may be perceived as demanding for some, but it will not cause any harm to the participants. The study is voluntarily and the participants can withdraw from the study at any point without providing any justification

Reference list

1. O'Leary C, Wieneke P, Buckley S, O'Regan P, Cronin CC, Quigley EM, et al. Celiac disease and irritable bowel-type symptoms. The American journal of gastroenterology. 2002;97(6):1463-7.
2. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. Gastroenterology. 2014;146(1):67-75.e5.
3. Gibson PR, Shepherd SJ. Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach. Journal of gastroenterology and hepatology. 2010;25(2):252-8.
4. The Rome Foundation. Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders 2015 [cited 2015 01.05.2015]. Available from: http://www.romecriteria.org/criteria/.
5. Shepherd SJ, Lomer MC, Gibson PR. Short-chain carbohydrates and functional gastrointestinal disorders. The American journal of gastroenterology. 2013;108(5):707-17.

Appendix 11: REC approval

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Hege Holde Andersson	22845514	01.07.2015	2015/915 REK sør-øst B
			Deres dato:	**Deres referanse:**
			12.05.2015	

Vår referanse må oppgis ved alle henvendelser

Jan Hatlebakk
Helse Bergen HF

2015/915 Effekt av FODMAP-reduksjon som tillegg til glutenfri kost ved coeliaki

Forskningsansvarlig: Helse Bergen HF
Prosjektleder: Jan Hatlebakk

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 08.06.2015.
Vurderingen er gjort med hjemmel i helseforsikringsloven (hfl) § 10, jf. forskningsetikkloven § 4.

Prosjektleders prosjektbeskrivelse
«Ca.30% av pasienter med coeliaki blir på ikke fri for symptomer som smerter, oppblåsthet og endret avføringsmønster. Dette ligner sykdommen irritabel tarm og vi vil se om de har nytte av kost som er redusert i innhold av kullhydrater som fermenteres i tykktarm. Pasienter som har diagnosen coeliaki og som oppfyller kriteriene for irritabel tarm, vil bli randomisert til enten: (1) kost redusert i slike karbohydrater, eller (2) kvalitetssikret glutenfri kost. Begge grupper blir veiledet av masterstudenter i klinisk ernæring. Pas fyller i kostliste i 4 dager før og under diett, tar blodprøver for coeliakistatus, leverer avføringsprøver og deltar i pusteprøver for å måle nivå av fermentering i tarmen. Diett følges i 6 uker og symptomer og livskvalitet sammenlignes i spørreskjemaer. En gruppe av nydiagnostiserte coeliakipasienter vil bli bedt om å delta med kostliste, pusteprøve og avføringsprøve før og etter 6 uker på standard glutenfri kost, for å se om fermentering endres.»

Komiteens vurdering
Komiteen har ingen forskningsetiske innvendinger til at prosjektet gjennomføres.
Under punkt **5.7** Håndtering av data etter prosjektslutt i søknadskjema skriver prosjektleder at data skal slettes etter prosjektslutt. Komiteen gjør oppmerksom på at avidentifiserte opplysninger skal som hovedregel lagres i 5 år etter prosjektslutt av dokumentasjonshensyn, og skal deretter slettes eller anonymiseres.

Biobank
I søknadskjema står det at det biologiske materialet skal oppbevares i en tidligere godkjent generell forskningsbiobank; Forskningsbiobank for mage-tarmsykdommer. I informasjonsskrivet til deltagerne står det imidlertid at; Avføringsprøver og blodprøver kastes etter analyse, det vil si at det ikke opprettes en biobank. Sekretariatet i REK-sør øst har vært i kontakt med prosjektleder for å oppklare hvordan materialet skal oppbevares. I e-post til sekretariatet 19.06.2015 skriver prosjektleder at Prøvene skal samles gjennom prosjektperioden for så å bli analysert samlet i februar 2016. Siden det humant biologiske materialet skal

oppbevares i mer enn 2 måneder er det behov en spesifikk forskningsbiobank i prosjektet. Komiteen oppretter derfor en spesifikk forskningsbiobank «*Effekt av FODMAP-reduksjon som tillegg til glutenfri kost ved coeliaki.*» Ansvarshavende er Jan Hatlebakk. Biobanken planlegges å vare til 24.06.2016.

Komiteen ber om at informasjonsskriv og samtykkeerklæring revideres slik at det fremkommer at det humant biologiske materielt skal oppbevares i en forskningsbiobank. Det reviderte skrivet må sendes komiteen til orientering.

Ut fra dette setter komiteen følgende vilkår for prosjektet:

1. Informasjonsskrivet revideres i tråd med det ovennevnte og sendes komiteen til orientering.

Vedtak
Komiteen godkjenner prosjektet i henhold til helseforskningsloven § 9 og § 33 under forutsetning av at ovennevnte vilkår oppfylles.

I tillegg til ovennevnte vilkår, er godkjenningen gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden.

Tillatelsen gjelder til 24.06.2016. Av dokumentasjonshensyn skal opplysningene likevel bevares inntil 24.06.2021. Opplysningene skal lagres avidentifisert, dvs. atskilt i en nøkkel- og en opplysningsfil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato.

Komiteen godkjenner også oppførelsen av en spesifikk forskningsbiobank som beskrevet i søknaden.

Biobankregisteret blir underrettet ved kopi av dette brev.

Hvis forskningsbiobanken opphører, nedlegges eller overtas av andre, skal det søkes REK om tillatelse, jf. helseforskningsloven § 30.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder "*Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren"*

Sluttmelding og søknad om prosjektendring
Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK. Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst B. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst B, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Komiteens avgjørelse var enstemmig.

Med vennlig hilsen

Geir Olav Hjortland
nestleder REK sør-øst B

Hege Holde Andersson
komitésekretær

Appendix 12: Abstract submitted for ESPEN congress, Copenhagen, September 2016

ESPEN 2016 Abstract Submission

Topic: Liver and gastrointestinal tract

Abstract Submission Identifier: ESPEN16-ABS-1496

PREVALENCE OF DYSBIOSIS AND EFFECT OF LOW FODMAP DIET IN CELIAC DISEASE PATIENTS WITH IBS-LIKE SYMPTOMS

Ida Strindmo [1], Kamilla Nuland[1], Gudrun Elise Kahrs[2], Jan Gunnar Hatlebakk[3]

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If you think another topic than the one selected at first would suit your abstract, please choose below: Nutrition and chronic diseases

Presentation Method: Oral or Poster presentation

Please indicate your professional occupation: Dietitian

The presenting author fulfills the above conditions and wants to apply for a travel award: Yes

I confirm that the presenting author is under the age of 35: Yes

Rationale: A subgroup of celiac disease patients have IBS (irritable bowel syndrome)-like symtoms despite following a gluten free diet (GFD). We wanted to compare the microbiota in these patients with an IBS- and a healthy population, and look at changes during a low FODMAP (fermentable oligo-, di-, monosaccharides and polyols) diet vs. a more strict GFD.

Methods: 40 celiac disease patients with IBS-like symptoms confirmed by the Rome III-criteria and IBS-SSS (symptom severity scale) were compared to Norwegian IBS and healthy cohorts, and randomized: Group A followed a more strict GFD for 6 weeks, whilst patients in group B reduced FODMAPs in their GFD. Faecal samples at baseline (BL) and 6 weeks. IBS-SSS at BL, 3 and 6 weeks. The faecal samples were analysed by Genetic Analysis for bacteria and Dysbiosis Index (DI) 1-5, where DI>2 is clinically relevant. Statistics: T-test, Mann-Whitney U, Fisher's linear discriminant analysis.

Results: FODMAPs were reduced from 12 to 2g/day (p=0.0001) in group B and IBS-SSS improved in both groups. 45% of the patients had dysbiosis at BL, compared to 73% in IBS (p<0.0091) and 16% in healthy controls (p<0.0007), with a mean score of 2.5±1.1 vs. 3.0±1.0 and 1.7±0.7, respectively. Group B had significantly more Bacilli and Prevotella than healthy controls. In group A (18F/2M, age 39±15), dysbiosis stayed constant on diet, but more patients had severe dysbiosis (DI>3), 15% vs. 25% (p=0.85). In group B (15F/5M, age 44±12), fewer patients had dysbiosis after diet, 60% vs. 50% (p=0.79). Responders to low FODMAP diet had less Lactobacilli and Firmicutes (Clostridia), and more Atopobium at BL.

Conclusion: Celiac disease patients with IBS-like symptoms had less severe dysbiosis than an IBS-population, but more than healthy controls. We found that the level of Lactobacilli, Firmicutes (Clostridia) and Atopobium predicted response to the lowFODMAP diet.

Disclosure of Interest: None Declared

Keywords: coeliac disease, microbiota

Appendix 13: Abstract submitted for UEG Week, Vienna, October 2016

PREVALENCE OF DYSBIOSIS AND EFFECT OF LOW FODMAP DIET IN CELIAC DISEASE PATIENTS WITH IBS-LIKE SYMPTOMS

I. Strindmo[1], K. Nuland[1], G. E. Kahrs[2,3] and J.G. Hatlebakk[4]

[1]Clinical Institute 1, University of Bergen, [2]Department of Occupational Medicine, and Sections of [3]Clinical Nutrition and [4]Gastroenterology, Department of Medicine, Haukeland University Hospital, Bergen, Norway[3].

Rationale: A subgroup of celiac disease patients have IBS (irritable bowel syndrome)-like symtoms despite following a gluten free diet (GFD). We wanted to compare the microbiota in these patients with an IBS- and a healthy population, and look at changes during a low FODMAP (fermentable oligo-, di-, monosaccharides and polyols) diet vs. a more strict GFD.

Methods: 40 celiac disease patients with IBS-like symptoms confirmed by the Rome III-criteria and IBS-SSS (symptom severity scale) were compared to Norwegian IBS and healthy cohorts, and randomized: Group A followed a more strict GFD for 6 weeks, whilst patients in group B reduced FODMAPs in their GFD. Faecal samples at baseline and 6 weeks. IBS-SSS at BL, 3 and 6 weeks. The faecal samples were analysed by Genetic Analysis for bacteria and Dysbiosis Index (DI) 1-5, where DI>2 is clinically relevant. Statistics: T-test, Mann-Whitney U, Fisher's linear discriminant analysis.

Results: FODMAPs were reduced from 12 to 2g/day (p=0.0001) in group B and IBS-SSS improved in both groups. 45% of the patients had dysbiosis at baselline, compared to 73% in IBS (p<0.0091) and 16% in healthy controls (p<0.0007), with a mean score of 2.5±1.1 vs. 3.0±1.0 and 1.7±0.7, respectively. The patients had significantly more *Bacilli* and *Prevotella* than healthy controls. In group A (18F/2M, age 39±15), dysbiosis stayed constant on diet, but more patients had severe dysbiosis (DI>3), 15% vs. 25% (p=0.85). In group B (15F/5M, age 44±12), fewer patients had dysbiosis after diet, 60% vs. 50% (p=0.79). Responders to low FODMAP diet had less *Lactobacilli* and *Firmicutes (Clostridia),* and more *Atopobium* at baseline.

Conclusions: Celiac disease patients with IBS-like symptoms had less severe dysbiosis than an IBS-population, but more than healthy controls. We found that the level of *Lactobacilli, Firmicutes (Clostridia)* and *Atopobium* predicted response to the lowFODMAP diet.

Conflicts of interest: None