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How does a low-FODMAP diet affect the gut microbiota composition in patients with irritable bowel syndrome?

Hvordan påvirkes tarmflorasammensetningen til pasienter med irritabelt tarmsyndrom av en lav-FODMAP diett?

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As my dad says *"Always do your best, but you´re doing that already"*

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ABSTRACT

Background: Irritable bowel syndrome (IBS) is a disorder characterized by abdominal pain, diarrhea, constipation and general discomfort. It is also characterized by a change in the composition of microorganisms in the intestine, which is referred to as gut microbiota dysbiosis. This disorder is regarded as a multifactorial disorder, although the pathophysiology remains controversial. Dietary strategies have been employed to reduce symptoms of IBS. However, diets, particularly the low-FODMAP diet, which appears to reduce symptoms, may not be optimal with respect to a healthy gut microbiota composition.

Aim: To determine to which extent dietary strategies used against IBS, particularly the low-FODMAP diet, alter gut microbiota in IBS patients, and to further discuss whether such changes are beneficial or not

Methods: A literature search was conducted using various terms, some examples; ´irritable bowel syndrome or IBS´, ´nutrition or diet´, ´RCT or randomized controlled trial or epidemiology or pilot´

Results: A diet low in fermentable carbohydrates change the gut microbiota composition, however whether this is beneficial or not to IBS patients is difficult to determine due to different findings and the short duration of these studies. Despite this, there is evidence to state that the gut microbiota has changed. This includes a probable switch from carbohydrate to protein metabolism by bacteria belonging to the genera *Bacteorides, Porphyromonas, Clostridium* and *Adlercreutzia*, despite not confirmed by changes in BCFAs or protein metabolites. In addition, it is a decrease of the lactate-producing probiotic bacteria, and of the mucus-associated *A. muciniphila*. Furthermore, there are increased levels of the mucus-associated bacteria *R. torques* as well as both a decrease and an increase of *Roseburia* spp. Finally, an increase of both gas-producing and gas-consuming bacteria were seen in non-responders, while responders were depleted for these. The degradation of proteins by the gut microbiota might have detrimental effects, due to the observed association between increased genotoxicity and protein metabolites in previous studies. However, further studies are needed, specifically on the long-term effect of carbohydrate restriction on the gut microbiota composition in IBS patients. Further research is also needed of the reintroduction phase of a fermentable carbohydrate restriction diet, to examine whether the changes in the gut microbiota composition in IBS patients following a low-FODMAP diet are being reversed or persists. Finally, further studies on the gut microbiota to non-responders in comparison to responders are needed.

Conclusion: A low-FODMAP diet alters the gut microbiota composition in patients with IBS, and it seems like this diet result in a trend toward adversely effects on the hosts´ health, despite not confirmed by changes in BCFAs. However, whether this diet is harmful for the host over time or not needs further research.

SAMMENDRAG

Bakgrunn: Irritabelt tarmsyndrom (IBS) er en lidelse karakterisert av mageknip, diaré, forstoppelse, flatulens, oppblåsthet og generell ubehag, i tillegg til en dysbiose, en endring i sammensetningen av tarmbakterier. IBS er en multifaktoriell lidelse, men årsakene er enda uklare. Forskjellige dietter har blitt foreslått for å redusere symptomene som oppstår hos IBS pasienter, spesielt en lav-FODMAP (Fermenterbare Oligo-, Di-, Monosakkarider And Polyoler) diett, men det kan tyde på at denne dietten er lite gunstig med hensyn på tarmflorasammensetningen hos IBS pasienter.

Mål: Å finne ut i hvilken grad koststrategier som reduserer symptomer hos IBS pasienter, spesielt en lav-FODMAP diett, påvirker tarmflorasammensetningen hos IBS pasienter, og ytterligere da diskutere om slike endringer er gunstige eller ikke.

Metoder: Et litteratursøk ble utført i PubMed og Cochrane, og følgende søkeord ble benyttet: ´irritable bowel syndrome or IBS´, ´nutrition or diet´, ´RCT or randomized controlled trial or epidemiology or pilot´

Resultater: I denne masteroppgaven ble det vist at en restriksjon av fermenterbare karbohydrater endrer tarmflorasammensetningen. Om dette er fordelaktig for vertens helse eller ikke, er vanskelig å bestemme, grunnet at studiene som ble inkludert i denne masteroppgaven observerte mye forskjellig. I tillegg var disse studiene korttidsstudier (1-4 uker). Uansett, en slik diett kan ha vist en trend mot et metabolismebytte hos tarmbakteriene hos IBS pasienter, fra karbohydrat- til proteinmetabolisme, til tross for at dette ikke ble støttet av målingene av BCFAs eller andre proteinmetabolitter. Spesielt av bakterier som hører til slektene *Bacteroides, Porphyromonas, Clostridium* og *Adlercreutzia* spp. Når tarmbakterier degraderer proteiner i den distale colon (tykktarm) produseres endeprodukter som har blitt assosiert med økt genotoksisitet hos mus. Videre forskning trengs, før en med sikkerhet kan si at en low-FODMAP diett gir negative effekter. Videre ga denne dietten en reduksjon av laktat-produserende probiotiske bakterier, og av den mucus-assosierte bakterien *A. muciniphila*. Det var og en økning av den mucus-assosierte bakterien *R. torques*, så vel som en reduksjon og en økning av *Roseburia* spp. Gassproduserende bakterier så vel som bakterier som konsumerer gass var økt hos non-responders, mens responders hadde lite av disse bakteriene. Det trengs videre forskning, spesielt på langtidseffekten av en lav-FODMAP diett på tarmflorasammensetningen hos IBS pasienter. I tillegg trengs det studier på effektene av reintroduksjonsfasen av en lav-FODMAP diett, for å undersøke om endringene som er vist i tarmflorasammensetningen hos IBS pasienter vedvarer eller reverseres. Det trengs og mer forskning på tarmflorasammensetningen hos non-responders sammenlignet med tarmflorsammensetningen hos responders.

Konklusjon: En lav-FODMAP diet endrer tarmflorasammensetningen hos pasienter med IBS, og det kan se ut som at denne dietten heller mot negative effekter for vertens helse. Om dette er effekter som er skadelige for verten over tid eller ikke trenger videre forskning.

ABBREVIATIONS

BCFAs: Branch-Chain Fatty Acids

BAM: Bile-Acid Malabsorption

DCA: Deoxycholic Acid

FGID: Functional GastroIntestinal Disease

FISH: Fluorescence *in situ* hybdridization (abbreviation used in Tables)

FODMAP: Fermentable Oligo-, Di-, Monosaccharides And Polyols

FOS: Fructo-oligosaccharides

GI: Gastrointestinal

GOS: Galacto-oligosaccharides

HFM: High-FODMAP Diet (abbreviation used in Tables)

IBS: Irritable Bowel Syndrome

IBS-C: Constipation predominant irritable bowel syndrome

IBS-D: Diarrhea predominant irritable bowel syndrome

IBS-M: Mixed irritable bowel syndrome (diarrhea and constipation)

IBS-U: Unsubtyped irritable bowel syndrome

LBHT: Lactulose Breath Hydrogen Test

LFSD: Low Fermentable Substrate Diet

LFM: Low-FODMAP diet (abbreviation used in Tables)

OTU(s): Operational Taxonomic Unit(s)

PCR: Polymerase Chain Reaction

pHBA: p-hydroxybenzoic acid

PI-IBS: Postinfectious IBS

RCT: Randomized Controlled/Clinical Trial

SCFAs: Short-Chain Fatty Acids

SIBO: Small Intestinal Bacterial Overgrowth

TAD: Typical Australian Diet (abbreviation used in Tables)

WGTT: Whole Gut Transit Time

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1 INTRODUCTION

1.1 IRRITABLE BOWEL SYNDROME

1.1.1 Definition, diagnoses and classification

In 2012, the World Gastrointestinal Organization (WGO) practice guideline defined irritable bowel syndrome (IBS) as "a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit. Bloating, distention and disordered defecation are commonly associated features" [1]. IBS is the most common functional gastrointestinal disorder (FGID), and it is a complex disorder that mostly affects the large intestine, but also, in part, the small intestine. Other symptoms include flatulence, bloating, a feeling of incomplete evacuation and mucus. This is in addition to psychiatric comorbidities, which is commonly seen among IBS patients, particularly anxiety and stress [2–4]. In addition, some patients may have increased fatigue, as well as limitations in their physical capabilities [5]. This may lead to increased absence from work and more frequent consultations with a physician compared to healthy individuals. In fact, IBS is the most commonly diagnosed disorder by gastroenterologists [6,7]. All these taken together might give IBS patients an impaired quality of life.

Gastroenterologists diagnose IBS patients using the Rome III criteria, often referred to as the current "gold standard". It is a symptom-based diagnostic tool, since there is currently no clear diagnostic marker for IBS [8]. Diagnosing IBS by the Rome III criteria requires the presence of recurrent abdominal pain, in addition to one or a combination of other symptoms, including altered stool frequency, relief of pain following defecation, and/or altered stool form or apperance. These symptoms have to occur >3 days per month during the previous three months. In addition, the symptoms must have been recurring for more than 6 months prior to the diagnosis [9].

IBS can be divided into subtypes using the Bristol Stool Scale (Table 1); predominant constipation IBS (IBS-C), predominant diarrhea IBS (IBS-D), or a mix between diarrhea and constipation (IBS-M). There is also an un-subtyped IBS (IBS-U), where the patients do not have diarrhea or constipation. IBS-C is determined if >25% of stools correspond to score 1 or 2 (table 1), IBS-D is determined if >25% of stools correspond to score 6 or 7, and IBS-M is

determined if 25% of stools correspond to score 1 or 2 and score 6 or 7 (Table 1). If the patient has an abnormal stool consistency that does not meet the criteria of the other subtypes, the individual will be classified as IBS-U [10]. However, patients may move from one classification to another over time [1,11].

Table 1: The Bristol Stool Scale[10]. Transit time is slow at score 1 but increases with higher score

Score	Description
1	Separate hard lumps, like nuts
2	Sausage-shaped but lumpy
3	Like a sausage but with cracks on the surface
4	Like a sausage or snake, smooth and soft
5	Soft blobs with clear-cut edges
6	Fluffy pieces with ragged edges, a mushy stool
7	Watery, no solid pieces, entirely liquid

The Rome Criteria was introduced in late 1980s by The Rome Foundation group to classify and diagnose FGIDs. In 2000, the Rome Criteria was updated to the Rome Criteria II, and further to the Rome Criteria III due to increased interest in FGIDs by gastroenterologists, psychologists and the public. The Rome III Criteria was also regarded as a vital tool for researchers to gain a better understanding of FGID, including IBS [12].

The Rome III criteria relies on the organs where the symptoms are most likely to be produced, and fall in order from the esophagus to the anus. The FGIDs are classified in six major domains for adults; bowel (category C) contains sub-categories such as functional bowel disorders that include IBS (C1), functional bloating (C2), functional constipations (C3) and functional diarrhea (C4). Symptoms like pain and change in bowel habit distinguish IBS from other GI-disorders [12].

1.1.2 Prevalence of IBS

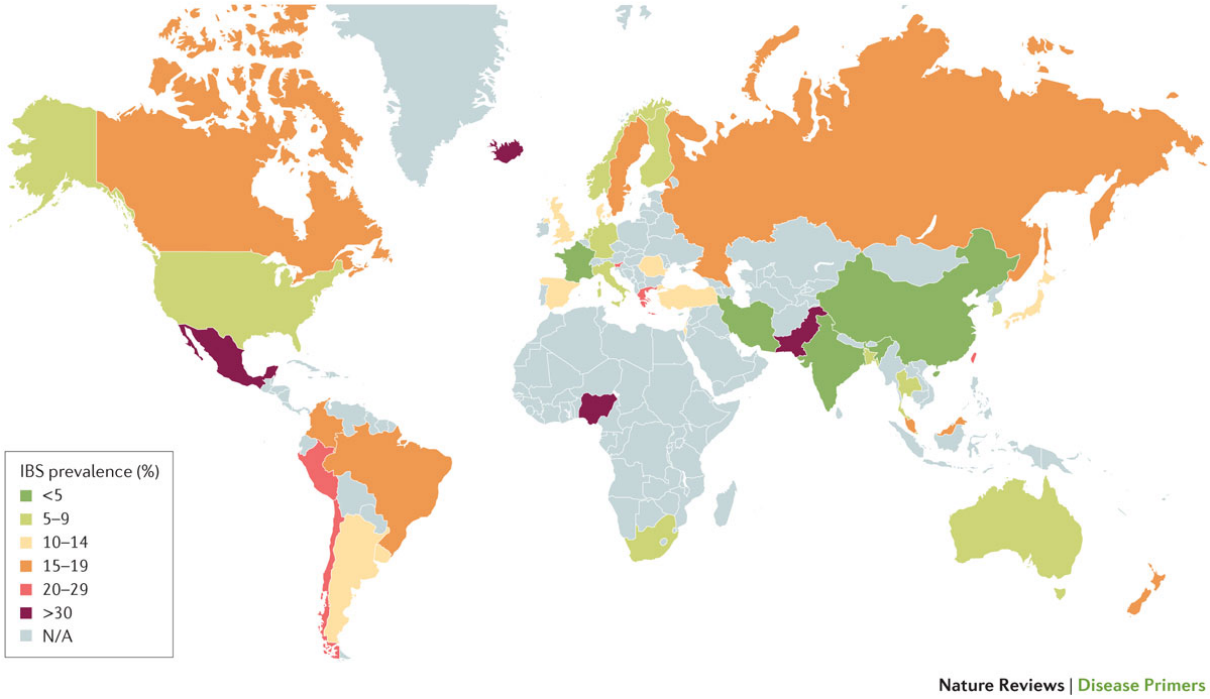

Figure 1: Worldwide prevalence of IBS [13]

The prevalence of IBS (Figure 1) varies worldwide from 1.1-45%. The pooled global prevalence rate are 11,2%, and are based on population studies from various countries worldwide [13]. However, the prevalence is unevenly distributed across continents and regions of continents. Prevalence rates are for instance 5-10% in most of the European countries, USA (including Alaska), South-Africa and Australia, and slightly higher in Russia, Canada and Brazil (10-14%). The lowest prevalence rates are registered in India, China, Iran and France (<5%). Most of the population data of IBS patients in African and Asian countries, in contrast, are unavailable (N/A), and this might be due to, for instance: poor health care systems or cultural differences [13]. For example, it is no general global definition of the IBS symptoms across countries, despite the existence of the Bristol Stool Scale. For example, the description of constipation in Asia is "a sense of incomplete evacuation", whereas the Bristol Stool Scale describes constipation as passing hard stools, whether there is normal frequency of bowel movements or not (Table 1) [1].

Prevalence also varies between subtypes of IBS. Globally, the most commonly subtype was IBS-D with a prevalence rate of 40% of all the reported IBS cases, and the least common

subtype was IBS-M with a prevalence rate of 23% [14]. IBS-M and IBS-D dominate in Bangladesh and India, IBS-M dominates in Brazil, and IBS-C is most common in parts of Asia, North America and Western Europe [1].

Differences in prevalence within a population is also evident. In most countries higher prevalence of IBS is found in young adults (<50 years) and a decreased prevalence with advancing age. This is, however, in contrast to countries such as Japan (prevalence rate 10-14%), India (prevalence rate <5%) and Iran (prevalence rate <5%), where the prevalence seem to increase with advancing age [1,14]. Globally, the prevalence of IBS in children varies from 6-14% [15,16].

1.1.3 Etiology of IBS

IBS was first introduced as a concept in 1950 in the *Rocky Mountain Medical Journal*, and it was suggested that IBS was caused by a psychosomatic or mental disorder. Until 1985, diet (in e.g. food intolerance), psychological factors, local organic disorders (in e.g. hemorrhoids) and motility disturbances were suggested to play the main roles in the pathophysiology of IBS. In addition it was suggested that luminal distention was caused by "air traps" and accumulation of gas, and not due to increased amount of intestinal gases [17–23].

In 1990 it was proposed that psychiatric conditions including mood and anxiety disorders were associated with IBS [24], as well as depression [25]. Later, stress was suggested to be the cause of these psychiatric conditions [3,9,26]. Stress might result in decreased gastric emptying, increased intestinal motility and increased abdominal discomfort (visceral hypersensitivity). In 1998, it was suggested that some IBS patients had increased excretion of breath hydrogen, and this was later proposed to be associated with small intestinal bacterial overgrowth (SIBO) in a subset of IBS patients [27]. SIBO is characterized by a quantitative increase of both aerobic and anaerobic coliform bacteria in the small intestine and have further been proposed to contribute to some of the symptoms in a subset of IBS patients. These include abdominal pain, bloating and altered bowel function [28–31]. In 2015, a study confirmed that SIBO was detected in a subset of patients with IBS, and reported increased levels of the genera *Escherichia/Shigella* spp., *Aeromonas* spp. and *Klebsiella* spp in the proximal small intestine of patients with irritable bowel syndrome [32].

Another hypothesis was the possible alterations of the gut microbiota, which have been observed in animals [33] and can further lead to increased permeability of intestinal epithelial cells, referred to as leaky gut. This is a state where lumen content leaks through the epithelial barrier into underlying tissue. Also metabolites from underlying tissue can leak into the lumen. This may in turn lead to a low-grade inflammation caused by pro-inflammatory cytokines produced by the innate immune system [9]. This in turn can lead to downregulation of tight junction proteins essential for the epithelial barrier [13]. Heredity may also play a role in IBS. The degree of similarity seen amongst monozygotic twins (22%) and in dizygotic twins (9%) indicate that there is a genetic component. However, the similirities seen in the twin studies might be due to environmental factors. To which extent genetics play a role in the pathophysiology of IBS is currently incompletely understood [33].

The neurotransmitter serotonin has also been proposed to play a role in the pathophysiology of IBS. Lower levels of serotonin are found in IBS patients compared to healthy subjects. Low serotonin levels are associated with certain symptoms in patients with IBS, such as reduced small intestinal motility, fibromyalgia and chronic fatigue/ME [34]. Furthermore, mast cells, which is primarily associated with allergic reactions and secretion of histamine, are more abundant in IBS patients compared to healthy controls. These cells are often found in connective tissue in the intestine around enteric neurons and have been associated with abdominal pain in IBS patients [35].

Furthermore, malabsorption of bile acid (i.e.Type 2 BAM) have been proposed to contribute to diarrhea in >25% of diarrhea predominant IBS patients in Western countries [36,37]. This can cause a state where the bile acid concentrations are altered in the colon; high levels of secondary bile acids can cause diarrhea and low levels can cause constipation [36,37].

Finally, one of the the strongest risk factor for IBS is acute gastroenteritis, with a 6- to 7-fold increased risk of developing IBS, referred to as post-infectious IBS (PI-IBS) [38]. This is because an enteric infection by bacteria, in e.g. *Campylobacter jejuni*, and possibly parasites (in e.g. *Giardia lamblia*), might disturb the gut microbiota. If changes in the gut microbiota persist after the infection, this can lead to PI-IBS [39]. Furthermore, antibiotics are often given to people with enteric infections, and these medications can also create a dysbiosis in the gut microbiota composition. Dysbiosis may last up to two years following an antibiotic treatment, as

demonstrated in mice, which may indicate that dysbiosis can enhance the disturbed gut microbiota in patients with PI-IBS [40]. An average incidence of developing PI-IBS is estimated to be 10% following an acute gastroenteritis [38].

Together, these factors might indicate that changes in the interaction between the gut microbiota and host factors (e.g. environmental factors and diet) are important in the pathophysiology of IBS. However, whether these factors are a consequence or a cause of IBS is not known.

1.2 GUT MICROBIOTA

1.2.1 Definition and function

The gastrointestinal tract (GI) extends from the oral cavity to the anus. The GI tract contains a variety of microorganisms, mainly bacteria, referred to as ´gut microbiota´. This is a complex ecosystem with an estimated 100 trillion microbial cells. The lowest numbers are found in the stomach (10/mL), and it is gradually increasing from proximal to the distal end of small intestine (10^2-10^8/mL). Finally, the colon consists of 10^{12} microbes/mL [41,42].

The relationship between the host and the gut microbiota is often mutualistic, which means that both host and bacteria are benefiting from each other. The gut microbiota contributes in maintaining homeostasis within the host by aiding digestion of nutrients, protecting against pathogens, regulating gut motility, and developing gut immunity, whereas, the host provides the microbiota with a nutrient-rich and protected environment [3,43]. In addition the gut microbiota produce certain vitamins (K and B) and short-chain fatty acids (SCFAs) from carbohydrate metabolism, the latter important as energy for colon enterocytes [3,42–45].

1.2.2 Taxonomy

Gut microbiota belonging to the kingdom bacteria consist of 17 families, 50 genera and more than 1000 species and they exhibit different functions and mechanisms [41]. Gut bacteria is divided in the taxonomic classes: phylum, class, order, family, genus and species, and some of the bacteria belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobioa are shown in figure 2-5. The most common phyla in the gut microbiota composition are Firmicutes and Bacteroidetes [41].

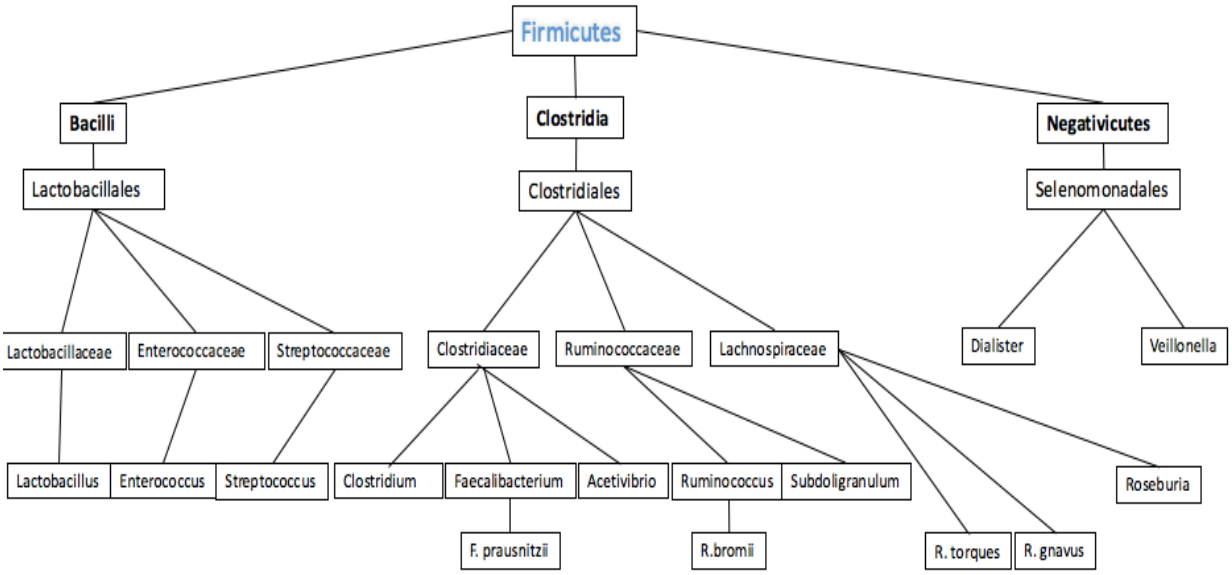

Figure 2: Phylogenetic tree of some of the bacteria belonging to the phylum Firmicutes (according to NCBI). Phylum, class, order, family, genus, species

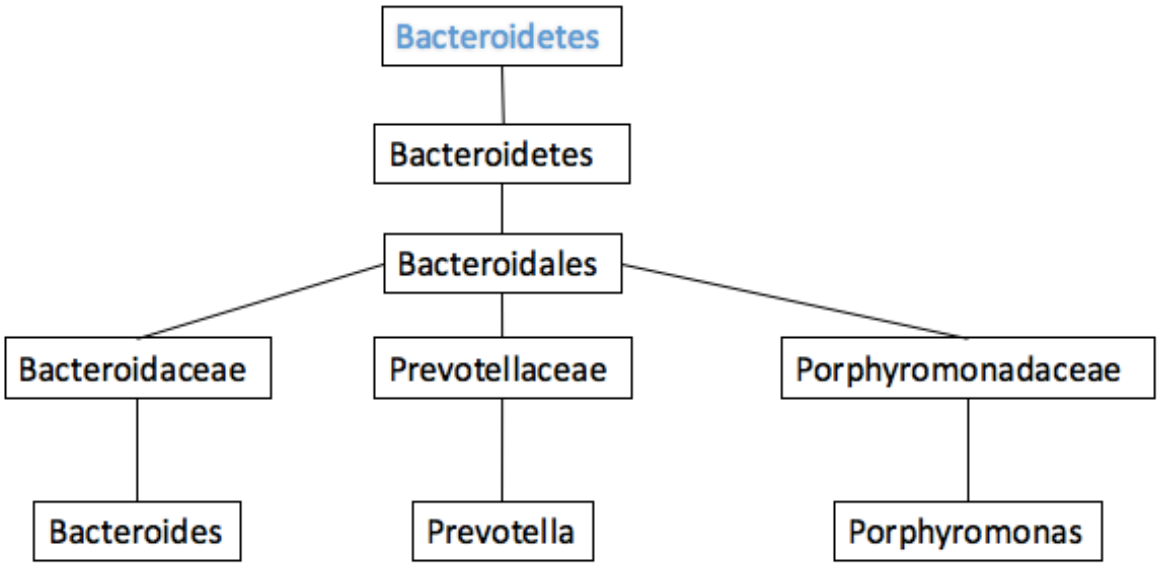

Figure 3: Phylogenetic tree of some bacteria belonging to the phylum Bacteroidetes (according to NCBI). Phylum, class, order, family, genus

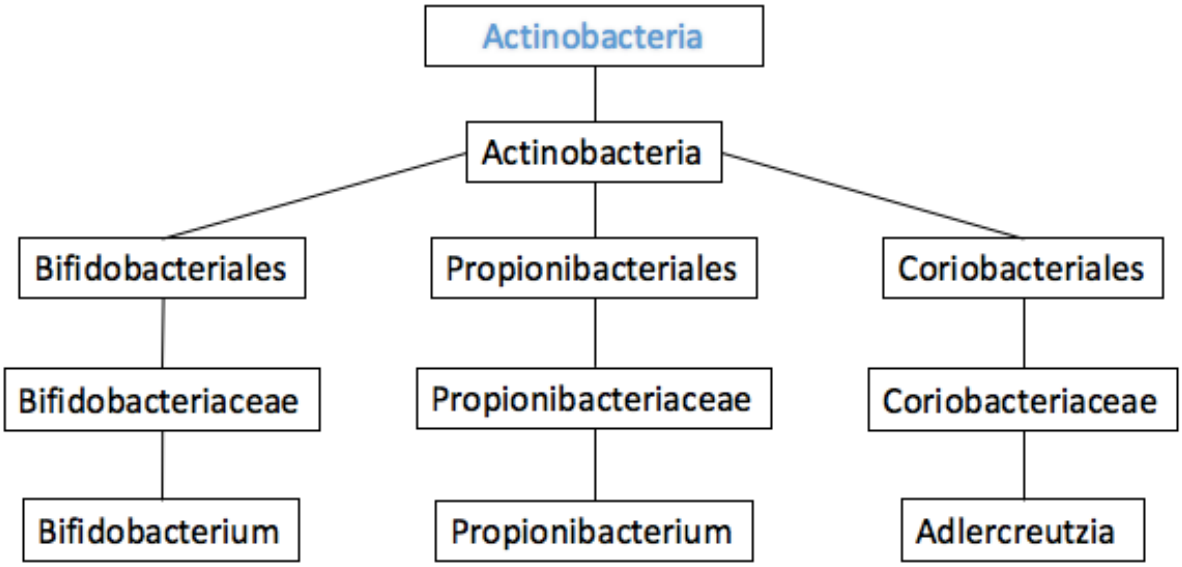

Figure 4: Phylogenetic tree of some bacteria belonging to the phylum Actinobacteria (according to NCBI). Phylum, class, order, family, genus

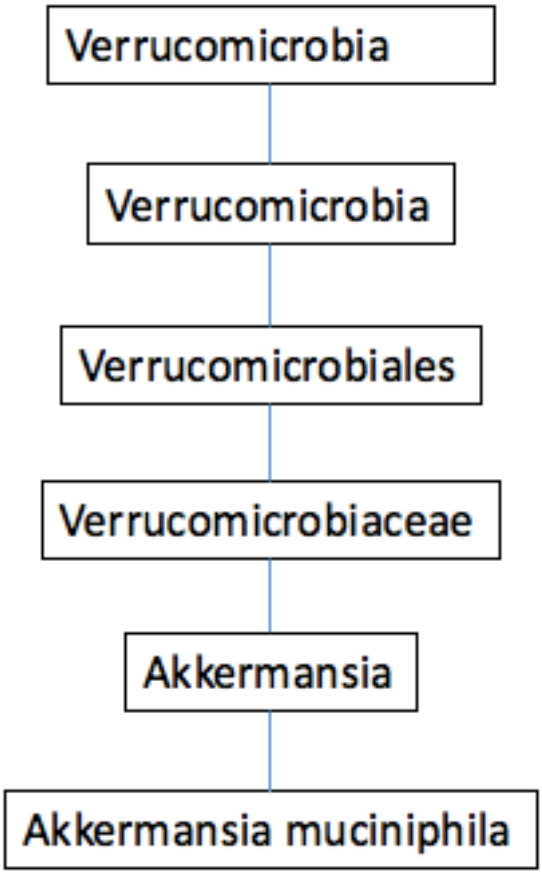

Figure 5: Phylogenetic tree of some bacteria belonging to the phylum Verrucomicrobia (according to NCBI). Phylum, class, order, family, genus, species

To classify the gut microbiota multiple techniques are available, however it is preferable that these techniques are cheap, rapid and gives accurate identification of the gut microbiota in their normal environment. Earlier, culture-based methods were widely used, but since many gut bacteria cannot be cultivated in the lab, results from such studies does not give accurate information about the gut microbiota in their natural environment or quantity. Now, it is standard to assess gut bacteria by sequencing bacterial DNA. There are several methods for this, but in general bacteria are collected, DNA is extracted and variable regions of the gene encoding 16SRNA are sequenced. This gives both the possibility to identify bacteria on different taxonomic levels as well as quantify the relative abundance [46,47].

1.2.3 What characterizes a healthy gut microbiota?

A diverse and a stable gut microbiota is associated with health, and can be defined by "the presence of classes of microbes that enhance metabolism, resilience of infection and inflammation, resistance to cancer or autoimmunity, endocrine signaling, and brain function" [48]. This is referred to as normobiosis, a term used when microbiota associated with health benefits dominates in number over potentially harmful ones. Dysbiosis, in contrast, is a term used when the ecosystem in the gut is dominated by one or more potentially harmful microorganisms, thus creating a transient or a permanent imbalance in the gut microbiota [44].

In a healthy state, the colonic microbiota are dominated by the phyla Firmicutes and Bacteroidetes, followed by the phyla Actinobacteria, Verrucomicrobia and Proteobacteria (figure 2-5) [49,50]. This profile usually remains stable, but the distribution at the Order level and beyond varies. The genera *Bacteroides, Bifidobacterium, Streptococcus, Enterobacteriales, Enterococcus, Clostridium, Faecalibacterium, Eubacterium, Roseburia, Lactobacillus and Ruminococcus* have been considered as the predominant fecal bacteria and are associated with health and proper gastrointestinal function [48,51]. In addition the species *Faecalibacterium prausnitzii* is considered as a key member of the colonic microbiota [50]. The colonic microbiota also consists of pathogenic bacteria within the phylum Proteobacteria, including *Campylobacter jejuni, Salmonella enterica, Vibrio cholera* and *Escherichia coli*. However, the abundance of this phylum is usually low. Based on several studies, a healthy gut microbiota may be characterized by a low abundance of the phylum Proteobacteria, and a high abundance of the genera *Ruminococcus* spp., *Bacteroides* spp., *Prevotella* spp and *Clostridium clusters* (IV and XIVa) [51,52]. The healthy gut microbiota that are associated with the mucosa, referred to

as ´mucosa-associated´ microbiota, are dominated among others by the genera *Akkermansia* and *Ruminococcus* [51], [53].

Large inter-individual differences and small intra-individual differences have been observed in the gut microbiota composition, indicating that a core microbial population exists [44]. This core microbial population are involved in central carbohydrate metabolism, and in some cases protein metabolism, including production of SCFAs and branch-chain fatty acids (BCFAs) [48].

1.2.4 Gut microbiota and diet interaction

Gut microbiota composition is affected by diets. Consequently, the gut microbiota can adapt to various dietary challenges, and change its fermentation. This can occur in conditions such as fermentable carbohydrate restriction, where the gut microbiota may switch from carbohydrate to protein metabolism. Some examples of this are when individuals ingest high amounts of plants (e.g. wheat), which contains dietary fiber such as resistance oligosaccharides (fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS)), they may be enriched with bacteria belonging to the genera *Bacteroides, Prevotella* and *Bifidobacterium*. Conversely, individuals that ingest diets containing high amounts of animal protein and fat may be enriched with e.g. *Bacteroides* spp. [48]. This shows that the gut microbiota can adapt to various dietary challenges and can change its metabolism.

As dietary challenges alter the gut microbiota composition, it is anticipated that the concentrations and types of fermentation products change as well. The gut microbiota ferment dietary carbohydrates or proteins that have escaped the digestion in the upper gastrointestinal tract. This leads to the production of various metabolites, including SCFAs, BCFAs and gases (Table 5). The most common SCFAs are butyrate, acetate and propionate. These, and in particular butyrate, are rapidly absorbed, and are used as energy source for colon enterocytes as well as for peripherally tissue (in e.g. the liver). SCFAs are associated with a numerous health-promoting properties by offering resistance to infection, anti-inflammatory properties (through G-proteins) and inhibition of pathogenic invasion by reducing pH [33,48,54,55]. In addition, particularly butyrate and propionate, have beneficial effects on the glucose- and energy homeostasis [56–58]. However, abnormal or elevated levels of butyrate, acetate and propionate may be negative for host health in some disorders of the gut, e.g. IBS. This includes increased

contractions in distal parts of the small intestine (ileum) as well as contribution to abdominal pain [59]. Table 2 gives an overview over the gut microbiota metabolism and their end-products.

The bacteria that ferment dietary carbohydrates and indigestible carbohydrates are called "saccharolytic" bacteria, and includes members of the genera *Bifidobacteria*, *Bacteroides*, *Ruminococcus*, *Lactobacillus*, *Clostridium, Roseburia*, *Coriobacteriaceae, Dorea, Subdoligranulum,* in addition to the species *F. prausnitzii, Ruminococcus bromii* and *Acetivibrio cellolyticus* [48,60–62]. Bacteria belonging to the genera *Lactobacillus* and *Bifidobacteria* are lactate-producing, probiotic bacteria and ferment carbohydrates such as lactose and oligosaccharides (fructo- and galacto-oligosaccharides), respectively, as well as ferment nutrients that have been degraded by *Bacteroides* spp. [63]. However, some of these saccharolytic bacteria can adapt to dietary changes and alter their metabolism, for instance by degrading proteins and amino acids. This includes members of the genera *Bacteroides*, *Clostridium*, *Coriobacteriaceae*, *Adlercreutzia* and *Porphyromonas* [54,57]. Bacteria that utilize the end-products of sugar metabolism of other gut bacteria are called "asaccharolytic bacteria" (e.g. *Bifidobacteria* spp. or *Propionibacterium* spp., see table 2) [57].

Protein metabolism may be associated with detrimental effects in contrast to what is the case with carbohydrate metabolism. Proteins and amino acids are mostly fermented in the distal colon and this part of the colon is often depleted for carbohydrates and the pH is therefore higher, hence leading to more efficient protein fermentation [48]. This leads to production of ammonia (NH_3), amines, phenols, indoles, sulfides, thiols, and BCFAs (isobutyrate and isovalerate), many of these (except BCFAs), are associated with genotoxicity in the host [64,65].

Some colonic microbiota can also produce gas following fermentation of either carbohydrates, proteins or metabolites from other GI-bacteria (Table 2). This relates to members of the genera *Prevotella, Collinsella, Coriobacteriaceae* and *Dorea*, to mention a few. If the composition of the colonic microbiota is disturbed, these metabolites can give symptoms such as bloating, distention and/or abdominal pain, typical for IBS patients. Conversely, bacteria belonging to the genera *Adlercreutzia* and *Dialister* have the ability to consume gas, thus the amount of these bacteria increase as gas-producing bacteria grows [60].

Table 2: Mechanisms of some of the members in the colonic microbiota[53,60–62]

Colonic microbiota	Ferments/utilizes/degrades	End-products	
		Short-chain fatty acids/metabolites	Gases
Adlercreutzia spp.	Hydrogen gas , protein metabolism	Eqoul (a isoflavondiol = nonsteroidal estrogen), BCFAs	
Acetivibrio cellolulyticus	Cellulose	Acetate (lactate + glucose in minor amounts)	Ethanol, CO2, H2, methane
Akkermansia muciniphila	Mucin-degrader. Polyphenols, fructo-oligosaccharide, polyamines	Galactose, N-acetylglucosamine, disaccharides and small oligosaccharides	
Bifidobacteria spp.	Oligosaccharides that have been released from more complex polysaccharides by *Bacteroides* spp. (In e.g. Lactose). In addition to mono-, manno- and fructo-oligosaccharides	Acetate, lactate	
Bacteroidales/Bacteroides spp.	Proteins, and complex sugar polymers (in e.g. Lactose)	Acetate, succinate, propionate, formate	Hydrogen gas
Clostridium spp.	Undigested carbohydrates + peptides and amino acids	Butyrate, BCFAs	NH4+ (to *Bacteroides* spp.)
Coriobacteriaceae spp.	Protein and glucose	Acetate, format, lactate, BCFAs	Hydrogen gas
Dorea spp.	Glucose		Hydrogen gas, CO2
Dialister spp.	Hydrogen gas, CO2	Acetate, propionate	
Eubacterium rectale	Complex glycan or simple carbohydrates and amino acids	Butyrate, BCFAs	
Faecalibacterium rectale	Glucose, fructose, fructo-oligosaccharides, N-acetylglucosamine and pectin	Lactate, butyrate, formate	
Lactobacillus spp.	Lactose and other carbohydrates	Lactate, (ethanol, carbon dioxide of some species under some conditions)	Ethanol, carbon dioxide
Prevotella spp.	Cellulose and xylans	SCFA	H2S
Propionibacterium spp.	Lactate, succinate	Propionate, produces vitamin B12	
Porphyromonas spp.	Amino acids (nitrogenous substrates)	BCFAs	
Roseburia spp.	Starch and inulin	Butyrate	
Ruminococcus spp.	Cellulose, mucin-degrader	Butyrate, products of mucins	
Ruminococcus bromii	Resistant starch	Acetate	Ethanol
Ruminococcus torques	Mucin-degrader, glucose, lactose	Galactose, N-acetylglucosamine, disaccharides and small oligosaccharides	From glucose: ethanol, hydrogen and CO2

Ruminococcus gnavus	Mucin-degrader, arabinose, maltose, xylose, glucose	Galactose, N-acetylglucosamine, disaccharides and small oligosaccharides	From glucose: ethanol, hydrogen and CO2
Subdoligranulum spp.	Glucose	Butyrate, lactate. Minor acetate and succinate	

1.2.5 Gut microbiota and IBS

Several studies have established that there are significant differences between the gut microbiota in IBS patients compared to healthy controls [41,66]. These changes have largely been characterized as dysbiosis and linked to the pathophysiology of IBS [41,44].

Most studies have demonstrated a reduced bacterial diversity in IBS patients. Also altered proportions of specific bacteria and a difference in the variation of the gut microbiota composition is seen [41]. For instance the phyla Firmicutes and Proteobacteria are increased, whereas the phyla Bacteroidetes and Actinobacteria are decreased (table 3) [67–70]. Furthermore, genera *Bacteorides* spp. [52,70], *Bifidobacteria* spp. [71–73] and *Faecalibacterium* spp. are less abundant in patients with IBS [70]. Increased relative abundances have been seen of the genera *Ruminococcus* spp., *Clostridium* spp., *Dorea* spp., *Subdoligranulum* spp., *Dialister* spp., *Clostridium* cluster XIVa., *Roseburia* spp., *Coprococcus* spp. [38,52,70], *Lactobacillus* spp. and *Veillonella* spp. [59,73,74]. A recent study indicates that patients with IBS might have a microbial signature. Casen and coworkers suggested that the phyla Firmicutes, Proteobacteria and Actinobacteria, in addition to the species *Ruminococcus gnavus* were the predominant bacteria contributing to the dysbiosis seen in patients with IBS [44]. Table 3 sums up the gut microbial changes in patients with IBS.

Table 3: Overview over the changes of the gut microbiota in patients with IBS

Increased	Decreased
Firmicutes	Bacteroidetes
Proteobacteria	Actinobacteria
Ruminococcus spp.	*Bacteroides* spp.
Clostridium spp.	*Bifidobacteria* spp.
Dorea spp.	*Faecalibacterium* spp.
Subdoligranulum spp.	
Clostridium cluster XIVa - *Roseburia* spp. - *Coproccus* spp.	
Lactobacillus spp.	
Veillonella spp.	
Dialister spp.	

Patients with IBS have demonstrably altered colonic fermentation, and some studies have observed an association between abdominal symptoms and abnormal concentration of organic acids (in e.g. p-hydroxybenzoic acid (pHBA), succinate, lactate) and SCFAs. An increase in hydrogen gas production has also been observed in IBS patients. Studies on SCFA-concentration in IBS patients are inconsistent. Both increased and decreased levels are found [75, 59].

Breath tests are used widely to assess certain functions of the gut microbiota. For instance, it is used to detect hydrogen- or methane levels in expired air as a measure of gas producing bacteria following administration of carbohydrate(s) (e.g. lactulose). The test was originally designed to detect small intestinal bacterial overgrowth (SIBO). If the breath hydrogen- or methane levels are increased, this can indicate SIBO, e.g. in patients with irritable bowel syndrome [31]. However, this test have been criticized as it has shown to have a high rate of false positive results [29,31].

1.3 DIETARY TREATMENT AND MANAGEMENT OF IBS

The pathophysiology of IBS is not fully understood, and a cure does not exist yet. However, evidence exist to suggest that the gut microbiota and their metabolites play a role in the pathophysiology and dietary treatment strategies have been exploited to reduce symptoms in IBS patients. Other treatment options, except dietary strategies, may be pharmacological treatments. This includes antispasmodics and antidiarrheal for diarrhea, fiber supplementation for constipation, or supportive therapy with low-dose antidepressants to normalize GI-motility. In addition to the antibiotics rifaximin and the anti-inflammatory agent mesalamine, have shown some efficacy in reducing the symptoms in subsets of patients with IBS, particularly IBS-D patients [76].

A low-FODMAP (Fermentable Oligo-, Di-, Monosaccharides And Polyols) diet has received a lot of attention for its effectiveness in reducing symptoms in patients with IBS [77–83]. Probiotics and prebiotics are also treatment options as they manipulate the gut microbiota. Probiotics are live microorganisms which normally resides in the GI-tract and might confer a health benefit to the host when it is ingested [11,45]. Prebiotics are selectively fermented ingredients that induce the growth or activity of several bacteria in the gastrointestinal tract, thus contributing to health benefits of their host [84,85]. Several studies have shown that some probiotics effectively alleviates the symptoms in patients with IBS, particularly abdominal pain and bloating [86–89]. Further, the gut microbiota can also be manipulated with fecal transplantation [41,90]. The long-term effect of the low-FODMAP diet, pro- and prebiotics, and fecal transplantation remains unclear [4,41,76].

1.3.1 Low-FODMAP diet

About two-thirds of all IBS patients report that their symptoms are associated with food, particularly poorly absorbed carbohydrates, including fructose, lactose, sorbitol and other sugar alcohols [11,91,92], but foods containing high amounts of fat, biogenic amines or lectins (proteins that binds carbohydrates) might also contribute to symptoms in IBS patients. The same has been reported concerning foods containing preservatives (e.g. benzoic acid or sulfite), spicy foods (onion, garlic), as well as food that trigger histamine release [91]. However, the low-FODMAP diet have been suggested as a treatment strategy for reducing the symptoms that occur in IBS patients following ingestion of poorly absorbed fermentable carbohydrates.

The low-FODMAP diet is the first reported diet to be effective in alleviating GI-symptoms in the majority of IBS patients [93]. As the name implies the low-FODMAP diet is low in dietary carbohydrates that are poorly absorbed in the small intestine, including lactose, polyols, fructans and galacto-oligosaccharides. These are osmotically active due to their small size, and are rapidly fermented by the gut microbiota that reside in the colon, thus leading to increased gas production [94–96]. Increased gas production is not necessarily painful to healthy individuals, but it can be painful to IBS patients, and this can be explained by more sensitive intestines (hypersensitivity) likely due to the activation of cells around enteric neurons in the intestine. Furthermore, the malabsorption may be explained by the absence or reduced concentration of digestive enzymes in the small intestine [7], which leads to luminal distension due to fermentation in the colon, and accounts for symptoms in patients with IBS [11]. Some FODMAPs are also prebiotic (e.g. oligosaccharides) and it has been suggested that a reduced production of some SCFAs, due to reduced intake of prebiotics, may reduce symptoms in IBS patients [52].

As early as in the 1980s and 1990s there was evidence to suggest that some short-chain carbohydrates, particularly lactose, sorbitol and fructose, were poorly absorbed, and played a role in the induction of IBS symptoms. This was observed in studies on dietary restriction that caused fewer GI-symptoms [77–79,83]. Further examination suggested that fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and multiple sugar alcohols (e.g. mannitol), also played a role in the induction of GI-symptoms, due to the incomplete absorption [97].

In 2006, the first research trial on the role of a restricted FODMAP diet was conducted in IBS patients. The focus was to restrict fructose/fructans diet. The initial study found that 74% of all IBS patients following this diet showed decreased symptoms [81]. This was confirmed by Shepherd et al. (2008), who concluded that a diet low in fructose and/or fructans may reduce GI-symptoms in IBS patients with fructose-malabsorption [80]. Subsequently, Staudacher et al. (2011) confirmed these findings in a controlled study in IBS patients, where a low-FODMAP diet was compared with a standard UK diet. Eighty six percent of the participants with IBS reported gastrointestinal symptomatic improvement following the low-FODMAP diet, compared to the UK diet [82].

Furthermore, high-quality evidence from prospective studies and randomized controlled trials supports the efficacy of a low-FODMAP diet in alleviating GI-symptoms in IBS patients,

including pain, bloating and diarrhea [98]. Seventy five percent of IBS patients have reported an improvement in symptoms after following a diet low in FODMAPs [63].

The low-FODMAP-diet consists of two phases, the elimination phase and the reintroduction phase. The first phase is to consider the patient´s degree of benefit, and reduce symptoms. This is often achieved through an elimination phase, generally lasting 6-8 weeks. However, the length of this period is individual and might last for a longer or shorter period. It is recommended to be guided by a dietitian and the purpose is to be symptom-free prior to the onset of the second phase; the inclusion phase [93].

During the elimination phase, patients with IBS must avoid multiple food items containing high FODMAPs, some examples are shown in Box 1. During the reintroduction phase (second phase), the aim is to reintroduce food items to determine which food items that create symptoms. Reintroduced food items are determined on individual basis, but wheat, fiber or onion-containing foods are the most common foods to reintroduce [93].

Box 1 High FODMAPs to avoid and some food items they are presented in	
Oligosaccharides (FOS, GOS)	Wheat, rye, garlic, onions, legumes
Lactose	Some dairy products
Fructose (particularly in excess of glucose)	Pears, apples, honey, high-fructose corn syrup
Polyols (sugar alcohols)	Pears, apples, artificially sweetened gums, confectionary

It is vital to consult a dietician during both phases of the diet to tailor a nutrition plan and to determine which type and what amounts of FODMAPs are tolerated. The aim is to prevent nutrient deficiency, without having GI-symptoms [93].

2 AIM

Diets low in FODMAPs, have been used widely to aid IBS patients to reduce symptoms such as bloating/distension, abdominal pain and diarrhea/constipation. However, such diets reduce the intake of prebiotics by up to 50%, hence reduce the amount of total carbohydrates available for colonic fermentation. Evidence suggest that this diet alter the colonic microbiota and further may be unfavorable to colonic health. This is particularly what this thesis aims to investigate, in addition to discuss if these effects really are unfavorable to colonic health or not. Particularly, the questions asked were:

- What changes occur in the gut microbiota composition in IBS patients following a dietary challenge with fermentable carbohydrate restriction with emphasis on low-FODMAP diets?
- Are the potential changes in gut microbiota influencing the hosts´ health?
- Are these putative effects positive or adverse to the host, and do they constitute a risk factor for the hosts´ health?

3 METHODS

3.1 Study selection

Original articles were primarily identified through selective searches using PubMed. Cochrane library were also used; however, this did not give any different results. The first search was performed using PubMed in January 2016, while an updated search was performed on April 29th 2016. Search terms used are shown in Box 2, briefly "IBS" or irritable bowel syndrome or abdominal pain", "microbiota or microbiome", "nutrition or diet" and "RCT or randomized controlled trial or epidemiology or pilot". The inclusion criteria are shown in Box 3, and included: original articles, either RCTs, epidemiology or pilot studies, that explored how a diet low in fermentable carbohydrates may affect the gut microbiota composition in both adults and pediatric patients with irritable bowel syndrome (IBS) compared with diet(s) high in FODMAPs. One pilot study on pediatric patients was included due to few studies on this issue. The exclusion criteria, shown in Box 4, were if the IBS patients received antibiotics, prebiotics or probiotics during the trial, because these criteria most likely affect the gut microbiota. Other pharmacological, complementary and alternative treatments (e.g. acupuncture, homeopathy, herbalism) were also exclusion criteria, as were other diseases or disorders (in e.g. diabetes and inflammatory bowel disease) and other diets than a diet low in fermentable carbohydrates. In this thesis the focus was on how a diet low in fermentable carbohydrates affected the gut microbiota composition. Only studies published in English were included, and search criteria were not restricted to year of publication. Figure 6 gives an overview over the study selection.

Box 2 Search terms
IBS or irritable bowel syndrome or abdominal pain Microbiota or microbiome Nutrition or diet RCT or Randomized controlled trial or epidemiology or pilot

Box 3 Inclusion criteria
Randomized clinical trials
Epidemiology trials
Pilot trials
Diagnosed with IBS (using either Rome II or Rome III)
Compared the effect of a diet low in fermentable carbohydrates on gut microbiota composition with a diet high in fermentable carbohydrates

Box 4 Exclusion criteria
Use of antibiotics, prebiotics, probiotics prior and/or during the trial
Pharmacological treatment
Complementary and alternative treatment (e.g. acupuncture, homeopathy, herbalism)
Other diseases or disorders (e.g. diabetes, inflammatory bowel disease)
Other diets than low in fermentable carbohydrates

The search gave 31 results (figure 6), of which 27 were excluded because they did not meet the inclusion criteria: Two studies were reviews, one study was about prebiotic-supplementation, four studies were about probiotics, 14 studies did not investigate IBS, five studies did not have a dietary intervention and one study did not investigate the gut microbiota following a diet low in fermentable carbohydrates. Thus, four of the results were of interest because they met the inclusions criteria. The characteristics of these four studies that were included is shown in table 4.

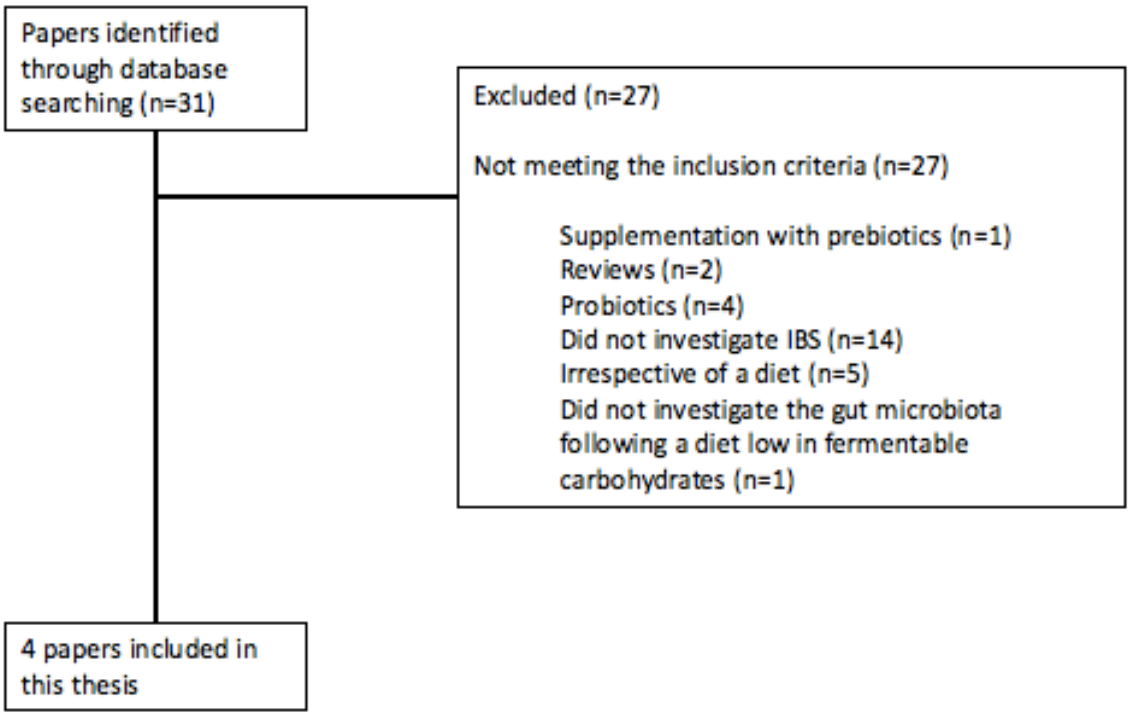

Figure 6: Flow chart of the study selection

3.2 Quality of the studies using the Jadad scale

Assessment of the quality of randomized controlled trials (RCTs) is relatively new, and it exist several scales and checklists to assess the quality of RCTs. In this thesis, the Jadad scale was used (Table 5). This scale consists of three items; randomization (max. two points), blinding (max. two points) and account of all patients (max. 1 point), and can be given maximum five points in total. The RCTs can initially be given maximum two points for the item ´randomization´, this includes one point if the word ´randomization´ is mentioned in the RCTs and one point if the randomization is suitable. In contrast, one point can be removed if the method of randomization in the RTCs is unsuitable. Further can the RCTs be given two points for the item ´blinding´ if the word ´blinding´ is mentioned in the trials, and if the method of blinding is suitable. One point can, same as for the item ´randomzation´, be removed if the method of the blinding is unsuitable. Finally, the item ´account of all patients´ in RCTs can be given maximum one point if the number of patients in the RCTs and the reason why some patients in the RCTs have been eliminated (if there is any eliminations) are stated [99].

Table 4: The characteristics of the four included studies

Study nr.	Study design	Study duration	Subjects			Interventions	Method	Exclusions criteria	*Reference*
				Age (mean)	Gender				
1	Randomized, controlled trial	4 weeks	Adults with IBS** (n = 35) (Rome III)	Intervention: 36,4 ± 11,6 Control: 35,5±9,1	Intervention: 11 females (69%), 5 males Control: 12 females, 7 males	Habitual diet (control) vs. LFM-diet	16S rRNA sequencing using FISH	Pregnancy or lactation, use of pro- or prebiotics, or bowel preparation in 4 weeks prior to the study or use of IBS medications 4 weeks prior to the study or during the study	*Staudacher et al. (2012)* Study 1
2	Randomized, controlled single-blinded cross-over trial	3-week intervention Longer than or 3-week wash-out period 3-week intervention	Adults with IBS (n = 27) vs. Healthy controls (n=6)*** (Rome III)	IBS: 43 (29-54). Controls: 31 (23-61)	IBS: 21 females, 6 males. Controls: 5 females, 1 male	Habitual diet vs. TAD (control) vs. LFM-diet	16S rRNA sequencing using qPCR	Celiac disease, previous abdominal surgery, diabetes, inability to understand english, use of probiotics or antibiotics for 2 weeks prior to the study or during the study	*Halmos et al. (2015)* Study 2
3	Randomized, prospective, single-blinded paralell study	3-weeks	Adults with IBS (n=37); LFM(n=18), HFM(n=19) (Rome III)	LFM: 50,28 (26-77). HFM: 51,47 (24-83)	LFM: 15 females, 3 males. HFM: 17 females, 2 males	LFM-diet vs. HFM-diet	16S rRNA gene profiling	No history of gastric, small bowel or colonic surgery, active IBD, celiac disease, pregnancy or use of antibiotica (including use 4 weeks prior the study), stool bulking agents, narcotic analgesis or lactulose (a prebiotic). In addition; if the patients were on specific diets such as: LFM-diet, Palaeolithic diet, gluten-free diet or Atkins diet	*McIntosh et al. (2016)* Study 3
4	Uncontrolled pilot study	1-week	Children with IBS (n=8) (Rome III)	7-17 years	Not mentioned	Habitual diet vs. LFSD	16S rRNA using 454-pyrosequencing	Use of antibiotics or probiotics within 3 months	*Chumpitazi et al. (2014)* Study 4

*** = patients with bloating and/or IBS-D were included in the study, **** = healthy controls and IBS patients combined, LFM: low-FODMAP diet, HFM: high-FODMAP diet, TAD: Typical Australian Diet, LFSD: Low Fermentable Substrate Diet*

4 RESULTS

4.1 Quality of studies and compliance to dietary interventions

In the four articles that was found eligible, 113 participants were included. Three of the studies [63,96,100] were randomized controlled trials, while the last one was an uncontrolled intervention pilot study (Table 4) [101]. The quality of the RCTs was generally good. Study 2 and 3 scored 5 of 5 on the Jadad scale, whereas Study 1 received score 3 (Table 5), mainly because it was not single- or double blinded. All the four studies recruited patients with Rome III IBS (Table 4).

Study 1, 2 and 4 assessed the dietary intake both prior to dietary interventions (baseline) and after the dietary interventions [63,96,101]. Study 3 only offered dietary guidance and provided menus, but did not measure the actual intake [100]. In study 1, 3, and 4 [96,100,101], the compliance to the intervention diets were recorded and they found good compliance. Furthermore, study 1 assessed compliance via weekly contact with the participants on the low-FODMAP group, through phone calls or emails, in addition to collect dietary diaries. Study 1 found that the intake of total carbohydrates, starch and fermentable carbohydrates following the intervention were lower in the low-FODMAP group compared with the control group (habitual diet) [96]. Study 3 also collected dietary diaries and at the end of the interventions the FODMAP content was scored in a blinded fashion using a score, made for this study, for low-FODMAP ranging from 1-6. The low-FODMAP content got a mean score of 3.5, suggesting good compliance to the diet. In addition it was observed a positive correlation between the global symptom scores and their level of FODMAP ingestion [100]. Finally, study 4 reported good compliance to the diet through the assessment of nutrient intake during both the habitual diet and the low fermentable substrate diet (LFSD). They found a lower total fermentable carbohydrate intake between the habitual diet and the LFSD, but no differences in numbers of fermentable items ingested between non-responders and responders were found [101]. The high-FODMAP diet (Australian diet) and the low-FODMAP diet in study 2 only differed in FODMAP-content, however the authors did not report compliance to the diet [63]. It was reported no significant differences in energy, protein or fat content between the low-FODMAP and the high-FODMAP diets in neither of the studies [63,96,100,101] (besides a lower total calorie-intake in pediatric participants in study 4) [101].

4.2 Effect of fermentable carbohydrate restriction on IBS symptoms, microbial metabolites and the gut microbiota composition

4.2.1 Low-FODMAP diet and effect on IBS symptoms

All the four studies (study 1-4) reported a reduction in symptoms in patients with IBS following a diet low in fermentable carbohydrates. The symptoms that were reduced in all the four studies were bloating, abdominal pain, flatulence, abdominal distension and tiredness. Study 4 divided the participants in responders (>50% symptom improvement) and non-responders (no or little symptom improvement) following the LFSD. In addition this study reported that responders had a trend toward lower pain frequency than non-responders, but this was not statistically significant [101].

Study 1, 2 and 4 assessed the whole gut transit time (WGTT) [63,96,101]. Study 2 and 4 did not observe any changes in the intestinal transit in IBS patients following a fermentable carbohydrate restriction [63,101]. However, study 4 reported that non-responders and responders had a trend toward fewer bowel movements, probably due to lower calorie intake on the LFSD compared to habitual diet [101]. Study 1, in contrast, observed lower stool frequency (p=0.008), and more stools with normal consistency (p=0.02) in the low-FODMAP group. This can indicate that this diet can normalize the stool output in pediatric IBS patients. Intriguingly, it was no difference in stool consistency or the severity of self-reported diarrhea (p=0.56) between the low-FODMAP group and the high-FODMAP group, despite the restriction of the osmotically active fermentable carbohydrates [96].

4.2.2 Gut microbial products/metabolites

Study 1, 2 and 4 measured fecal SCFA levels to compare changes before and after low-FODMAP interventions. Surprisingly neither of the studies observed any significant changes (Table 5) [63,96,101]. Intriguingly, study 2 observed an elevated pH of 0,2 units (p=0.008) in feces (Table 5) despite no change in SCFA-production [63]. Similarly, study 4 did not observe any changes in SCFA-production [101]. The authors suggest this could be due to the rapid absorption and use of SCFAs by colonic enterocytes. They also speculate that a possible change from carbohydrate- to protein metabolism, or a change of other gut bacteria could explain the elevated fecal pH associated with low-FODMAP intake. Study 2 and 4 also measured colonic branch-chain fatty acids (BCFAs) to evaluate whether a shift towards bacterial protein

metabolism was evident [63,101]. Neither of the studies observed any changes of the major BCFAs isovalerate and isobutyrate (Table 5).

Study 3 was the only study that measured metabolites in the urine and observed reduced levels of histamine following a low-FODMAP diet (Table 5) [100]. Histamine is an important signaling molecule that may explain some of the IBS symptoms, and is abundant in mucosal mast cells of IBS patients [102]. Relative increases of urinary azelaic acid and p-hydroxybenzoic acid (pHBA) were also reported. pHBA, a phenolic derivative of benzoic acid, is produced when gut bacteria ferment polyphenols from plant sources [100]. Azelaic acid is known to have anti-inflammatory properties and are found in foods such as wheat, rye and oat seeds [100]. However, the authors of study 3 did not identify the mechanism(s) causing the reduction in urinary histamine levels or the increase in urinary azelaic acid or pHBA.

Study 3 and 4 were the only studies that measured gas production prior to- and following the diet low in fermentable substrates (Table 5). Study 3 observed a reduction in the amount of breath hydrogen gas in the low-FODMAP group [100], which might explain the reduction of symptoms, while no significant changes in breath hydrogen or methane production were observed in study 4 [101]. However, non-responders in study 4 had a trend towards an increased breath hydrogen production following a diet low in FODMAPs. This may indicate that the gut microbiota composition in non-responders at baseline are different compared to responders, particularly containing bacteria with less saccharolytic capacity and more gas-producing bacteria.

4.2.3 Gut microbiota composition at baseline

All the four studies (study 1-4) measured the gut microbiota composition at baseline, however only two studies reported the results of this (study 2 and 4, see Table 5) [63,101]. The other two studies (study 1 and 3) refer only to changes in various strains and families [96,100].

4.2.4 Gut microbiota following a fermentable carbohydrate restriction diet

Firmicutes

Study 2, 3 and 4 (Table 5 and 6) observed multiple changes in the phylum Firmicutes, mostly in the order *Clostridiales*, in the low-FODMAP groups compared to the diets high in

FODMAPs [63,100,101]. Study 2 reported a decrease in total abundance of the probiotic genus *Lactobacillus* spp. (p=0.003) in IBS patients. These bacteria are saccharolytic, i.e. they ferment indigestible carbohydrates. Study 1, in contrast, did not observe any of these differences [96]. Study 4 observed higher levels of *Dialister*-like OTUs in the gut microbiota community of non-responders (accounted for 5% of the non-responder communities) compared to baseline, whereas responders were depleted for *Dialister* spp. (Fig. 7) [101]. Bacteria belonging to the *Dialister* genus are gas consumers, i.e. they consume hydrogen gas and carbon dioxide to produce acetate and propionate (Table 2 and 4). This may imply that in non-responders there is an increased growth of gas producing bacteria leading to increased growth of *Dialister* spp., despite no change in SCFA-production in non-responders in study 4 [101].

Furthermore, study 2 (Table 5 and 6) particularly observed a decrease of *Faecalibacterium prausnitzii* (p<0,001), and a decrease in *Roseburia* spp. (p<0,001) following a low-FODMAP diet (Fig. 7). These are butyrate-producing bacteria. Relative abundance of *Clostridium* cluster XIVa and XIV, in contrast, were increased. Additionally *Ruminococcus gnauvus* was decreased (p=0.002), while *Ruminococcus torques* was increased (p =0,001)(Fig. 7) [63]. Both of these bacteria are known mucus degraders. Study 3 observed an increase in bacterial richness of the order *Clostridiales* (p=0.023) in IBS-D and IBS-M patients following a low-FODMAP diet. This was particularly noted of genera within the *Clostridiales* family XIII *Incertae sedies* (p=0.008), in addition to the genera *Roseburia* (p=0.038)(Fig. 7) and *Clostridium* spp. (p=0.045) [100]. On the other hand, study 1 did not observe any changes in either *Clostridium* cluster XIVa or *F. prausnitzii* [96]. Furthermore, study 4 observed an enrichment of OTUs resembling the saccharolytic and hydrogen gas-producing bacterium *Acetivibrio cellulolyticus* in pediatric non-responders compared to responders. An enrichment of OTUs within the saccharolytic genus *Subdoligranulum* was reported in pediatric responders compared to non-responders following a diet low in fermentable substrates [101]. The increase in *Acetivibrio* sp. can imply that these bacteria contributed to a trend towards an increased hydrogen-gas production in non-responders and can also have contributed to the high levels of *Dialister* spp.

Bacteroidetes

Study 3 and 4 observed changes in the phylum Bacteroidetes in the low-FODMAP groups (Table 5 and 6), i.e. an increase of members within the order *Bacteroidales* [100,101]. Study 4, particularly, observed an increase of the genus *Bacteroides* spp. in pediatric non-responders,

whereas the family *Prevotellaceae* spp. were increased in pediatric responders [101]. This was in contrast to study 1, that did not observe any difference in *Bacteroides-Prevotella*-group [96]. Furthermore, study 3 observed an increase of members belonging to the genus *Porphyromonas* (p=0.01)(Fig. 7) in patients with IBS-D and IBS-M. Members of this genus are known to utilize the end-products of protein metabolism, particularly amino acids, of other GI-microbiota to produce BCFA, so called "asaccharolytic bacteria" [100]. The members of the genus *Bacteroides* are associated with both carbohydrate and protein metabolism. The increased abundance in the genera *Bacteroides* spp. and *Porphyromonas* spp. might indicate that a diet low in fermentable carbohydrates in patients with IBS, results in a switch in the gut microbiota metabolism, from carbohydrate to protein- and amino acid metabolism, but that was not supported by measurements of BCFAs in feces from non-responders in study 4 [101].

Actinobacteria

All the four studies have observed changes in the phylum Actinobacteria (Table 5 and 6). Study 1, 2 and 3 particularly observed a decrease in the order *Bifidobacteria* spp. (p<0.001, 0.001 and 0.048, respectively)(Fig. 7) [7,63,100]. Study 2 and 3 (but not study 1) included IBS-C patients and observed a decrease in relative and total abundance of the probiotic genus *Bifidobacterium* spp. in the low-FODMAP groups [63,100]. An effect like this is most likely due to the restriction of fructo-oligosaccharides and galacto-oligosaccharides, as these works as growth factors to the bacteria in this genus. Furthermore, study 3 observed a decrease in the family *Propionibacteriaceae* spp. (p=0.043) of the microbiota community in IBS-D and IBS-M patients [100]. Members of this genus are known to produce propionate from lactate and succinate, and are considered as health promoting. Finally, study 3 and 4 observed an increase in the order *Coriobacteriales* spp. Study 4, particularly, observed an increase of *Coriobacteriaceae* spp. in pediatric responders[101] and study 3 observed an increase of the protein-degrader genus *Adlercreutzia* spp. (p-value not specified)(Fig. 7) in IBS-D and IBS-M patients [100]. The increase of these taxa may indicate that these bacteria have adapted the new diet, and switched from carbohydrate- to protein metabolism, but in study 3, measurements of BCFAs or other protein metabolites were not performed, and hence does not confirm this suggestion.

Verrucomicrobia

Study 2 observed a decrease in the phylum Verrucomicrobia (Table 5 and 6), particularly the mucosa-associated bacterium *Akkermansia muciniphila* (p<0.001)(Fig. 7)in IBS patients

following the low-FODMAP diet compared to the Australian diet (high-FODMAP diet) [63]. This bacterium is known to degrade mucins as well as to stimulate mucus-production. No significant changes in this phylum were observed in any of the other studies (study 1, 3 and 4) [7,100,101].

Table 5: Impact of a diet low in FODMAPs on the gut microbiota composition in IBS patients (study 1-4)

Study nr.	Impact on microbiota*						Jadad score	*Reference*
	Baseline microbiota following a low-FODMAP diet	Following a diet low in fermentable carbohydrates	SCFA (including BCFA)	pH	Gas production	Urine metabolites		
1	Not reported	Decreased relative and total abundance of *Bifidobacteria* spp. in the low-FODMAP group vs. Habitual diet.	No significantly changes in fecal SCFA-production	No significantly change in pH	Not measured	Not measured	3	*Staudacher et al. (2012)* Study 1
2	The participants (both IBS and healthy controls, combined) were enriched with *F. Prausnitzii, Clostridium* cluster XIVa, *Roseburia* spp., *Lactobacilli* spp. and *Bifidobacteria* spp.	Total abundance: Decrease in *Bifidobacterium* spp. vs. TAD and habitual diet. Decreased *A. muciniphila, Roseburia* spp., *R.gnavus* and *Lactobacillus* spp. vs. TAD. Decreased *F. Prausnitzii* and *C.*cluster XIVa vs. TAD and habitual diet. Relative abundance: decreased *A. muciniphila* , increased *R. torques* and *C.* cluster XIVa vs. TAD. Increased *C.* cluster XIV vs. TAD and habitual diet.	No significantly changes in fecal succinate or other SCFA, or BCFAs	Significantly 0,2 units higher on the LFM compared with the habitual diet and the TAD	Not measured	Not measured	5	*Halmos et al. (2015)* Study 2
3	Not reported	The entire cohort Decrease in *Bifidobacterium* spp. Decreased levels of *Enterococcus* spp. Increased richness and diversity in Actinobacteria vs. HFM IBS-D and IBS-M : Higher bacterial richness and diversity in Actinobacteria vs. the HFM-group. Higher bacterial richness in Firmicutes and in *Clostridiales* spp. vs. HFM-group. An increase in *Adlercreutzia* spp. vs. HFM-group. Decrease in *Propionibacteriaceae* spp. vs. baseline. Increase in *Clostridiuales* family XIII *Incertae sedies* vs. baseline. Increase in *Porphyromonas* spp. vs. baseline. *Roseburia* spp. and *Clostridium* spp. increased with lower FODMAP-content.	Not measured	Not measured	Following 10 hrs. Fasting, LBHT and ingestion of Kristalose: Slightly less H2 production on the LFM-diet. No differences in methane production between LFM and HFM-diet	Eightfold reduction in urinary histamine in the LFM-group. Relative increase in azelaic acid and pHBA	5	*McIntosh et al. (2016)* Study 3
4	Responders: Enriched with members belonging to *Ruminococcaceae* spp., particularly *Sporobacter* - and *Subdoligranulum*- like OTUs compared to non-responders. Non-responders: Enriched with *Bacteroidales* -like OTUs and one OTU resembling *Ruminococcaceae* spp. compared to responders	Responders: Enriched with members in *Subdoligranulum, Prevotellaceae* and in unclassified *Coriobacteriaceae* and a decrease in *Dialister*. Non-responders: enriched with microbes resembling *Acetivibrio cellolyticus* and several OTUs in *Bacteroides* and *Dialister*	No significantly changes in SCFA- or BCFA-production	Not measured	Following 14 hourly breath tests at the last day of the LFSD periods: No significantly change in H2-production between the baseline and LFSD periods. No significantly change in H2- or methane production between responders and non-responders	Not measured	Not a RCT	*Chumpitazi et al. (2014)* Study 4
	** Following the diet low in dietary fermentable carbohydrates (except the baseline column)*		*LFM: low-FODMAP diet, HFM: high-FODMAP diet, TAD: Typical Australian Diet, LFSD: Low Fermentable Substrate Diet. LBHT: Lactulose Breath Hydrogen Test*					

Summary

To summarize, the global symptom scores were reduced in participants in the low-FODMAP groups (except non-responders in study 4). The four studies all observed changes in some or all the phyla Firmicutes, Actinobacteria, Bacteroidetes and Verrucomicrobioa (Table 5) following the low-FODMAP diet. The largest effect was seen in Actinobacteria in study 1, 2 and 3, with a consistent decrease in *Bifidobacterium* spp. However, there are large variations between the studies. Finally, it was no change in SCFA- or BCFA-production (measured in study 1, 2 and 4), despite changes in bacteria that could indicate so (e.g. increased levels of *Clostridium* XIVa and *Bacteroides* spp. in study 2 and 4, respectively). Figure 7 gives an illustrative overview over the changes of the gut bacteria following a fermentable carbohydrate restriction and the same goes for table 6 gives, but it is a taxonomic overview.

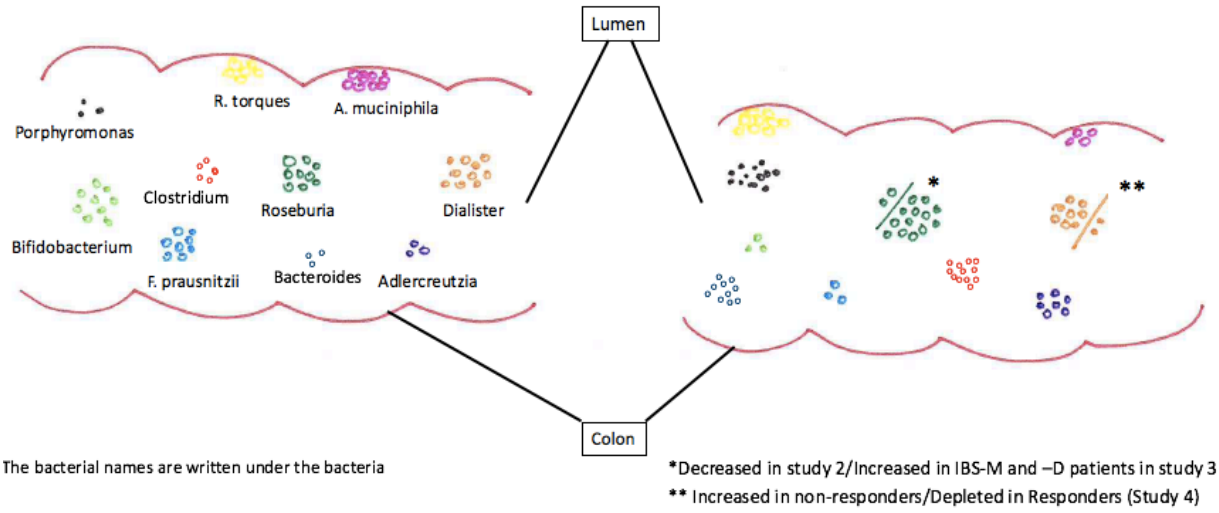

Figure 7: Some of the gut bacteria prior to and following the fermentable carbohydrate restriction (illustrative)

Table 6: A taxonomic overview over the change in the gut microbiota after dietary interventions in the four included studies

Impact on the gut microbiota in IBS patients following a diet low in fermentable carbohydrates							
Study nr.	Phylum	Class	Order	Family	Genus	Species	Study
1	Actinobacteria	Actinobacteria ↑	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium ↓		Staudacher et al. (2012)*
2	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium ↓		Halmos et al. (2015)
	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Faecalibacterium	*F. prausnitzii* ↓	
				Lachnospiraceae	Roseburia spp. ↓		
					Ruminococcus el. Blautia	*R. torques* ↑	
				Ruminococcaceae	Ruminococcus	*R. Gnavus* ↓	
		Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus spp. ↓		
	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	*A. muciniphila* ↓	
3	Actinobacteria ↑		Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium ↓		McIntosh et al. (2016)**
			Coriobacteriales	Coriobacteriaceae	Adlercreutzia spp. ↑		
			Propionibacteriales	Propionibacteriaceae ↓			
	Firmicutes ↑	Clostridia	Clostridiales ↑	*Clostridiales* family XII *Incertae sedis* ↑	Unclassified		
			Clostridiales	Lachnospiraceae	Roseburia spp. ↑		
					Clostridium ↑		
	Bacteroidetes	Bacteroidetes	Bacteroidales	Porphyromonadaceae	Porphyromonas ↑		
4	Actinobacteria Ψ	Actinobacteria	Coriobacteriales	Coriobacteriaceae ↑			Chumpitazi et al. (2012)***
	Firmicutes Ψ	Clostridia	Coriobacteriales	Ruminococcaceae/Clostridiaceae	Subdoligranulum ↑		
		Negativicutes	Selenomonadales	Veillonellaceae	Dialister ↓		
	Bacteroidetes Ψ	Bacteroidetes	Bacteroidales	Prevotellaceae ↑			
	Firmicutes ☩	Clostridia	Clostridiales	Clostridiaceae	Acetivibrio	*A. cellolulyticus* ↑	
		Negativicutes	Selenomonadales	Veillonellaceae	Dialister ↑		
	Bacteroidetes ☩	Bacteroidetes	Bacteroidales	Bacteriodaceae	Bacteroides ↑		
	Ψ : Responders, ☩: non-responders, * = excluded IBS-C patients, ** = IBS-D and IBS-M patients, *** = pediatric IBS patients, ↑: increase/enriched with, ↓: decrease/depleted in						

5 DISCUSSION

5.1 Overall quality of the included trials

5.1.1 Quality of the four included studies

The overall quality of the three RCTs (study 1-3)[63,96,100] described is good, based on scores obtained on the Jadad scale. Study 2 and 3 got score two out of two on the item ´blinding´ [63,100]. Blinding are important due to potentially placebo-effects, which is challenging when measuring symptoms following a dietary intervention but perhaps not so challenging when measuring the gut microbiota composition. It was difficult to decide whether study 2 and 3 should be given score one or two on the item ´blinding´, as the studies not explained the appearance of the meals. However, the participants were given meals home weekly (study 2)[63] as well as received menus (study 3)[100], and they were free to pick the specific food items from the menus. A low-FODMAP diet may not be so unlike a high-FODMAP diet in appearance, due to only quantity restriction on a lot of food items. This was the basis for the score on the item ´blinding´ for study 2 and 3.

A strength for study 1, 2 and 4 is that nutrient intake was assessed at baseline [63,96,101]. Study 3, in contrast did not analyze baseline dietary intake [100], and this can have affected the results in IBS patients following a low-FODMAP diet, particularly when interpreting changes in gut microbiota based on changes in food intake.

The short length of study 4 (one week) [101], may be a weakness since changes in gut microbiota often takes longer time. However, a previous study observed changes only 24 hours after a dietary challenge [103]. This supports that changes in the gut microbiota can be seen one week after a change in diet. Study 4 also had few participants (n=8) which may indicate that the results are less robust, compared to the other studies with a higher number of participants (study 1-3) [63,96,100]. Study 4 was not placebo-controlled in comparison to study 1-3. However, as the participants´ parents in study 4 learned how to follow the LFSD, this can have reduced the effect of no placebo-controls on the symptom-scores following the LFSD. A placebo-controlled study with more pediatric participants and longer duration would be preferred to get results that are more reliable.

Study 1 did not investigate the effect of a fermentable carbohydrate restriction in IBS patients with predominant constipation (IBS-C) [96]. Due to this, study 1 can only be applied to those IBS patients without constipation as their major symptom. The same is partly true for study 3, because most of the effect from a low-FODMAP diet on gut microbiota composition were reported in IBS-D and IBS-M patients (excluded the one IBS-C patient that participated to make a more uniform model) [100]. Study 4, in contrast, included mostly IBS-C patients (6 of 8 participants), in line with this, most of the responders had IBS-C [101].

A limitation of study 3 was that the participants were told that the diets may affect symptoms, but clues beyond this were not given. The participants were recruited in the study prior to the use of a low-FODMAP diet at the authors' clinic, and at the end of the study the participants asked the dietician about the nature of the diet. This can indicate that the blinding was successful. However, whether some of the participants in the study were aware of the nature of the diet is unknown. It is, as mentioned, unlikely that the low-FODMAP diet has given placebo effects on the gut microbiota composition. Furthermore, this study only collected feces samples from day 0 (baseline) and at day 21 (last day on the dietary intervention), and more periodic samples during the intervention could have given more accurate results [100].

5.1.2 Adherence to the diet

The dietary advice that were given to the low-FODMAP group in study 1 were most likely followed, despite no blinding, due to lower total fermentable carbohydrate intake in IBS patients at follow-up compared with the controls that ingested their habitual diet [96]. Since gut bacteria that ferments fermentable carbohydrates were reduced in IBS patients in study 2 following a low-FODMAP diet, the low-FODMAP group most likely exclusively ingested the diet that was given [63]. As the FODMAP content ingested in the low-FODMAP group in study 3 got a mean score of 3,5 (range 1-6) and the authors concluded good compliance, it is suggested that the participants followed the low-FODMAP diet. Additionally, it was observed a positive correlation between global symptom scores and FODMAP consumption, that further support the good compliance to the diet [100]. As the pediatric patients in study 4 ingested lower total fermentable substrate food items during the dietary intervention compared to the baseline period, they most likely followed the dietary advices given prior to the study [101].

The subjects on the low-FODMAP diets were compared with subjects on various high-FODMAP diets, and it was no reported significant differences in energy, protein or fat content between the low-FODMAP diets and the high-FODMAP diets in neither of the studies. Due to this, the various high FODMAP diets will be specified as diets high in FODMAPs, unless otherwise is specified. For more specific details, see table 4 and Table 5.

5.2 Effect of a fermentable carbohydrate restriction of the gut microbiota composition and microbial metabolites in IBS patients

5.2.1 Changes at the phyla level

Study 1 and 2 did not report richness or diversity of Firmicutes, Verrucomicrobia or Actinobacteria, despite both decreases and increases of bacteria within these phyla [63,96]. However, the increased richness and diversity of Actinobacteria (despite the reduction of *BIfidobacterium* spp.), and the increased bacterial richness of Firmicutes in study 3 [100], in addition to the decreased abundance of Bacteroidetes in study 4 [101] are partly similar with other not-dietary interventions of the gut microbiota in IBS patients. These other studies have found an increase of Firmicutes, a decrease in Actinobaceria and Bacteroidetes [67–70]. Additionally, a healthy gut microbiota is dominated by the phyla Firmicutes and Bacteroidetes, followed by the phyla Actinobacteria, Verrucomicrobia and Proteobacteria [49,50]. These findings show that a diet low in fermentable carbohydrates alters the gut microbiota composition in IBS patients, compared to diets high in FODMAPs. Intriguingly, neither of the studies (1-4) reported anything about the Firmicutes:Bacteroidetes ratio. However, a restriction of fermentable carbohydrates seem to increase Firmicutes, and decrease Bacteroidetes, i.e. disturb the Firmicutes:Bacteroidetes ratio, and therefore may not be that beneficial to host health. Further studies are needed.

5.2.2 Probiotic bacteria

The decrease of the probiotic, lactate-producing *Bifidobacterium* spp. in IBS patients in study 1, 2 and 3 [63,96,100] are not surprising. Carbohydrates that work as growth factors for these bacteria, particularly galacto-oligosaccharides and fructo-oligosaccharides [48,84], were restricted on the low-FODMAP diet. The same goes for the decrease of *Lactobacillus* spp. in IBS patients in study 2 [63], as the disaccharide lactose was restricted on the low-FODMAP diet. Furthermore, the concentration of *Bifidobacteria* spp. in the low-FODMAP group seemed to be negatively

correlated with baseline concentrations in study 1 [96]. This can indicate that IBS patients with highest concentrations of *Bifidobacteria* spp. at baseline, had the greatest decrease following a diet low in fermentable carbohydrates. However, as the gut microbiota composition in IBS patients have been associated with decreased levels of *Bifidobacteria* spp. and *Lactobacillus* spp. compared to healthy controls [71–73,104], this can indicate that the low-FODMAP diet decrease the levels of these bacteria in IBS patients even more. Intriguingly, a previous study observed an association between low levels of *Bifidobacteria* spp. and increased abdominal pain in healthy individuals [105], and whether the long-term effect of reduced levels of these bacteria in IBS patients are beneficial to hosts´ health or not, remains unclear. These findings, taken together, indicate the need of further long-term studies, perhaps a *Bifidobacteria* spp. or *Lactobacillus* spp. probiotic supplement combined with a low-FODMAP give positive results on both the symptoms and the concentration of probiotic bacteria in patients with IBS.

5.2.3 Butyrate-producing bacteria

F. prausnitzii and *Roseburia* spp. were in high levels in IBS patients at baseline in study 2 [63], but were decreased following the low-FODMAP diet. This might indicate that the low-FODMAP diet have unfavorable effects on the gut microbiota composition since both of these bacteria are associated with a healthy gut microbiota [50]. However, the decrease of these bacteria in IBS patients following a low-FODMAP diet are not surprising as they are saccharolytic, and usually ferment the fermentable carbohydrates that are restricted on the low-FODMAP diet to butyrate and other SCFAs. The baseline levels of these bacteria are not consistent with previous studies (not dietary interventions), that have reported reduced levels of *Faecalibacterium* spp. [70] and an increase of *Roseburia* spp. in IBS patients [50]. This difference might be due to the combination of healthy controls and IBS patients at baseline in study 2, that may have given an overestimation of *F. prausnitzii* levels at baseline in the same study, despite the small number of healthy controls (n=6).

On the other hand, study 3 reported that the low-FODMAP diet increased the abundance of *Roseburia* spp. [100], which can indicate that this diet does not lead to unfavorable effects on the gut microbiota composition anyhow. Since we do not know the nature of the baseline diet in study 3, we can only speculate whether this is due to the low-FODMAP diet. For instance, if the habitual diet to the IBS patients in this study contained higher levels of starch and inulin,

this may have been a contributing factor to the increased levels of *Roseburia* spp., due to the saccharolytic capacity of this bacterium [106].

Additionally, it could be expected that changes in butyrate-producing bacteria would lead to correspondent changes in butyrate-production but this was not the case in either study 2 or study 3. It is speculated whether the reduction in symptoms are due to the reduced or increased levels of butyrate-producing bacteria. Other studies have shown that abnormal levels of butyrate is associated with visceral hypersensitivity (enhanced abdominal discomfort) in rats, and elevated concentrations of other SCFAs is associated with enhanced contractions in the distal small intestine (ileum) [52].

As the responders in study 4 were enriched with *Subdoligranulum* spp., *Sporobacter* spp. and *Phascolarctobacterium* spp. at baseline, and non-responders were not (Table 5) [101], this can indicate that these genera can function as biomarkers, and perhaps in the future they can be used to tailor a personalized dietary strategy in IBS patients to reduce symptoms.

5.2.4 SCFA-production

The concentrations of the fecal SCFA, in IBS patients in study 1, 2 and 4 [63,96,101] was unchanged between baseline diets, high-FODMAP diets and low-FODMAP diets. The stability of the SCFA-concentrations in IBS patients following a low-FODMAP diet in study 1 can be explained by the continued high total carbohydrate intake in the low-FODMAP group. In study 2 and 4, the stability of SCFA-concentrations can be explained by the increased levels of bacteria that produces SCFA (e.g. *Clostridium* cluster XIVa, *R. torques* and *Bacteroides*) as well as by the reduced levels of SCFA-producers (e.g. *F. prausnitzii*), in IBS patients following the fermentable carbohydrate restriction. However, an analysis of SCFA-concentration from feces samples may not be reliable, as the colonic SCFA are produced in the proximal colon, and approximately 95% of all SCFAs are rapidly absorbed by the colon enterocytes and further utilized [95]. A biopsy of the epithelial cells in the proximal colon or blood analysis would be more appropriate to estimate changes in SCFA-concentrations.

As other studies have reported both decreased and increased levels of SCFAs in IBS patients [59,74,75], it is difficult to say what generally is the case in patients with IBS. However, these studies were not dietary interventions, and it is therefore difficult to judge whether changes in

SCFAs corresponds with IBS symptoms following a change in diet. Elevated concentrations of acetate and propionate have been associated with abdominal symptoms and negative emotions in patients with IBS [59], however this was not the case in the four included studies. Anyway, normal concentrations (70-140 mM in proximal colon, 20-70 mM in distal colon) of SCFAs are associated with a healthy colon [48,54–56]. Further studies on the colonic microbiota, in e.g. analysis of the whole profiles of colonic SCFA and organic acids (succinate, lactate) in patients with IBS are needed, to determine their role in the colon.

5.2.5 Protein-associated bacteria

The increase of *Clostridium* spp. in study 3 after the low-FODMAP intervention [100] are surprising as these are saccharolytic bacteria and mostly ferment the carbohydrates that are restricted on the low-FODMAP diet [51]. *Clostridium* spp. has also been observed in increased levels in IBS patients in other studies, however these studies was not dietary intervention [52,70]. Therefore, the increase of this genus in study 3 can be explained by the other property they have besides being saccharolytic, particularly to degrade proteins [54]. This can further indicate that the low-FODMAP diet led to a switch in the metabolism of members of *Clostridium* spp. from carbohydrate- to protein metabolism, despite no difference in protein intake between diets and despite no measurements of protein metabolites. If this is the case, the low-FODMAP diet might result in detrimental effects, due to the association between accumulation of some protein metabolites (e.g. amines) and increased intestinal tumorigenesis in rats [64]. However, the analysis of the baseline diet was not conducted, in study 3 was not conducted, and this may have affected the results, in e.g. if the patients ingested high amount of proteins during their habitual diet. Further studies on the protein metabolites in association with restricted fermentable carbohydrate intake, and which effects these have, are further needed.

Bacteroides spp., *Coriobacteriaceae* spp. and *Adlercreutzia* spp. were increased in non-responders, responders [101], and IBS-D and- M patients [100], respectively, following a diet low in fermentable carbohydrates. These bacteria have the capacity to break down proteins. The same goes for the members of *Porphyromonas* spp., that was increased in IBS-D and -M patients following a low-FODMAP diet in study 3 [100]. These findings might support the suggestion that a diet low in fermentable carbohydrates may lead to a switch in the gut microbiota metabolism, to protein- or amino acid metabolism, despite no change in the production of BCFAs or other protein metabolites in participants in study 4 after a LFSD. However, further studies are needed,

specifically on the long-term effect of fermentable carbohydrate restriction on the gut microbiota composition in IBS patients.

Alternatively, increased levels of *Bacteroides* spp. in non-responders indicate that a LFSD increase the levels of this genus in pediatric non-responders toward a bacterial abundance associated with health. This is because *Bacteroides* spp. have been observed in low levels in IBS patients [67–70], and is associated with a healthy colonic environment [48,51]. However, as the concentrations of SCFA did not differ between baseline and follow-up in study 4, the most probably explanation may be, as mentioned above, that the gut bacteria have adapted the conditions with carbohydrate restriction and switch its metabolism to protein metabolism.

5.2.6 Mucus-associated bacteria

As *R. torques* are known mucin-degraders of the mucosa layer in the colon, the increase of this bacteria in IBS patients following a low-FODMAP diet in study 2 [63], can indicate that this diet may lead to an impairment of the intestinal barrier of IBS patients. Additionally, this bacterium can have pro-inflammatory properties, due to the expression of certain proteins that are recognized by specific components of the humoral immune system [40,52]. As a low-grade inflammation and elevated levels of *R. torques* have been observed in IBS patients in earlier not-dietary intervention studies [9,52], this can indicate that a low-FODMAP diet contributes to a further low-grade inflammation in the mucosa of IBS patients. However, as *R. gnavus*, also known to degrade mucins, was decreased in IBS patients following a low-FODMAP diet in the same study, the hypothesis that this diet might have adverse effects on the intestinal epithelial barrier cannot be suggested with certainty. Alternatively can the increase of *R. torques* indicate a mucus-turnover in patients with IBS following a low-FODMAP diet, and this can also be supported by the decrease in *A. muciniphila* compared to the Australian diet (high-FODMAP diet), known to degrade mucins as well as stimulate the growth of the mucosa, in the same study [63]. However, as *A. muciniphila* is associated with a healthy gut microbiota [53,57,107], the decrease of this bacterium in IBS patients following a low-FODMAP diet can also indicate that the function of this bacterium is suppressed as *R. torques* increases. However, *R. torques, R. gnavus*, and *A. muciniphila* are important for other gut bacteria as they provide substrates through the degradation of mucins, for example in the absence of dietary glycan´s. As the microbiota differs gradually, from lumen to the mucosal surface, the evaluation of the mucosa-

associated microbiota from feces samples may be of little relevance, hence samples from mucosal biopsies should have been performed instead.

5.2.7 Gas-producing bacteria

As *Acetivibrio* sp. are known hydrogen producers[61], the increase of this genus in non-responders in study 4 [101] can explain the trend toward an increased breath hydrogen gas-production in these pediatric non-responders compared to responders, despite the increase in the gas-consumer *Dialister* spp. However, the controversial is why non-responders have a different gut microbiota composition than responders at baseline, and further don´t respond to the LFSD. Further studies of the gut microbiota composition at baseline in both non-responders and responders are needed, to perhaps tailor a personalized dietary strategy based on the residents of the gut.

6 CONCLUSION

Restriction of fermentable carbohydrates is an effective treatment strategy for IBS, as it results in decreased overall symptoms. Conversely, the gut microbiota composition seems to play a role in the efficacy of a diet low in fermentable carbohydrates in reducing symptoms in IBS patients, with future, long-term studies needed to help elucidate this further. However, this dietary therapy may indicate that some members of the gut microbiota change its metabolism, from carbohydrate to protein metabolism. Over a longer period, increased protein metabolism may have detrimental effects, as the accumulation of the end-products of protein metabolism in the colon, in e.g. phenols, indoles, thiols and amines, have been associated with increased genotoxicity. Furthermore, this diet also resulted in a significant reduction of *Bifidobacteria* spp. and *Lactobacillus* spp., which is known as probiotics and conduct health benefits to the host. The mucus-associated bacteria *Ruminococcus torques* was increased, whereas *Akkermansia muciniphila* was decreased compared to the Australian diet, which may indicate that this dietary strategy can lead to an even more impaired intestinal barrier in patients with IBS. Additionally, the low-FODMAP diet seemed to reduce the immune activation seen in IBS patients, via reduction in urinary histamine release, and due to this may be confer a health benefit to the host. In total, the low-FODMAP diet seem to result in a trend toward adversely effects on the hosts´ health. However, these results are not entirely conclusive as some effects can be beneficial to the hosts´ health, and as it was no observed changes in the production of BCFAs or other protein metabolites, thus further studies are needed until one can state with certainty that a low-FODMAP diet in IBS patients is harmful over time. Further studies are, in particular, needed to assess the long-term effects of a low-FODMAP diet on the gut microbiota composition in IBS patients as well as studies on the reintroduction phase of this diet. This is needed to examine if the changes in the gut microbiota observed in IBS patients in these four studies persists or are reversed by reintroduction of FODMAPs. Furthermore, more comprehensive studies of the gut metabolome are further needed to understand the role of all metabolites by the gut microbiota, such as protein metabolites and carbohydrates metabolites, and to understand which role the end-products have for hosts´ health.

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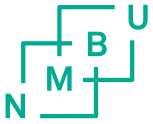

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