CARRIE ALEIDA MARIA WEGH

From healthy defecation to functional constipation in children & adults

A clinical and microbiological perspective.

PROPOSITIONS

1. The scientific reasoning behind many novel non-pharmacological therapies for the treatment of functional constipation in children is weak. (this thesis)
2. Pinpointing one microbial genus or species responsible for a multifactorial disorder in humans is like blaming one city for climate change. (this thesis)
3. Comparable to professional sports, academia needs a 'doping' authority to detect scientific misconduct.
4. More room for serendipity is needed in research projects.
5. An adversarial procedure between researchers and medical-ethical committees improves the process and speed to obtain medical-ethical project approval.
6. Education and society in general benefit from learning to identify viral misinformation.
7. Whether it is about gender, age or ethnicity; speaking out is often counter-productive but necessary.

Propositions belonging to the thesis, entitled

From healthy defecation to functional constipation in children and adults: a clinical and microbiological perspective

Carrie A.M. Wegh,
Wageningen, 11th of November 2022

From healthy defecation to functional constipation in children and adults

A clinical and microbiological perspective

Carrie A. M. Wegh

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From healthy defecation to functional constipation in children and adults

A clinical and microbiological perspective

Carrie A. M. Wegh

Thesis

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‘Het gaat niet om
wat je is ontnomen
maar wat je
doet met wat er
overblijft’
Co Wegh

TABLE OF CONTENTS

'Ondertussen denk ik: wat is de grens van de wetenschapsbeoefening? Iets zoeken dat door niemand nog gevonden is, maar het dan zelf ook niet kunnen vinden – mag dat nog wel het bedrijven van wetenschap heten, of alleen maar gebrek aan geluk? Of gebrek aan begaafdheid? Wie zal het zeggen? Een verschrikkelijke angst komt bij mij op: terug moeten keren met niets'

NOOIT MEER SLAPEN – WILLEM FREDERIK HERMANS

PART 1

Functional constipation in childhood, adulthood and its relation to the intestinal microbiota

General introduction and outline of the thesis

AIM OF THIS THESIS

The process of defecating involves a wide variety of well-orchestrated, coordinated sensorimotor functions but is also influenced by many other factors such as genetic, lifestyle, behavioral and psychosocial factors. Moreover, all processes involved in digestion, absorption, secretion and motility, including interactions of the intestinal microbiota, may be of influence and therefore of interest to further investigate, particularly when the going gets tough. To this end, this thesis aims to provide insight in the range from defining and measuring healthy defecation patterns to when it goes wrong in functional constipation (FC) in children and adults from both a clinical and a microbiological perspective. Additionally, this thesis aims to provide insight in the role of non-pharmacological and intestinal microbiota directed interventions in several other functional gastrointestinal disorders (FGIDs), such as functional abdominal pain disorders (FAPD), colic and irritable bowel syndrome (IBS). This thesis includes four parts: (1) a general introduction into FC in children, adulthood and its relation to the intestinal microbiota, (2) defining and measuring (healthy) defecation patterns, (3) non-pharmacological and intestinal microbiota directed interventions in FGIDs and health and (4) clinical studies in FC and other defecation disorders.

PHYSIOLOGY OF DEFECATING

The path towards defecation comprises four basic processes: digestion, absorption, secretion and motility. The complex process of food digestion starts in the mouth. Once food enters the mouth, digestion starts with mechanical breakdown of food and secretion of salivary amylase that can digest carbohydrates (**Figure 1**). Via the pharynx and esophagus, food enters the stomach where it is further digested into chyme upon the secretion of acid, pepsinogen, pepsin and intrinsic factor. The chyme is then transported to the small intestine. The small intestine consists of three regions: the duodenum, jejunum and ileum. First, in the duodenum chyme is mixed with pancreatic juice, which contains a wide variety of digestive enzymes and bicarbonate that neutralizes the acid from the stomach. In addition, bile, essential for fat digestion, is added to the chyme mix. In the duodenum a simultaneous process of secretion and absorption takes place, which continues in the jejunum and ileum. By this time the chyme contains very few digestible nutrients, as they have been absorbed earlier in the gastrointestinal (GI) tract. The chyme is further propagated towards the colon via the ileocecal sphincter. Beyond this junction is a blind-ended bulb called the cecum, which is attached to the vermiform appendix. The colon is divided into four major regions: the ascending, transverse, descending and sigmoid colon, which in the end leads to the rectum and internal and external anal sphincter. The cecum, the four colonic regions and rectum constitute what is often referred to as the large intestine. The first three segments of the colon are specialized to absorb water and ions from the chyme. This passive reabsorption is critical to maintain a normal fluid balance; we only consume approximately two

liters of water, but the GI tract processes roughly six to eight liters of water due to secretions from the stomach, intestine and accessory glands. Approximately 95% of the water that is initially present in the duodenum is normally absorbed by the time the chyme reaches the colon. However, when chyme enters the colon, it still contains a relatively large amount of water. Upon reabsorption of this water, the volume of the chyme is reduced to a large extent and is turned into a semisolid material, called feces. At the end of the colon, all consumed food and water is reduced to only approximately 100-200 grams per day. This fecal content is composed of two main components: on average 75% water and microbial biomass. This microbial biomass is the major component of dry mass (25-54% of the dry organic content) and the remainder contains undigested carbohydrates, fibers, proteins and fats [1, 2].

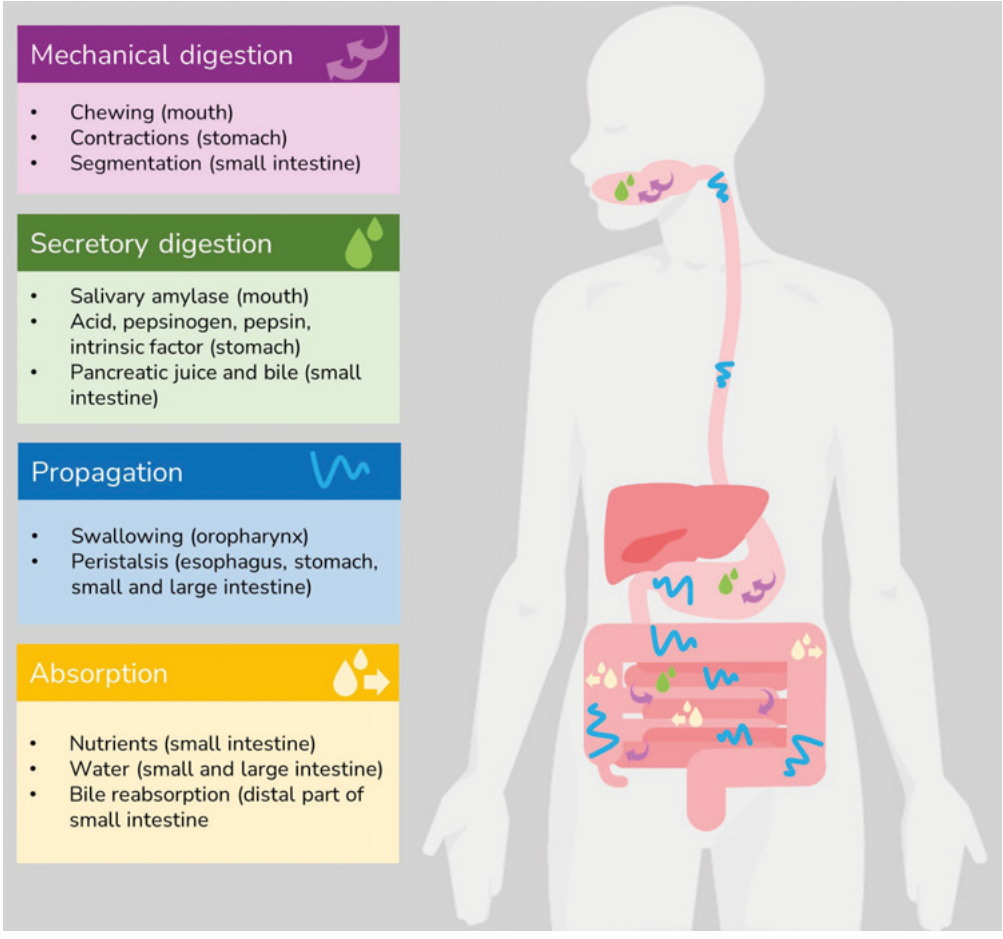

Figure 1 | The digestive system and its processes.

Although not exclusively found there, the colon hosts an essential, complex entity of microorganisms. The so-called intestinal microbiome is often referred to as additional organ of our body, due to its impact on host physiology. The human body is colonized by a very large number of microorganisms, including bacteria, archaea, viruses, fungi and other micro-eukaryotes, that live with us on or inside our body, but primarily reside in the GI tract [3]. Therefore, before we continue the journey through the GI tract, we need to take a pitstop at this mesmerizing microbial ecosystem that has a high level of interactions not only between microorganisms (microbe-microbe interactions or microbial community interactions) but also with the host (host-microbiota interactions) [4]. The number of microorganisms increases throughout the GI tract, reaching highest numbers in the colon [5]. This community of microorganisms resides in a complex and dynamic habitat where a mutualistic relationship between the intestinal microbiota and the host can be found. The intestinal microbiota plays an important role in GI health and, among others, protection against pathogens, nutrient metabolism, vitamin synthesis, and bioavailability of minerals [6]. Beside the involvement of specific processes, the microbe-microbe and host-microbiota interactions are vital for shaping a host-microbial symbiosis and establish a stable ecosystem that is health promoting and resilient to perturbations throughout life [7].

Going back to the main road and to continue the journey through the GI tract, feces is propagated through the colon towards the rectum. In general, the method by which food, chyme and feces is propagated through the GI tract is achieved by intrinsic peristaltic movements and complex motor patterns, of which the most well recognized might be the high-amplitude propagating colonic contractions, which are associated with a mass movement of colonic contents [8]. GI motility relies on several factors, such as food-induced stretch as well as changes in luminal chemistry. Moreover, it was found that the interaction between the enteric nervous system and microbiome, with a tissue-resident population of muscularis macrophages at the cross-roads, also highly influences GI motility [9]. Under the influence of these factors, the contents then reach the sigmoid colon and rectum, which serve primarily as storage depot for this fecal content. This fecal material will not leave the body immediately, despite contractions of the large intestine, due to the internal and external sphincter. Only relaxation of the pelvic floor and both sphincters, which are normally closed, allows for fecal material to be eliminated, a process called 'defecation', or the beautiful Dutch word for this: 'ontlasten' which is freely translated as 'to unburden' [8]. Concluding, human defecation, as simple as it might seem, involves well-orchestrated, integrated and coordinated sensorimotor functions by the central and peripheral nervous system. The nervous system communicates with the GI tract by neural activities. They include central spinal, peripheral somatic and visceral enteric activities. In addition, the pelvic floor and internal and external anal sphincters are of great importance for the act of defecating. Beside this complex system, many other factors play a role in defecation and include voluntary suppression of defecation, the posture during defecating, volume and consistency of stools, and the nature of the intraluminal contents [8].

NORMAL DEFECATION IN CHILDREN

HOW TO MEASURE DEFECATION PATTERNS IN YOUNG CHILDREN.

In the second part of this thesis, I focus on defining and measuring (healthy) defecation patterns. When describing normal defecation patterns, stool frequency and stool consistency are most often used for which a wide range of stool scales are available. The most well-known scale to score stool consistency in adults is the 7-point Bristol Stool Form Scale (BSFS) [10]. Also, for children many stool scales exist that can be used for toilet trained children including the BSFS and the 5-point modified Bristol Stool Form Scale (mBSFS) [11]. For non-toilet trained children, specific diaper scales were created such as the 4-point Amsterdam Infant Stool Scale (AISS), that also includes amount and color, and the 4-point Brussels Infant and Toddler Stool Scale (BITSS) [12, 13]. These stool scales all differ in terms of number of items, which makes data generated by different scales very difficult to compare. Additionally, toddlers might be in the process of toilet training, leading to some defecations being in a diaper and others on a potty or toilet. Therefore, we conducted a study to investigate whether one scale could be used for toddlers in all these different phases, as described in **chapter 3**.

WHAT IS NORMAL DEFECATION?

As opposed to our understanding of aberrant defecation patterns in children, there are no clear guidelines of what can be considered normal, only what can be regarded as 'not normal'. Several studies have been conducted to investigate healthy defecation patterns in children, however there is no worldwide overview on this. Therefore, a systematic review was conducted to describe healthy defecation patterns in children 0-4 years of age in **chapter 4.** But what if the going gets tough?

GASTROINTESTINAL DISORDERS IN CHILDREN AND ADULTS

ORGANIC- AND FUNCTIONAL GI DISORDERS

GI disoders can be divided into two categories: organic diseases and functional disorders. By definition organic diseases are diseases that can be measured, validated, quantified and monitored by biomarkers, as is the case in e.g. inflammation or tissue damage. In contrast, in FGIDs, an impairment of normal functioning of processes occurs that cannot be explained by structural or biochemical abnormalities [14]. The clinical expression of FGIDs varies with age (**Figure 2**) and depends on an individual's stage of development in terms of physiologic, autonomic, affective and intellectual development [14, 15]. The difference in FGIDs between children and adults is apparent whenever diagnosis relies on e.g. pain indication by the patient. For example, irritable bowel syndrome (IBS) diagnosis relies on pain indication and therefore can only be diagnosed at a later age [16].

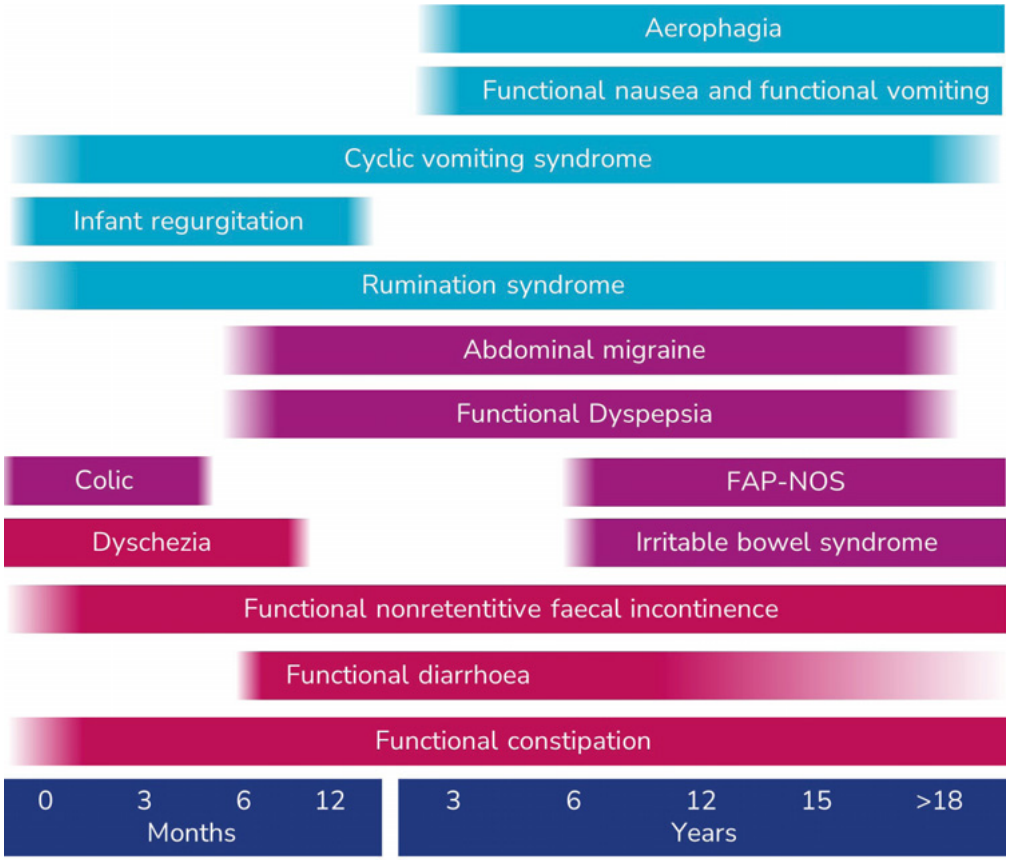

Figure 2 | Age of presentation of functional nausea and vomiting disorders (blue), functional abdominal pain disorders (purple) and functional defecation disorders (red). Adapted from Drossman et al. 2016 [15] . FAP-NOS: functional abdominal pain - not otherwie specified

In general, FGIDs can be divided into three main categories: (1) functional nausea and vomiting disorders, (2) functional abdominal pain disorders (FAPDs) and (3) functional defecation disorders (FDD) (**Figure 2**). The first category, functional nausea and vomiting disorders, are not within scope of this thesis, but include disorders such as infant regurgitation, infant rumination syndrome and cyclic vomiting syndrome for neonates and toddlers, and for children and adolescents cyclic vomiting syndrome, functional nausea and functional vomiting, rumination syndrome and aerophagia [14, 15].

With regards to the second category, FAPDs, most disorders are diagnosed later in life, except for infant colic. Other FAPDs include functional dyspepsia, IBS, abdominal migraine and functional abdominal pain – not otherwise specified (FAP-NOS) [14, 15]. In **chapter 5 and 7** we looked at the effect of different fibers, prebiotics and probiotics on colic and FAPD symptoms.

Table 1 | Rome IV criteria for functional constipation

Children <4 years of age [14]	**Children with a developmental age of >4 years [17]**	**Adult population [18]**
Must include 1 month of at least 2 of the following in infants up to 4 years of age: 1. 2 or fewer defecations per week 2. History of excessive stool retention 3. History of painful or hard bowel movements 4. History of large-diameter stools 5. Presence of a large fecal mass in the rectum In toilet-trained children, the following additional criteria may be used: 1. At least 1 episode/week of incontinence after the acquisition of toileting skills 2. History of large-diameter stools that may obstruct the toilet	Must include 2 or more of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome 1. 2 or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years 2. At least 1 episode of fecal incontinence per week 3. History of retentive posturing or excessive volitional stool retention 4. History of painful or hard bowel movements 5. Presence of a large fecal mass in the rectum 6. History of large diameter stools that can obstruct the toilet After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.	1. Must include 2 or more of the following fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis. a. Straining during more than one-fourth (25%) of defecations b. Lumpy or hard stools (BSFS 1–2) more than one-fourth (25%) of defecations c. Sensation of incomplete evacuation more than one-fourth (25%) of defecations d. Sensation of anorectal obstruction/blockage more than one-fourth (25%) of defecations e. Manual maneuvers to facilitate more than one fourth (25%) of defecations (e.g., digital evacuation, support of the pelvic floor) f. Fewer than 3 spontaneous bowel movements per week 2. Loose stools are rarely present without the use of laxatives 3. Insufficient criteria for irritable bowel syndrome

Lastly, which is also the main focus of this thesis, FDDs comprise the third category. FDDs include several disorders that can be diagnosed already early in life such as functional diarrhea, infant dyschezia and FC, whereas during childhood and adolescence only FC and functional nonretentive fecal incontinence (FNRFI) are diagnosed, according to the Rome IV criteria [14, 17, 18]. This thesis focusses mainly on FC.

FUNCTIONAL CONSTIPATION

FC is defined as constipation without an organic etiology and is diagnosed according to the Rome criteria [14, 17, 18]. These Rome criteria are symptom-based criteria, which were first developed for adults in 1989 during a consensus meeting of experts in FGIDs. In the past decades, these criteria have been updated several times and are now used for both clinical and research purposes. The first criteria for the pediatric population were published in 1999. The most recent revision are the Rome IV criteria which include criteria for both the pediatric and adult population and were published in 2016 (**Table 1**) [14, 17, 18].

FC is a common disorder in all age groups, that shows some similarities in children and adults but also has differences when it comes to epidemiology, symptomatology, pathophysiology, diagnostics and therapeutic management. Symptoms for both children and adults include infrequent and hard stools. **Chapter 2** of this thesis gives an overview of physiology, evaluation, management and treatment of FC in children.

EPIDEMIOLOGY, PATHOPHYSIOLOGY AND DIAGNOSIS OF FC IN CHILDREN AND ADULTS

EPIDEMIOLOGY

The implementation of the Rome criteria has resulted in a more uniform definition of FC and an improved understanding of its prevalence in children and adults. In children 0 to 18 years of age the reported prevalence of FC ranges from 0.5%-32.2%, with a pooled prevalence of 9.5% (95% CI 7.5-12.1%) [19]. Some studies suggested that constipation is more common in boys, however, no statistically significant difference in sex prevalence was found in a meta-analysis [19, 20]. Long-term follow-up studies have shown that 25% of children who have received treatment for FC as a child still experience symptoms of constipation as adult [21].

In adults, a meta-analysis reported a prevalence of 14% and that FC was more common in women than in men (OR 2.22, 95% CI 1.87-2.62) [22]. Additionally, the prevalence in adults seems to increase with age and is higher in elderly compared to young adults. This may be explained by a degeneration of epithelial, muscle and neural cells of the colon and pelvic floor [23]. FC can therefore be regarded, both in children and adults, as a common and bothersome disorder.

PATHOPHYSIOLOGY

Constipation in children rarely has an organic cause and is found to be functional in more than 95% of the cases [24]. Organic etiologies of constipation in children and adults may include intestinal, anorectal, metabolic, endocrine or neuropathic conditions. The differential diagnosis of organic causes of constipation differs depending on the age of the onset of symptoms. For

example, in an infant with a history of delayed meconium passage, Hirschsprung's disease, anorectal malformations and spinal cord defects should be excluded. In contrast, in adolescents an eating disorder should be excluded, and in an elderly patient degenerative diseases or polypharmacology should be carefully considered [25].

In both children and adults, the pathophysiology is considered multifactorial where genetic factors, lifestyle factors and psychological disorders may play a role. Moreover, intestinal microbiota composition has been suggested as a factor [25].

With regards to genetic factors, genetic predisposition may have a role in the etiology of FC as it seems to occur more often in certain families [26, 27]. The importance of heredity is further supported by a study in twins, which revealed that 59% of childhood constipation is explained by a genetic predisposition to produce mainly hard stools, rather than being caused by a low fiber intake [28]. However, studies in adults could not confirm a familial clustering of FC, and therefore authors suggested that familial aggregation might reflect associations with lifestyle, diet and environmental factors prevailing in certain families, rather than resulting from actual genetic factors [29]. Indeed, to date studies have failed to identify mutations in specific genes associated with FC [30].

Table 2 | Alarm symptoms in adults and children with constipation, adapted from Vriesman *et al.* 2020 [25]

Adults	Children
History • Change in bowel habits • Unexplained iron deficiency anemia • Recent sudden onset of symptoms • Blood in the stools • Unintentional weight loss • Family history of colon cancer or inflammatory bowel disease • Rectal tenesmus	**History** • Delayed passage of meconium • Early onset (<1 month old) • Positive family history for Hirschsprung's disease, celiac disease or hypothyroidism • Blood in the stools • Ribbon stools • Fever • Bilious vomiting • Smearing of feces
Physical examination • Abdominal or rectal mass • Cachexia • Jaundice • Lymphadenopathy • Abnormal thyroid gland	**Physical examination** • Failure to thrive • Severe abdominal distention • Abnormal anal or cremasteric reflex • Abnormal position of anus or gluteal cleft • Extreme fear of anal exam • Scars on anus • Anal fissures or hematoma • Abnormal neurological exam • Hair tuft on spine • Sacral dimple

Several lifestyle factors might also play a role in the etiology of FC. For example, dietary factors can play an important role in the pathophysiology of FC of both children and adults. In infants, the transition from human-milk-feeding to formula feeding or the introduction of solid foods can be a trigger for the onset of FC [31]. Moreover, a low intake of fiber or fluids is known to predispose to constipation at all ages [32, 33]. Additionally, low physical activity has been suggested to be an important risk factor for FC in children and adults as well [22, 34].

DIAGNOSIS

FC is a clinical diagnosis made, as described above, according to the Rome IV criteria (**Table 1**). In children and adults presenting with constipation, a thorough medical history and complete physical examination can be sufficient to establish the diagnosis. Identification of alarm symptoms is of importance as these raise the suspicion of underlying organic conditions. The role of physical examination, laboratory testing, radiography, colonic transit time (CTT), magnetic resonance imaging (MRI) and manometry (anorectal and colonic) in the diagnosis of FC in children is described in **chapter 2**. The workup in adults does not differ significantly from that in children except for the differences in alarm symptoms (**Table 2**).

MANAGEMENT, AN OPPORTUNITY FOR INTESTINAL MICROBIOTA DIRECTED INTERVENTIONS, AND PROGNOSIS OF FC

MANAGEMENT

In **chapter 2** we give an extensive overview of all four steps in the treatment of FC: (1) education, (2) disimpaction, (3) maintenance therapy to prevent re-accumulation of feces, and (4) follow-up [35]. In both children and adults, the first step in management of FC is nonpharmacological management. For children the first part of step 1 includes education, a toilet training program, a reward system, keeping a bowel movement diary and dietary and lifestyle recommendations (**Figure 3**). Some of these steps are of course not part of the first treatment in adults, such as the reward system, but education and dietary and lifestyle recommendations are also for adults very relevant.

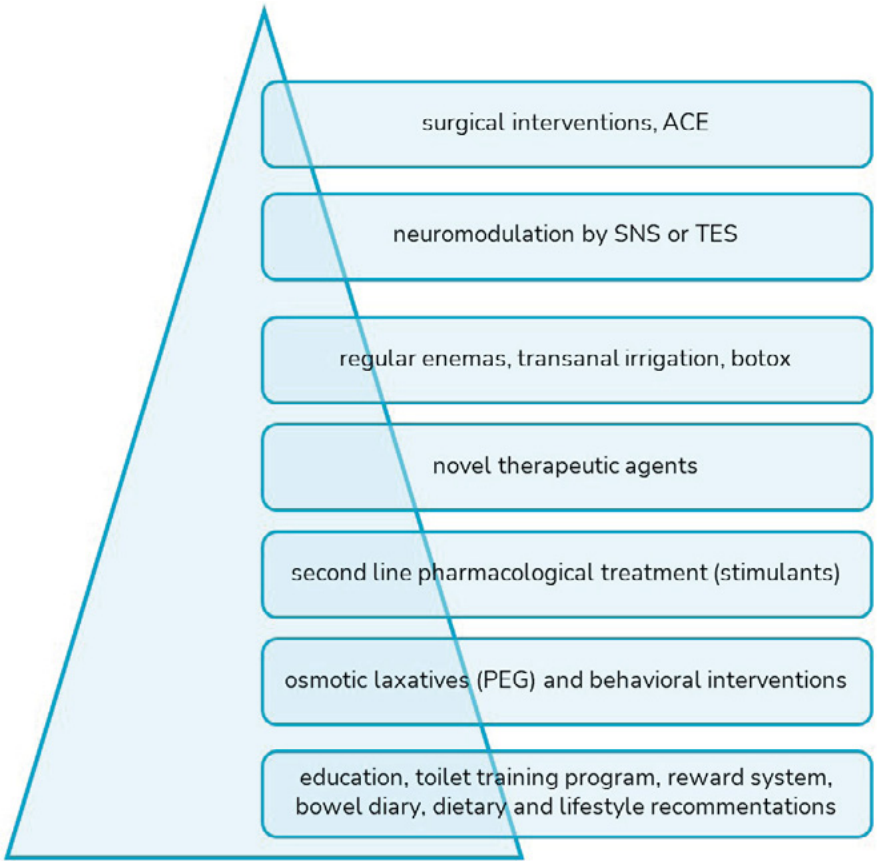

Figure 3 | Treatment pyramid for FC. FC is usually treated in a step-up approach, starting with non-pharmacological interventions and osmotic laxatives (bottom of the pyramid). If these measures are unsuccessful, use of more invasive modalities may be necessary (towards the top of the pyramid). Abbreviations: PEG: polyethylene glycol, ACE: antegrade continence enemas, SNS: sacral nerve stimulation, TES: transcutaneous electrical stimulation.

INTESTINAL MICROBIOTA DIRECTED INTERVENTIONS IN FC AND OTHER FGIDS

Before going towards pharmacological treatments, there is a vast amount of non-pharmacological treatments that have been and are currently investigated as treatment alternative for FC. In fact, more than one third of parents of children with FC seek help in the form of complementary and/or alternative medicine [36]. Therefore, we investigated the role of non-pharmacological and intestinal microbiota directed interventions in FGIDs in part 3 of this thesis (**chapter 5, 6 and 7**). With a focus on FC, a wide variety of complementary and alternative medicine is discussed in this thesis, including fiber supplementation, and intestinal microbiota directed interventions.

The intestinal microbiota

The intestinal microbiota is a collection of microorganisms with a tremendous number of organisms, estimated to be ~10^{13}-10^{14}, residing in the intestines [37]. The intestinal microbiota fulfils a number of important functions including: digestion of food; synthesis of products that can be of benefit to the host, such as amino acids, short chain fatty acids (SCFAs) and vitamins; training of the immune system; and preventing GI infections [38, 39]. In general, the composition can vary greatly between individuals, whereas the function is rather similar and stable, indicating that different microbial species can fulfil similar functions [40]. There are numerous factors that may influence intestinal microbiota composition, including the mode of delivery and of feeding during infancy, diet, use of antibiotics and other types of medication, lifestyle and host genetics [41, 42]. How these factors affect the intestinal microbiome, how the host and intestinal microbiota interact and what the role of the intestinal microbiome is in health and disease are among the main topics of intestinal microbiome research [43].

Methods to analyze the intestinal microbiome

Methods to investigate the composition of the intestinal microbiota, but also the functions and activities of the intestinal microbiome have changed drastically over the past decades. The rise of culture-independent methods has led to an immense increase in microbiome research; whole microbial communities can be characterized at once by sequencing DNA-fragments of these microbial cells. These methods do, however, require complex bioinformatic solutions to disentangle which DNA-fragment belongs to which microbial species, genus or other taxon. Here, taxon refers to the hierarchical classification of life that ranges from domain, (kingdom), phylum, class, order, family, genus, to species, subspecies or strain (**Figure 4**).

The highest level, 'Domain', comprises three categories: the Eukarya, the Bacteria and the Archaea. The Eukarya, to which, amongst others, plants, fungi and animals belong, have a more complex cell structure compared to Bacteria and Archaea. Toward the other end of the taxonomy, the taxon of species is further specified by the subspecies/strain level. This level is highly relevant to the microbial world since different strains can show different properties by themselves, but can also have a different effect on the human body. Many methods exist to analyze the intestinal microbiota and intestinal microbiome. These terms might sound very similar, but they are not. **Table 3** shows a list of terms used in the microbial ecology field that are being used throughout this thesis with their definition. This fast-changing field requires consensus on these definitions, for which several consensus papers have been published. Despite small differences in exact wording, this table gives a good indication of the general idea of the terms and the current consensus definition.

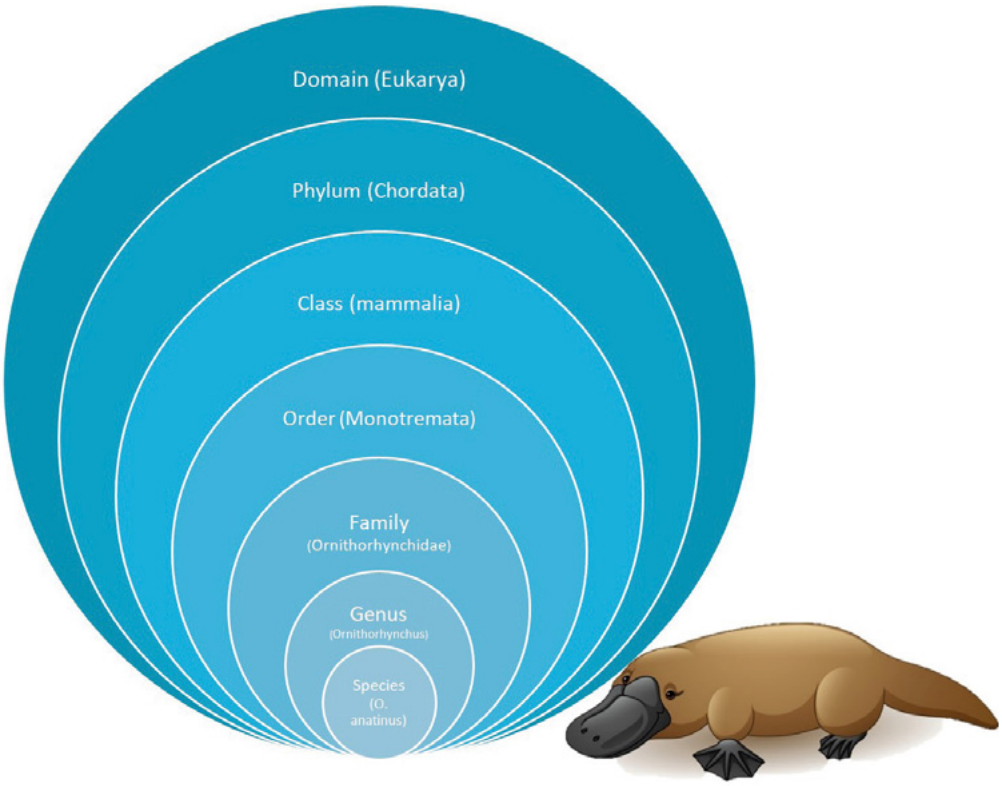

Figure 4 | Taxonomy with a platypus as example: all life is ranked and classified via this taxonomic system, including bacteria and other microorganisms. Below species there is another hierarchical level: subspecies/strain, which is very relevant in the microbial world.

In **chapter 8** of this thesis, we used a DNA-sequencing technique where the 16S ribosomal RNA (rRNA) gene, which encodes the RNA of the small subunit of the prokaryotic ribosome, was used to characterize intestinal microbiota composition. The 16S rRNA gene is of great use for composition analyses since the gene is present in all prokaryotic cells, and it has conserved and variable regions. The conserved regions are highly similar between species and can be used as targets for primers used for PCR amplification, whereas variable regions can be used to distinguish taxa. Software tools match sequenced DNA reads in a sample to known sequences in a reference database of the 16S rRNA regions. Thereby, the presence and relative abundance of the taxa in this sample can be determined [52]. The reason to analyze these relative abundances, is to investigate first whether there is a correlation between a specific composition pattern or specific taxa in relation to, among others, health and disease. The next step could be to investigate whether there is a causal relationship by conducting a human intervention trial to explore whether steering the composition also leads to a different health status.

Table 3 | Definitions of words often used in microbial ecology

Metabolome	All metabolites produced in any given strain or single tissue at a given point in time [44].
Metagenome	The collection of genomes and genes from the members of a microbiota [44].
Metatranscriptome	This term refers to the suite of RNAs expressed by members of a microbiota at a given point in time [44].
Metaproteome	This term refers to the comprehensive characterization of the entire protein complement of environmental or clinical samples at a given point in time [44].
Microbiome	This term refers to the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eukaryotes), their genomes (i.e., genes), viruses, and the surrounding environmental conditions [44].
Microbiota	The assemblage of microorganisms present in a defined environment [45].
Prebiotics	A substrate that is selectively utilized by host microorganisms conferring a health benefit [46].
Probiotics	Live microorganisms which when administered in adequate amounts confer a health benefit on the host [47].
Postbiotics	Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host [48]. Bioactive compounds produced by food-grade microorganisms during a fermentation process, including microbial cells, cell constituents and metabolites [49].
Synbiotics	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host [50].

The role of the intestinal microbiota in FC

A vast amount of disorders and diseases are associated with differences in intestinal microbiota composition patterns compared to healthy individuals. As described before, there are many factors that can influence intestinal microbiota composition. In adults, one of the main drivers of intestinal microbiota composition is long-term dietary intake [39, 53, 54]. The plasticity of the microbiota in response to the diet raises to the possibility to direct the intestinal microbiota towards a composition with potential beneficial health effects.

Children and adults with FC have been shown to have a different intestinal microbiota composition compared to healthy individuals [55-57]. The oldest study, in children with FC, found significantly higher levels of *Clostridium* and *Bifidobacterium*. Among *Clostridium* species, *C. sporogenes*, *C. paraputrificum*, *C. fallax*, and *C. innocuum* were predominant [58]. Another study used similar methods to investigate the difference in microbiota composition in adults with FC and found reduced levels of *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Clostridium* species and an increased level of *Enterobacteriaceae*, such as *Escherichia coli*, as well as *Staphylococcus aureus*

and several fungi [59]. A more recent study used 16S rRNA gene pyrosequencing to investigate differences in intestinal microbiota composition in adolescents with FC and found significantly lower relative abundances of *Bacteroidetes*, in particular *Prevotella*, and an increased abundance of several species belonging to the *Firmicutes*, including members of *Lactobacillus*. The authors also found that the relative abundances of *Lactobacillus* and *Bifidobacterium* species were not reduced [60]. A more recent study in adults used real-time or quantitative polymerase chain reaction (qPCR) and found that patients with FC had significantly lower amounts of *Bifidobacterium* and *Bacteroides* [61]. Lastly, one study in children with FC used an intergenic spacer (IS) region microbiota profiling technique and found no disease-specific separation when using principal coordinate analysis (PCoA) when analyzed with all phyla together or per phylum. However, patients with FC and controls could be discriminated by ridge regression with 82% accuracy. Most discriminative species that were increased in FC were *Bacteroides fragilis*, *Bacteroides ovatus*, *Bifidobacterium longum*, *Parabacteroides* species while *Alistipes finegoldii* was decreased in patients with FC [57]. These findings are inconsistent, possibly also further influenced by the big differences in age between the patient populations included in these trials, as intestinal microbiota composition changes throughout life [62]. It is therefore very difficult to draw any conclusions as to which species, genera or phyla might be involved in FC in children and adults. However, results from clinical studies on prebiotics, probiotics, synbiotics and fecal microbiota transplants suggest that the composition and/or functionality of the intestinal microbiota may play a big role in constipation symptoms [55]. It is therefore worthwhile to investigate how intestinal microbiota composition is associated with this disorder among different ages. Identifying potential causative relationships between microbiota profiles, specific species, genera or phyla can pave the way for the development of intestinal microbiota directed interventions to cure functional constipation [55].

Ways to (re)direct the intestinal microbiota by fibers and prebiotics

One promising way to direct the intestinal microbiota in FC is by prebiotic fibers. Fibers can be defined in many ways, due to differences in scientific, regulatory and biochemical perspectives. Moreover, many differences exist in their biological, chemical and physiological characteristics and thereby they constitute a highly diverse group [63]. A commonly used definition of dietary fiber is as follows: 'dietary fiber is made up of carbohydrate polymers with three or more monomeric units (MU), which are neither digested or absorbed in the human intestine and includes: non-starch polysaccharides (NSP) from fruits, vegetables, cereals, and tubers whether intrinsic or extracted, chemically, physically and/or enzymically modified or synthetic (MU≥10); (2) resistant (non-digestible) oligosaccharides (RO) (MU 3–9); and (3) resistant starch (RS) (MU≥10). When extracted, chemically, physically and/or enzymically modified or synthetic, generally accepted scientific evidence of benefits for health must be demonstrated to consider the polymer as dietary fiber.' [64, 65]. Prebiotics fall within the group of dietary fibers, although the current definition of prebiotics additionally allows for non-carbohydrate substances. Prebiotics are defined as: 'a substrate that is selectively utilized by host microorganisms conferring a health benefit.' (**Table 3**) [46].

Several aspects of prebiotic fibers may have an influence on FC symptoms. Firstly, FC is associated

with a low fiber intake, and other studies suggested that a low fiber intake may represent a risk factor for FC [14, 66-69]. Current guidelines recommend to have a normal intake of fiber, but do not recommend to use fiber supplements due to the conflicting evidence for the use of fibers as treatment for FC [14, 70]. Secondly, prebiotic fibers are fermented by intestinal microbiota that will degrade them and produce, among others, microbial biomass and SCFAs, which may stimulate bowel movement via an increase in osmotic pressure [71-75]. Subsequently both the increase in microbial biomass and an increase in osmotic pressure may lead to an increase in dilation of the intestinal wall which, in turn, can trigger the reflex action of the bowel peristalsis [71, 76, 77]. Therefore, supplementation of prebiotic fibers may improve bowel habit in children and adults with FC. In fact, prebiotic fibers such as inulin, fructo-oligosaccharides (FOS) or galacto-oligosaccharides (GOS) have been shown to relieve constipation symptoms in young adults and elderly [78]. A recent pilot randomized controlled trial showed improvement in stool consistency in children aged 2-5 years with FC after the consumption of inulin-type fructans [79]. Another study in children aged 4-16 years showed improvement in stool consistency and frequency after consumption of GOS [80]. However, evidence linking additional fiber intake to improved symptoms in children with FC is rather weak [35]. This is not only due to the low number of studies, but also small sample size of studies, overall poor quality of methods used, and incomplete reporting of results. Therefore, a large scale, well executed study is needed to investigate if inulin, GOS or FOS can successfully result in softer stools in children and adults with FC. Such a study was set-up in the framework of the research described in this thesis. The protocol for this study can be found in **chapter 9**. In addition, a study in adults was conducted to investigate whether inulin/FOS can be used in the treatment of FC in adults and to explore the effects of inulin/FOS on intestinal microbiota composition in these patients (**chapter 8**).

PHARMACOLOGICAL INTERVENTIONS, TRANSANAL IRRIGATION AND SURGICAL MANAGEMENT

Higher up in the FC treatment pyramid (**Figure 2**) we find the pharmacological treatments for FC, which consist of treatment with laxatives in three steps: disimpaction, maintenance and weaning.

Pharmacological interventions

Pharmacological treatment of children with FC has been extensively described in **chapter 2**, but in short starts with fecal disimpaction by e.g. a high dose of oral polyethylene glycol (PEG) or enemas. After successful disimpaction, maintenance therapy is advised to prevent reoccurrence of accumulation of stools. This disimpaction is less often required in adults, but the approach is similar [25]. Osmotic laxatives, and in particular PEG, are the first-choice maintenance therapy recommended for FC in children and adults, due to their effectiveness and perceived safety [35, 81]. If symptoms persist, stimulant laxatives such as bisacodyl or senna can be used, both in children and adults [35]. More recently, studies have been conducted with novel therapeutic agents, such as prosecretory agents, serotonergic agents, bile acids and cholinesterase inhibitors. Firstly, prosecretory

agents like lubiprostone, linaclotide and plecanatide modulate the epithelial channels of the gut leading to intestinal secretion of fluids, resulting in an improvement in GI transit [82]. Secondly, serotonergic agents, such as prucalopride, velusetrag and naronapride have a working mechanism based on increasing the release of acetylcholine, resulting in an increased fluid secretion and increased gut motility by initiating the gastrocolic reflex [83]. Thirdly, bile acids are of interest because endogenous deconjungated bile salts increase fluid secretion and colonic motility. Elobixibat, an inhibitor of the ileal bile acid transporter, has been shown to increase defecation frequency in adults with FC, but studies in children are lacking [84]. Lastly, cholinesterase inhibitors such as pyridostigmine increase the availability of acetylcholine and thereby increase gut motility. Pyridostigmine has been used in small cohorts of children and adults with slow-transit constipation or pediatric intestinal pseudo-obstruction, where it was found to be effective in several cases [85].

Transanal irrigation

Transanal irrigation (TAI) is an advanced medical treatment option designed to assist evacuating stools, which can also be used in the treatment of FC. This option is often used in children with FC who are unresponsive to pharmacological treatment [86]. During TAI, patients insert a catheter into the rectum via which water, with or without added laxatives, is infused into the colon to thoroughly wash out feces. It has been suggested that TAI can be effective in the treatment of fecal incontinence and constipation, with high parental satisfaction. However, therapy adherence, changes in quality of life and the extent to which children experience independence was not investigated yet. Therefore, a retrospective and cross-sectional study was set-up in children that use or have used TAI, as reported in **chapter 11**.

Surgical management

In severe cases of FC, and when other treatments have failed, surgery can sometimes be considered as last resort, as also described in **chapter 2**. There are currently no evidence-based guidelines for surgical management, but options include antegrade colonic enema, pelvic floor surgery, botox injections and colorectal resection [87, 88]. However, evidence is weak, and more studies are needed to identify subgroups of patients who may benefit from surgical interventions in the treatment of FC. Moreover, conservative management for patients with FC should first be considered before moving to these dramatic surgical interventions [87].

PROGNOSIS

Despite this workup with non-pharmacological, pharmacological and surgical management, approximately 40% of children referred to a pediatric gastroenterologist for their FC still has symptoms after five years [35]. Long-term follow-up studies have shown that 25% of children still experience symptoms of FC at adult age [21]. This underlines that there is still a need for more effective and better treatments of FC in both children and adults.

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Functional constipation in children

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ABSTRACT

Childhood constipation is a common and worldwide pediatric healthcare problem. The diagnosis is based on history and physical examination, in accordance with the pediatric diagnostic Rome IV criteria. Additional investigations are only indicated if the diagnosis is not clear or in order to rule out an underlying organic disease. Non-pharmacological management involves education, demystification, a toilet program with reward system, daily bowel diary, and in some cases additional cognitive behavior therapy is helpful. Pharmacological treatment with laxatives consists of disimpaction, maintenance treatment, and weaning of medication. Polyethylene glycol (PEG) is the treatment of first choice for both disimpaction and maintenance treatment. If PEG is not available or poorly tolerated other laxatives are available as second-line or additional treatment if treatment with PEG is insufficient. In children with intractable symptoms pelvic floor physiotherapy, sacral nerve stimulation and surgery can be considered.

Keywords: Constipation; Functional constipation; Children with constipation; Laxatives; Enemas; Fecal incontinence.

INTRODUCTION

Functional constipation is a common gastrointestinal disorder in children, accounting for 3%-10% of general pediatric outpatient visits and up to 25% of visits to pediatric gastroenterologists worldwide [1]. Symptoms include hard, large, infrequent and painful bowel movements, often accompanied by abdominal pain and fecal incontinence in toilet trained children. In approximately 95 % of children with constipation, no organic cause can be identified, these children suffer from functional constipation (FC) [1]. The prevalence of FC ranges between 0.7 and 29.6 % with a pooled prevalence of 9.5% and occurs more often in girls than in boys (ratio: 2.1:1) [1]. Three subtypes of FC are recognized: normal transit constipation, slow transit constipation and outlet obstruction [2]. The diagnosis of FC is based on the pediatric diagnostic Rome IV criteria for functional gastrointestinal disorders (**Table 1**) [3].

Table 1 | Rome IV criteria for functional constipation

Children <4 years of age	Children with a developmental age of >4 years
Must include 1 month of at least 2 of the following in infants up to 4 years of age: 1. 2 or fewer defecations per week 2. History of excessive stool retention 3. History of painful or hard bowel movements 4. History of large-diameter stools 5. Presence of a large fecal mass in the rectum In toilet-trained children, the following additional criteria may be used: 1. At least 1 episode/week of incontinence after the acquisition of toileting skills 2. History of large-diameter stools that may obstruct the toilet	Must include 2 or more of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome 1. 2 or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years 2. At least 1 episode of fecal incontinence per week 3. History of retentive posturing or excessive volitional stool retention 4. History of painful or hard bowel movements 5. Presence of a large fecal mass in the rectum 6. History of large diameter stools that can obstruct the toilet After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

PHYSIOLOGY

MECONIUM PASSAGE AND DEFECATION FREQUENCY

In more than 99 % of healthy term neonates, the first meconium passes within the first 48 h of life [3, 4]. Delayed passage of the first meconium beyond the first 48 h of life is suggestive for an organic defecation disorder (e.g., Hirschsprung's disease or anorectal malformations). During the first months of life, the defecation frequency may vary from child to child, and is influenced by their feeding mode; human-milk-fed children have a higher defecation frequency and softer stools than formula-fed infants [4-6]. In the first weeks of life, the defecation frequency lies

around 3-4 stools a day, this frequency gradually decreases over time until it is approximately once a day in children at the age of 2-4 years [4, 7-12]. This stabilization of the defecation frequency is correlated with maturation of the intestinal microbiota composition [13]. In older children, defecation usually occurs either daily or every other day [8, 10, 12, 14, 15].

PASSAGE AND DEFECATION FREQUENCY

The physiological dynamics of defecation are complex and rely on several intricate processes involving the autonomic and somatic nervous system, the pelvic floor muscles, and the internal and external anal sphincters. In the colon, feces is propelled by propagating colonic contractions. Several different colonic motor patterns have been described [16, 17], but the most well-recognized propagating motor patterns are high-amplitude propagating contractions (HAPCs) and low-amplitude propagating sequences (LAPCs). HAPCs typically occur upon awakening, following meals and can be induced by bisacodyl [18]. HAPCs can be fully propagating, when they reach the sigmoid colon, partially propagating when they stop at the level of the splenic flexure or at the descending colon and absent when there are no HAPCs observed in the entire colon and can be classified as normal or abnormal based on the morphology of pressure waves within the contraction sequence (**Figure 1**) [16]. LAPCs occur considerably more often during the day than at night and increase in frequency upon wakening and following meals, as with HAPCs. Differences were found in frequency of LAPCs in children with slow-transit constipation compared to healthy controls in the mean number of ascending, transverse and descending LAPCs [16, 19]. Besides these HAPC and LAPC patterns, other motility patterns have been described for children with functional constipation; these children lack a normal postprandial increase in retrograde propagating motor patterns. Moreover, during the preprandial phase, children with constipation showed greater numbers of antegrade propagating long single motor patterns [20]. However, the clinical significance of these findings is still unclear. Normally, anterograde colonic movements lead to filling of the rectum, which induces a relaxation of the internal anal sphincter, allowing feces to travel further down the anal canal; this reflex is known as the recto-anal inhibitory reflex (RAIR). Subsequently, sensory stimuli caused by rectal distention and by the contact between fecal material and the mucosa of the proximal part of the anal canal result in an urge to defecate. At this point, voluntary contraction of the external anal sphincter can postpone defecation, by moving the fecal load back, higher up in the anal canal and rectum, until the place and time are appropriate for defection. When defecation is initiated, voluntary relaxation of the external anal sphincter and the pelvic floor musculature (i.e., the puborectalis muscle and musculus levator ani) allows for an easy defecation process. In young children, this can be promoted by proper support of the feet when sitting on the toilet and a relaxed posture. Then, by gently increasing the intra-abdominal pressure, stools can be expelled from the rectum.

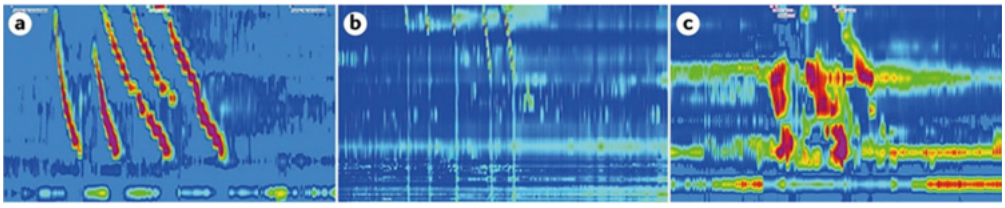

Figure 1 | Normally and abnormally propagating high-amplitude propagating contractions (HAPCs) have been identified by high-resolution manometry in children. **A |** In normal HAPCs, the amplitude is >75 mmHg and the contractions propagate distally to the rectosigmoid junction. The anal sphincter relaxes concurrently to the HAPC. **B |** In abnormally propagating HAPCs, the contractions do not propagate beyond the transverse colon. **C |** An abnormal configuration of HAPCs with multipeaked waveforms and prolonged duration. This configuration has been associated with histological evidence of colonic neuropathy [19]. Retrieved with permission under the terms of the Creative Commons CC BY from Corsetti, M., et al. (2019). First translational consensus on terminology and definitions of colonic motility in animals and humans studied by manometric and other techniques. Nature Reviews Gastroenterology & Hepatology, 16(9), 559-579.

PATHOPHYSIOLOGY

The pathophysiology of FC is incompletely understood; multiple factors are likely to play a role in its pathogenesis and may affect different phases of the physiological defecation dynamics (**Figure 2**).

AGE OF MANIFESTATION

FC occurs in children of all ages, but there are three phases in life when children seem to be more prone to develop constipation: (1) in infancy, concomitant with changes in feeding (e.g., change from human-milk-feeding to formula-feeding, introduction of solid foods); (2) around the time of toilet training; and (3) in school children who avoid going to the toilet at other places than home [21]. This suggests that both dietary and behavioral factors play an important role in the pathogenesis of FC.

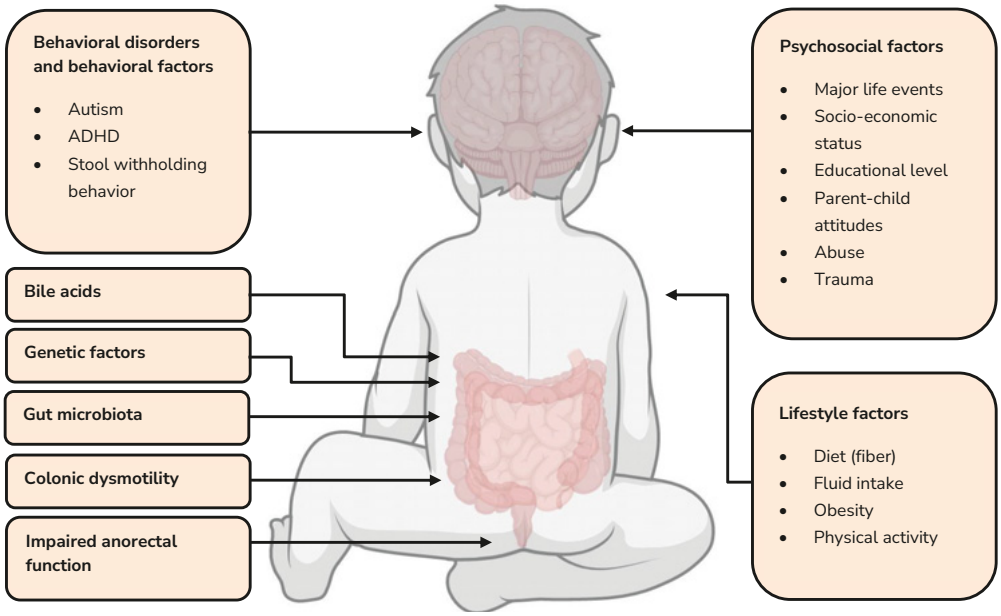

Figure 2 | Pathophysiological factors involved in functional constipation in children

STOOL WITHHOLDING BEHAVIOR

Probably the most important etiologic factor, especially in young children, is stool withholding behavior. This often occurs after a negative experience such as a hard, painful, or frightening bowel movement [22]. Stool withholding behavior can lead to the accumulation of a large fecal mass in the rectum that is difficult to evacuate, also known as fecal impaction. Fecal impaction may lead to overflow fecal incontinence which is the involuntary loss of soft stools that pass the solid, obstructing, fecal mass. Stool withholding can lead to a negative chain of events; due to a painful defecation experience, the child voluntarily retains the stools in an attempt to prevent another painful bowel movement, causing the stools to become harder and more difficult to evacuate, leading to more pain during defecation [23].

IMPAIRED ANORECTAL FUNCTION

Withholding behavior may eventually lead to dyssynergic defecation and occurs when the coordination of the muscles involved in defecation are inadequately coordinated during defecation [2]. This is caused by a paradoxical contraction of the muscles in the abdomen and pelvic floor or an inadequate anal relaxation leading to a poorly coordinated attempt at defecation, preventing stools to be expelled from the rectum and sustaining constipation [24, 25].

COLONIC DYSMOTILITY

Propagation of feces through the colon is an essential step in the physiology of defecation. Colonic motility dysfunction is thought to be present in a subset of children with FC with delayed transit time, and is supported by colonic manometry studies which report that HAPCs occur less frequently in patients with slow-transit FC compared to patients without constipation [20, 26]. It is not entirely clear whether this delay in colonic transit time (CTT) plays a causative role or if it is an effect of long-standing constipation and becomes a perpetuating factor, resulting in a detrimental causal sequence.

Studies utilizing colonic manometry have revealed that in children with intractable FC, several types of colonic dysmotility can be differentiated. In healthy humans, stretching of the stomach after a meal induces an increase in colonic motility via the enteric nervous system and the neuropeptides serotonin, gastrin, cholecystokinin, and prostaglandin E1. This response is better known as the gastrocolic reflex [27, 28]. Colonic manometry studies have shown that this reflex is impaired in a subset of children with FC, which may indicate an impaired extrinsic innervation [20, 29]. Furthermore, it has been shown that a small proportion of children with FC have incompletely propagating HAPCs or a general lack of HAPCs in response to a stimulant laxative, which likely implies an intrinsic (neurogenic or myogenic) pathophysiological process [30]. But it remains uncertain whether these findings are cause, effect, or a combination of both.

PSYCHOSOCIAL FACTORS

Although the precise underlying pathophysiological mechanisms are not always clear, psychosocial factors such as major life events, socioeconomic status, educational level, and parental child-rearing attitudes might play a role in the pathophysiology of FC [1, 31-33]. Furthermore, behavioral and developmental disorders such as autism spectrum disorders and attention deficit hyperactivity disorder (ADHD) are associated with a higher risk of childhood constipation [34-36].

GENETICS

Since FC seems to occur more often in certain families, a genetic predisposition might have a role in the etiology of childhood constipation [37, 38]. A twin study suggested that constipation in children is caused by a genetic predisposition to form hard stools and revealed that 59% of childhood constipation can be explained as a genetic or natural phenomenon [39]. However, studies have failed to identify mutations in specific genes associated with FC yet [40].

MICROBIOTA

The role of the intestinal microbiota in the pathophysiology of FC is incompletely understood. Intestinal microbiota differences have been identified between children with and without FC,

suggesting that intestinal microbiota may play a role in the pathogenesis of FC [41-43]. Causality in intestinal microbiota research remains a challenge. Diet is one of the main key drivers of intestinal microbiota composition, of which fibers are probably the most important in the light of FC. Literature suggests that fiber intake is different between healthy children and those with FC [44-47]. Only few studies investigated the actual intestinal microbiota composition in children with FC and findings were inconsistent [42, 48]. Some of the found associations can be explained by the effect of the intestinal microbiota's end products, such as short-chain fatty acids (SCFAs). One of these SCFAs is butyrate, which is the main energy source for colonocytes and might have a role in intestinal mucus production, increase colonic smooth muscle contraction, and has been associated with increased fecal water content [49, 50]. Another possible mechanism in which the intestinal microbiota may potentially influence gut motility is by the production of methane. Anaerobic fermentation of undigested polysaccharides produces hydrogen in the gut which in turn can be the substrate for methane production by intestinal methanogens [41, 51]. There is strong evidence from animal studies that methane delays intestinal transit, possibly acting as a neuromuscular transmitter. Indeed, methane production has been associated with constipation in adults [52, 53]. More studies are clearly needed to unravel the role of the diet and intestinal microbiota in the pathophysiology of FC in children and thereby find potential microbiota-based interventions such as pre-, pro-, syn-, or postbiotic treatments [54-56].

BILE SALTS

There has been an increasing interest in bile salt metabolism as a potential pathophysiological factor in FC; endogenous deconjugated bile salts have the potential to function as endogenous laxatives by increasing colonic motility and fluid secretion [57]. In a subset of children with FC, bile acid metabolism has been shown to be altered, leading to a decreased secretory activity. This suggests that bile acid metabolism may play a role in the pathophysiology of constipation in a subset of children [58]. Again, there is a role for the microbiota in this process; only a small portion of deconjungated bile acids end up in the colon, where they could exert their laxative effect, however the intestinal microbiota will influence the overall physiological effect through dehydroxylation, deconjungation and desulfation of bile acids [59, 60].

EVALUATION

The evaluation of a child with constipation should always aim to differentiate between FC and constipation due to an organic cause. The diagnosis of FC is a clinical diagnosis based on a thorough medical history and a complete physical examination. Additional investigations are usually not required (**Figure 3**) [61].

MEDICAL HISTORY

The medical history should address questions about defecation frequency, stool consistency, painful bowel movements, size of the stools, episodes of fecal incontinence, and a history of withholding behavior (**Table 1**). Keeping a daily bowel diary can be useful to gather reliable information about a child's bowel habits. The Bristol Stool Scale or the Modified Bristol Stool Form Scale for Children can be helpful in the assessment of stool consistency in the toilet or in a diaper [62, 63]. Special attention should be paid to questions about withholding behavior, as this behavior may not be recognized as such by parents and may even be wrongfully interpreted as straining to defecate. Questions regarding stool withholding behavior should therefore be clear and illustrated with examples.

In infants, withholding may be characterized by grunting, back arching, and tightening of the legs. In toddlers, squeezing the buttocks together, crossing the legs, standing on the toes, and rocking back and forth are distinctive signs of withholding. The medication history should include the use and efficacy of oral laxatives, enemas, colonic irrigation and other medications that potentially influence gastrointestinal motility.

ALARM SYMPTOMS

To differentiate between FC and constipation with an organic cause, alarm symptoms suggestive for an organic cause should be sought out (**Figure 4**) [2, 61]. Alarm symptoms indicative of an organic cause include delayed passage of meconium, which raises suspicion of Hirschsprung's disease or cystic fibrosis. Other important questions include the age of onset, a history of bloody stools without the presence of a fissure, failure to thrive, and severe abdominal distention. Furthermore, a history of smearing feces, detection of fissures and hematomas or abnormal behavior during physical examination (e.g. sexual acting out, extreme fear) should always raise suspicion of sexual abuse [65].

DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

Besides organic causes of constipation and devastating causes of FC such as sexual or physical abuse, the differential diagnosis should include harmless conditions that may be misinterpreted as FC; infrequent defecation in human-milk-fed infants and screaming or crying before or during defecation in infants can be worrying to parents but are often innocuous. Approximately 10% of human-milk-fed infants defecate once every 7–10 days, without any other symptom of FC and while still gaining weight normally. This is usually an innocent and self-limiting phenomenon related to human-milk-feeding, with hypotheses ranging from a better digestion of the fat in mother's milk compared to formula milk to a greater number of saccharolytic bacteria that can degrade unabsorbed and unabsorbable sugars and does not require any treatment [5, 66]. Infant dyschezia is a functional gastrointestinal disorder in young children that is defined as straining and crying for at least 10 minutes before successful or unsuccessful passage of soft stools in an

infant younger than 9 months of age without any other health problem [3]. Parents report that their child turns red or purple during defecation, but is usually passing soft stools several times daily. This is a self-limiting condition, which does not require any medication or intervention. It is thought to be caused by a lack of coordination between increased intra-abdominal pressure preceding defecation and relaxation of the pelvic floor [67].

PHYSICAL EXAMINATION

Assessment of weight and height is of key importance since detection of failure to thrive may be a sign of an organic cause of constipation. Physical examination primarily consists of examination of the abdomen, the perianal region, and the lumbosacral region.

Abdominal examination mainly focuses on the detection of a palpable fecal mass or scybala. Perianal inspection should be performed in all children; the physician should look for anatomic abnormalities, perianal feces, fissures, scars, and erythema. The presence of fissures can be a sign of hard or large stools but can also be a sign of sexual abuse. Hematomas in the perianal region are highly suspicious of abuse as well. Special attention should be paid to abnormal behavior during physical examination (e.g., sexual acting out, extreme fear) [65]. Although digital rectal examination provides valuable information on the presence of a rectal fecal mass, anorectal sensation, and sphincter tone, it is not necessary for the diagnosis of FC if a child already fulfils 2 or more Rome IV criteria (**Table 1**) [3]. If a child fulfils one of the Rome IV criteria, a digital rectal examination is recommended since it may help establish the diagnosis of FC. Examination of the lumbosacral region may reveal the presence of a dimple, a tuft of hair, or gluteal cleft deviation, indicative of an organic cause of constipation (e.g., spina bifida).

LABORATORY TESTING

Laboratory testing in children with constipation should only be performed in the presence of alarm symptoms as indication for an underlying organic disease, but it does not belong in the routine workup of children with FC. The need for routine screening for cow's milk allergy or hypercalcemia is not supported by current literature [61, 68]. Moreover, serological testing for celiac disease and thyroid function is only indicated in children with short stature, unexpected weight loss, persistent gastrointestinal symptoms or a positive first-degree family history [2]. The prevalence of celiac disease was not found to be higher in children with constipation compared to healthy matched controls, confirming that routine testing of children with constipation for celiac disease is not indicated [69].

ABDOMINAL RADIOGRAPHY

Evidence-based guidelines clearly state that FC is a clinical diagnosis, relying on history and physical examination. Despite this statement abdominal radiography is often used as an adjunct in the management of FC [70, 71]. Extensive literature has however shown that a plain abdominal

X-ray is not the appropriate tool to diagnose constipation. The sensitivity and specificity rates are unsatisfactory, and low inter- and intra-observer reliability have been reported for the different scoring systems (Barr, Leech, Blethyn) that are used to evaluate fecal loading based on abdominal X-rays [72-75]. Moreover, children are exposed to unnecessary radiation. Therefore, abdominal X-rays are only of added value in very limited cases for example when the medical history is unreliable (e.g. anorexia nervosa, factors that make rectal examination inappropriate or unreliable or too traumatic) [76].

COLONIC TRANSIT TIME

There is no evidence to support the routine measurement of colonic transit time in the diagnostic workup of FC, but can be a useful tool in children with fecal incontinence to discriminate between constipation-associated fecal incontinence or functional non-retentive fecal incontinence (FNRFI), a disorder characterized by fecal incontinence without signs of constipation [61]. The most widely used method to determine CCT is the radiopaque marker test performed by single or multiple ingestion of radiopaque markers and calculated by the amount of intra-abdominal markers as visualized on an abdominal X-ray once or at several specific intervals [77]. A colonic transit time <62 h is considered to be normal [78]. Patients are considered having slow-transit constipation when transit time exceeds 62 hours and when the markers are spread throughout the colon. When >50% of the markers are found in the rectosigmoid it is labeled as a rectal evacuation disorder, also known as outlet obstruction [79]. Another method to determine colonic transit time is radionuclide scintigraphy; after ingestion of radioactive isotopes, colonic transit is measured with a large-field-view gamma camera. Scintigraphy is a more novel technique than the radiopaque marker test, with the advantage of minimal radiation exposure, but its use is less widespread and more expensive than a radiopaque marker transit test. More importantly normative values are lacking in the pediatric population [2, 80-83].

CONTRAST ENEMA

A contrast enema is a useful tool to identify anatomic abnormalities of the anorectum; after infusion of contrast fluid into the rectum an abdominal X-ray is obtained, visualizing the distribution of contrast fluid in the distal gastrointestinal tract. Contrast enemas do not belong in the routine workup of children with FC but may be useful to evaluate the morphology of the colon to detect mechanical causes of constipation (e.g., anatomical abnormalities, dilated segments, or complications after colorectal surgery) [84].

ULTRASONOGRAPHY

Transabdominal ultrasonography has been used to measure the transverse rectal diameter [85, 86]. An increased rectal diameter (>30 mm) is often considered to be suggestive for fecal

impaction, but this cut-off value induces major overlap between children with FC and healthy controls [87, 88]. Although transabdominal ultrasonography is a promising technique for assessment of rectal diameter, there is currently insufficient evidence that the transverse diameter can be used as a reliable predictor of constipation and fecal impaction in children [61].

MANOMETRY

Manometry allows for measurement and quantification of intraluminal pressure and contact force in the gastrointestinal tract; this technique can be utilized to gain insights into gastrointestinal motility.

Anorectal Manometry

Anorectal manometry provides information about anorectal neuromuscular function. It can be used to assess the recto-anal inhibitory reflex, anal sphincter pressure, rectal sensation, and defecation dynamics; therefore it is a useful instrument to rule out Hirschsprung's disease and to detect anal sphincter achalasia or dyssynergia [61, 89]. The presence of a normal recto-anal inhibitory reflex is considered to be sufficient to reliably rule out Hirschsprung's disease. However, an absent recto-anal inhibitory reflex is not sufficient to diagnose Hirschsprung's disease; this requires confirmation with histochemical evaluation of a rectal biopsy to confirm absence of enteric ganglia (aganglionosis) [89]. High-resolution anorectal manometry in children with FC with or without fecal incontinence demonstrated lower pressures in the anteroposterior quadrants at rest and during squeezing in children with FC and FI than for children with FC without FI [90]. Interestingly, children with FC with or without FI showed lower resting pressures, lower maximum squeeze pressure and higher recto-anal inhibitory reflex (RAIR) values compared to children without lower GI symptoms [90]. The main drawback of the use of anorectal manometry for evaluating defecation dynamics in children is that patients need to be awake and cooperative during the test. In young children anorectal manometry is therefore sometimes performed with the use of sedation or general anesthesia. Some anesthetics, however, significantly lower the anal resting pressure [91]. The performance and analysis of anorectal manometry belongs in specialized centers and should not be routinely applied in children suspected of FC.

Colonic Manometry

Colonic manometry is a diagnostic test performed to differentiate between normal colonic motor function and colonic neuromuscular disorders in the evaluation of children with intractable constipation (**Figure 1A&B**). In colonic manometry the quality and frequency of HAPCs are identified, often in a fasting phase, during a meal, during the postprandial phase and during a provocative phase in which stimulant laxatives are administered. Abnormal HAPCs may indicate segmental or milder colonic dysfunctioning while absent HAPCs may

indicate a severe colonic motility disorder [92]. Many differences exist between centers in the type of catheter used, number of sensors and spacing between sensors, and the protocols for investigations, which makes comparison of data among groups difficult [89]. The introduction of high-resolution colonic manometry allows to not only focus on HAPCs, which are relatively rare events (<2% of all motor patterns) even in a healthy colon, but also on other propagating motor patterns [93]. However, these data originate from adult trials and clinical relevance, if any, of high-resolution manometry findings still need to be established in pediatrics [89]. Nevertheless, despite differences in execution of colonic manometry, it is considered a useful tool to rule out neuromuscular motility disorders of the colon associated with slow-transit constipation. But, motility testing, such as anorectal manometry and colonic manometry belongs in specialized centers, usually in an academic setting.

MAGNETIC RESONANCE IMAGING

To date, evidence does not support the use of magnetic resonance imaging (MRI) of the spine in patient with intractable constipation without other neurologic abnormalities [61]. One retrospective study found lumbosacral spine malformations in 9% of children with intractable constipation which was not associated with major neurologic symptoms [94]. In contrast another study, in children with defecation disorders including constipation, constipation-associated fecal incontinence and FNRFI, spinal cord abnormalities such as intradural lipoma or tethered cord were found in only 3% of affected children [95]. Recently, a feasibility study has been conducted in adolescents with FC to investigate if MRI could be a non-invasive alternative to colonic manometry. However, results did not overlap in the identification of HAPCs [96]. Therefore, MRI should not be included in the routine workup of children with FC and should only be considered when there is strong suspicion of neurologic disorders such as neurological findings in the lower extremity and midline defect in the skin of the lower back and gluteal cleft deviations.

MANAGEMENT

The ESPGHAN/NASPGHAN guideline includes four important phases in the treatment of FC: (1) education, (2) disimpaction, (3) maintenance therapy to prevent re-accumulation of feces, and (4) follow-up [61]. The management of FC in children consists of non-pharmacological and pharmacological treatment modalities. **Figure 5** represents a treatment pyramid for the management of children with FC.

EDUCATION

Education is the first step in the non-pharmacological treatment of FC [61]. This should include an explanation of the physiology of defecation, tailored to the developmental age of

the child. The negative chain of events that may have been prompted by a painful defecation experience should be explained to parents and, if possible, children. It is important to describe the pathophysiology of overflow incontinence and the pivotal role that withholding behavior plays in this process. Also, the role of parental child-rearing attitudes towards fecal incontinence, such as frustration and overprotection, should be discussed [31]. Lifestyle advice such as dietary recommendations, regular physical activity and advice on toilet training, toileting posture and behavior should be part of this step and in the presence of behavioral problems, behavioral therapy should be considered [61, 97].

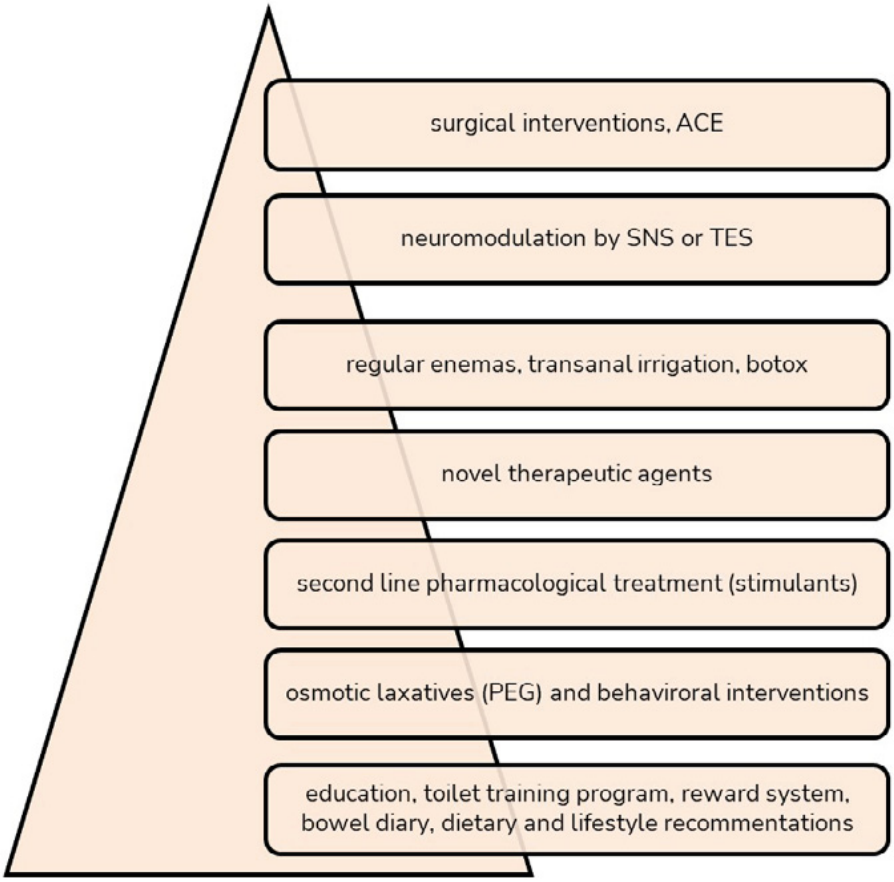

Figure 5 | Treatment pyramid for FC. FC is usually treated in a step-up approach, starting with non-pharmacological interventions and osmotic laxatives (bottom of the pyramid). If these measures are unsuccessful, use of more invasive modalities may be necessary (towards the top of the pyramid). Abbreviations: PEG: polyethylene glycol, ACE: antegrade continence enemas, SNS: sacral nerve stimulation, TES: transcutaneous electrical stimulation.

TOILET PROGRAM AND REWARD SYSTEM

Toilet training can be challenging for parents, but in case of delayed toilet training the child must be thoroughly assessed in order not to miss important diagnoses such as spinal cord abnormalities and constipation. In rare cases delayed toilet training may be a presentation of sexual abuse [98]. In toilet trained children, stasis of feces in the rectum can maintain constipation, therefore it is important to evacuate the rectum regularly. In children with a developmental age of ≥4 years, this can be established by introducing a toilet training program, with scheduled toilet sits throughout the day, usually after every meal and after coming home from school. The toilet sits are scheduled after a meal, to benefit from the gastrocolic reflex [27], which increases colonic peristalsis upon distention of the stomach [27]. During these times, it is advised to have the child pay attention to sensation and not divert their attention with reading or screen activities [27]. To motivate children to maintain this toilet training program, a reward system can be introduced. By rewarding the child with small gifts for completing toilet training, the child is positively reinforced to comply with therapy. A non-accusatory approach of both physicians and parents is of key importance since children may feel guilty or embarrassed, especially about episodes of fecal incontinence [61]. Only rewarding periods without fecal incontinence is therefore not recommended, this may increase feelings of guilt and can be experienced as punishment for having fecal incontinence.

DIETARY FIBER, FLUID, AND PHYSICAL ACTIVITY

Fiber

Insufficient fiber intake has been reported to be associated with FC, and advice on normal fiber and fluid intake and physical activity are the first steps in the treatment of FC [61, 99]. As stated in the ESPGHAN/NASPGHAN guidelines, there is currently insufficient evidence to support the use of supplementary fiber in excess of the daily recommended intake in children with FC [61]. Recent systematic reviews and a meta-analysis found limited high-quality studies and give no indications to change the current guidelines of ESPGHAN/NASPGHAN [100, 101]. However, since most children fail to meet the daily fiber recommendations (0.5 g/kg/d for children aged >5 years) fiber intake should be addressed [102, 103].

Fluid

Only few studies investigated the association between fluid intake and FC [104, 105]. These studies showed insufficient evidence for an advantageous effect of additional fluid intake on constipation symptoms. Indeed, extra fluid intake in children with FC in excess of a normal fluid intake is not recommended [61]. An exception should be made for extra fluid that is recommended for medication intake, such as polyethylene glycol, which needs to be dissolved in water.

Physical Activity

Although physical activity may be associated with a decreased risk of developing FC at the preschool age [106], no studies have been performed to assess the effect of increasing physical activity to treat symptoms of constipation in children [61, 107].

Probiotics

Studies on the use of probiotics have been conducted in children, but to date, there is insufficient evidence to support the use of probiotics in the treatment of childhood constipation [56, 108].

BIOFEEDBACK TRAINING

Biofeedback training utilizes reinforcing stimuli in an attempt to achieve a recognizable sensation and encouraging an appropriate learnt response. In theory, this may help children with dyssynergia to adapt their defecation dynamics. However, currently available evidence does not support the use of biofeedback training for the treatment of childhood constipation [61].

PELVIC FLOOR PHYSIOTHERAPY

Pelvic floor physiotherapy teaches how to perform pelvic floor muscle exercises and is described as potential treatment option for the treatment of children with FC in relation to dyssynergic defecation [109-112]. Three studies showed beneficial effects of pelvic floor physiotherapy in children with FC in addition to standard medical care [113-115]. Contradictory, a recent RCT in primary care did not find evidence to recommend physiotherapy for children with FC in primary care [116]. Before recommending pelvic floor physiotherapy in the treatment of FC or as addition to standard medical care, also taking cost-effectiveness into account, larger studies are needed.

TREATMENT

The pharmacological treatment of FC mainly consists of treatment with laxatives and involves three steps: disimpaction, maintenance treatment, and weaning. The pharmacological treatment options, including recommended dosages, are summarized in **Table 2** [2, 61, 64].

Table 2 | Pharmacological management of functional constipation in children [2, 61, 64].

Osmotic laxatives	
PEG 3350 (with electrolytes)/4000 (without electrolytes)	Maintenance: 0.3–0.8 g/kg/day in 1–2 doses
	Fecal disimpaction: 1–1.5 g/kg/day (max 7 days)
Lactulose	7 months–18 years: 1–2 g/kg/day, in 1–2 doses
Milk of magnesia (magnesium hydroxide)	2–5 years: 0.4–1.2 g/day, in 1 or more doses
	6–11 years: 1.2–2.4 g/day, in 1 or more doses
	12–18 years: 2.4–4.8 g/day, in 1 or more doses
Lactitol	1–6 years: 0.5–1 g/kg/day in 2–3 doses
	6–12 years: 10–30 g/day in 2–3 doses
	12–18 years: 20–60 g/day in 2–3 doses
Lubricants	
Mineral oil (liquid paraffin)	*Oral*
	3–18 years: 1–3 mL/kg/day, 1 or more doses/day (max 90 mL/day)
	Rectal
	2–11 years: 30–60 mL, in 1 dose/day
	>11 years: 60–150 mL, in 1 dose/day
Stimulant laxatives	
Bisacodyl (diphenylmethane)	3–10 years: 5 mg/day, in 1 dose/day (at night)
	>10 years: 5–10 mg/day, in 1 dose/day (at night)
Senna (anthraquinone)	2–6 years: 2.5–5 mg/day, in 1–2 doses/day
	6–12 years: 7.5–10 mg/day, in 1–2 doses/day
	>12 years: 15–20 mg/day, in 1–2 doses/day
Sodium picosulfate	1 month-4 years: 2.5–10 mg/day, in 1 dose/day
	4–18 years: 2.5–20 mg/day, in 1 dose/day
Rectal laxatives/enemas	
Bisacodyl	3–10 years: 5 mg/day, in 1 dose/day (at night)
	>10 years: 5–10 mg/day, in 1 dose/day (at night)
Sodium lauryl sulfoacetate	1 month–1 year: 2.5 mL/dose (=0.5 enema)
	1–18 year: 5 mL/dose (=1 enema)
Sodium docusate	<6 year: 60 mL
	>6 years: 120 mL
Sodium phosphate	1–18 year: 2.5 mL/kg/dose (max 133 mL/dose)

PEG: polyethylene glycol

DISIMPACTION, MAINTENANCE TREATMENT, AND WEANING

Fecal impaction occurs in approximately 50 % of children with FC [23, 64, 117]. To increase success, pharmacological treatment consists of two steps: fecal disimpaction followed by maintenance therapy [117].

Disimpaction can be achieved with enemas or temporary high-dosed oral polyethylene glycol (PEG) (1–1.5 g/kg/day) during 3–6 days [117]. High-dose PEG and sodium docusate enemas have been found to be equally effective for disimpaction and although high-dose PEG is associated with a higher risk of fecal incontinence during treatment compared with enemas, PEG is recommended as first choice for disimpaction because it is administered orally [64].

After successful disimpaction, maintenance therapy should be initiated to prevent the re-accumulation of feces [117]. The aim of maintenance treatment is to soften the stools and to facilitate easy and frequent bowel movements. Several laxatives are available for maintenance treatment (**Table 2**). PEG is the oral laxative of first choice in a dosage of 0.2–0.8 g/kg/day. Other therapeutic options are discussed below. Depending on the severity of symptoms, the effect of treatment should be evaluated 1–2 weeks after initiation of treatment. Maintenance treatment should be continued and FC symptoms should be resolved for at least 2 months before considering weaning in order to prevent a relapse [61, 117]. This means that children have a defecation frequency of ≥3 times per week and do not fulfill any other ROME IV criteria. It is recommended to evaluate symptoms again 2 months after cessation of treatment, to prevent or detect relapses.

PHARMACOLOGICAL AGENTS

Osmotic Laxatives

Maintenance treatment in children with FC usually consists of oral osmotic laxatives; these agents are poorly absorbed by the intestinal wall, causing osmotic water retention in the intestinal lumen. This softens the stools and increases peristalsis through intestinal distention (**Figure 6A**). A number of laxatives are commonly used in children, but PEG (macrogol) is the first-choice osmotic laxative in children with FC based on its effectiveness and safety profile [118]. PEG is more effective in increasing stool frequency than placebo, lactulose, and milk of magnesia (magnesium hydroxide) [119]. Even in young children (less than 2 years of age) the use of PEG has been proven to be effective and safe [118]. PEG combined with electrolytes can be prescribed to minimize the risk of disturbing the electrolyte balance due to osmosis (e.g., in young children). However, the addition of electrolytes affects the taste of the medication, which can result in problems with treatment compliance, but acceptance of PEG-based laxatives was found to be better than non-PEG laxatives [120]. Most commonly reported side effects include fecal incontinence (especially during disimpaction), flatulence, abdominal pain, nausea, and abdominal bloating [27].

Two other commonly used osmotic laxatives are lactulose and lactitol, both synthetic derivatives of lactose, which are fermented into SCFAs such as acetic, lactic and formic acid by the intestinal microbiota [64, 121]. Both agents result in intraluminal water retention and a decrease in intraluminal pH, which induces an increase in colonic peristalsis (**Figure 6**).

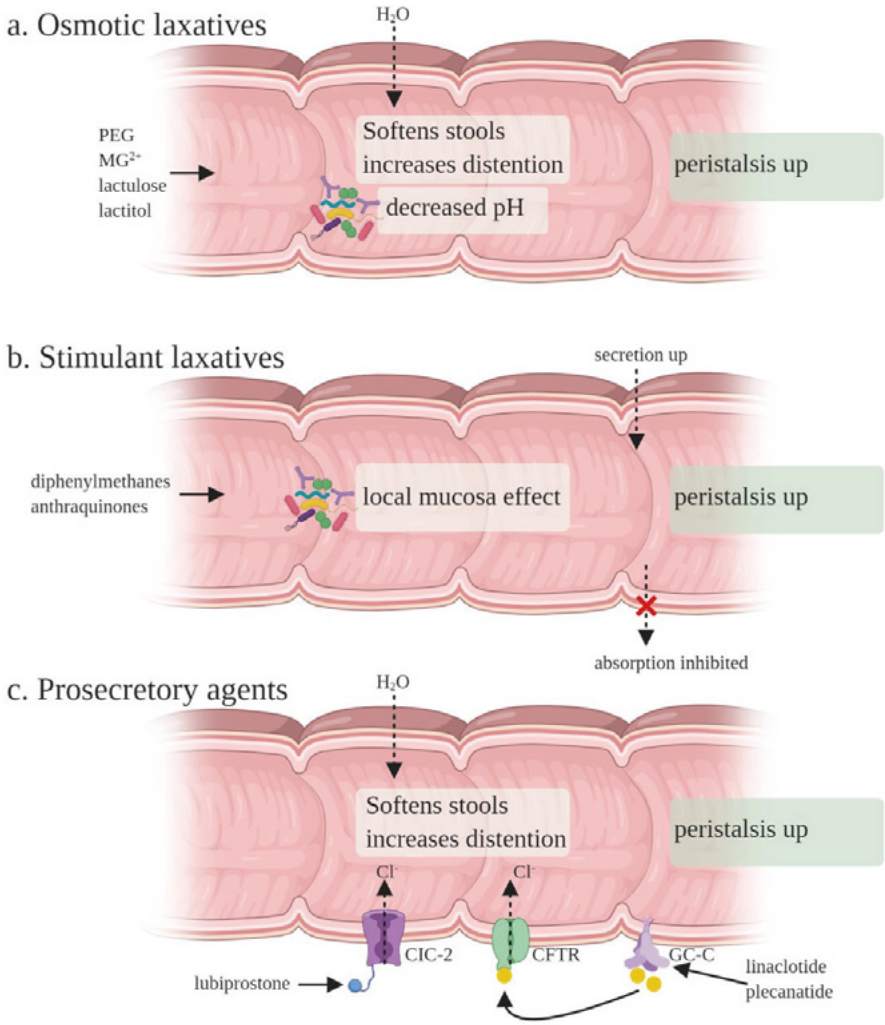

Figure 6 | Working mechanisms of different types of laxatives. **A |** Osmotic laxatives are poorly absorbed by the intestinal wall. This stimulates retention of water in the intestinal lumen, softening the stools, and increasing peristalsis through intestinal distension by increasing stool volume. In addition, fermentation of the disaccharides lactulose and lactitol by the intestinal microbiota results in a decrease in intraluminal pH, which induces an increase in colonic peristalsis. **B |** Stimulant laxatives are metabolized into active metabolites by the intestinal microbiota, these act directly on the intestinal mucosa stimulating peristalsis and influencing fluid regulation mechanisms. Diphenylmethane metabolites exert a local prokinetic effect and stimulate intestinal secretion. Anthraquinone metabolites stimulate colonic motility and water and electrolyte secretion, while they inhibit absorption of water and electrolytes. **C |** Lubiprostone and linaclotide both promote secretion of chloride-rich fluid in the intestine, softening stools and enhancing stool volume. Lubiprostone is a prostaglandin E1 derivative, which activates chloride channel subtype 2 (ClC-2). Linaclotide activates the luminal guanylin receptor (GC-C), this promotes production of cyclic GMP, which in turn activates CFTR channels. Created with BioRender.com

Bacterial fermentation of these agents also induces gas formation, which induces additional intestinal distension and increases peristalsis but may also result in side effects such as flatulence, abdominal pain, and abdominal bloating. Lactulose is less effective than PEG [119], but since it is considered to be safe for all ages, it is recommended in case PEG is not available.\

Mgnesium hydroxide (also referred to as "milk of magnesia" as suspension) is an antacid with an osmotic laxative effect. It is considered to be less effective than PEG in the treatment of childhood FC [119]. Side effects of magnesium hydroxide include diarrhea, hypotension, weakness, and lethargy [64].

Stimulant Laxatives

Stimulant laxatives have a different action mechanism than osmotic laxatives, these agents act directly on the intestinal mucosa, stimulating intestinal motility or increasing electrolyte and water secretion (**Figure 6B**). Based on expert-opinion, stimulant laxatives may be considered as additional or second-line treatment [118]. Bisacodyl and sodium picosulfate are diphenylmethanes. In the colon, these nonabsorbable agents are hydrolyzed to their active metabolites, which exert a local prokinetic effect and stimulate intestinal fluid secretion [121]. Bisacodyl can be administered orally and rectally, in the latter form its effect is observed rapidly after administration. Moreover, long-term use of bisacodyl was not associated with complications or development of tolerance to the medication, and patients were able to be weaned off the medication with minimal reported side effects [122]. Another stimulant and effective laxative is senna, which contains anthraquinones. This agent is also metabolized into their pharmacologically active metabolite by the intestinal microbiota and the metabolites stimulate colonic motility and the secretion of water and electrolytes, while they inhibit the absorption of water and electrolytes from the colon [121]. The most common side effects of stimulant laxatives are flatulence, abdominal pain, nausea, and diarrhea.

Lubricants

Mineral oil (or liquid paraffin) is a mixture of higher alkanes, often a derivative of petroleum that functions as a lubricant. It is not absorbed by the intestines and may also exert an osmotic effect when it is converted to fatty acids [123, 124]. A Cochrane systematic review found some evidence that mineral oil increased stool frequency, but also reported side effects such as abdominal pain, distention and watery stools [119]. Liquid paraffin is considered to be safe and effective in the treatment of FC in children [123], but a bothersome adverse effect is leakage of the agent from the anus, causing irritation, itching, and staining of clothing and furniture. Due to incidental reports of the severe side effect of granuloma following absorption and lipoid pneumonia after aspiration [123, 125, 126], liquid paraffin should be considered as an additional or second-line treatment and should not be administered to children under 3 years of age [118, 127].

Enemas

Rectally administered enemas used in the treatment of FC contain chemically active agents that increase gut motility, exert an osmotic effect, or both. They work rapidly, usually within minutes. Different kinds of enemas are available. Sodium lauryl sulfoacetate enemas bring about a redistribution of the water that is bound to feces and thereby soften the stools. These enemas do not have an osmotic effect and are therefore often used in infants. Sodium docusate enemas contain the lubricant docusate (sometimes with added sorbitol, a hyperosmolar agent) and sodium phosphate enemas contain a strong hyperosmolar phosphate solution. Adverse effects of enemas include abdominal pain and anorectal discomfort.

NOVEL THERAPEUTIC AGENTS

Prosecretory agents

Prosecretory agents such as lubiprostone, linaclotide, and plecanatide are therapeutic agents that modulate epithelial channels in the gut, promoting the intestinal secretion of fluids and thereby enhance stool volume, resulting in an improved gastrointestinal transit (**Figure 6C**) [128]. These agents have been found to be effective in the treatment of constipated adults [2], but data on the efficacy of these agents in the treatment of FC in children are scarce or not yet available.

Lubiprostone is a prostaglandin E1 derivative that induces intestinal fluid secretion by activating the chloride channel subtype 2 (ClC-2) and cystic fibrosis transmembrane conductance regulator (CFTR), enhancing the secretion of chloride-rich intestinal fluid [129]. Only one study in the pediatric population has been published. This open label, noncontrolled study showed after 4 weeks of treatment with lubiprostone an increased defecation frequency in 127 children with functional constipation [130]. Reported adverse events included nausea, vomiting, diarrhea, and abdominal pain [130].

Linaclotide and plecanatide promote intestinal fluid secretion by activating the guanylate cyclase C receptor, activating CFTR, leading to the secretion of chloride-rich intestinal fluid. To this date, no studies were found evaluating its use in children, but studies in adults with linaclotide found improvement in stool frequency and consistency, abdominal symptoms and global relief versus placebo [131, 132]. Similarly, no studies were found in children evaluating plecanatide, however in adults the use of plecanatide showed a substantial improvement in stool frequency and consistency compared with placebo [133].

Serotonergic agents

A number of 5-hydroxytryptaime 4 (5-HT4) agonists have been developed for the treatment of FC. Serotonin (5-HT) is a central and enteric neurotransmitter that binds to the 5-TH4 receptors in the gut, thereby increasing the release of acetylcholine which in turn results in an increased secretion and gut motility [134].

Prucalopride is a highly selective serotonin 5-HT4 receptor agonist which functions as a prokinetic agent. Only two published studies evaluated prucalopride in children with FC and showed, in an 8-week open-label controlled study in 37 children, improvement in stool frequency and consistency, and fecal incontinence frequency [135]. In contrast, another study, a RCT in 213 children with FC, did not find a statistically significant improvement in bowel movements or frequency of fecal incontinence [136]. Reported adverse events included headache, nausea, abdominal pain, and diarrhea [136].

Other serotonergic agents such as velusetrag and naronapride have not yet been investigated in children and have not yet been approved by the FDA or EMA.

Bile acids

As mentioned above, endogenous deconjugated bile salts have the potential to function as endogenous laxatives by increasing colonic motility and fluid secretion [57]. In adult women chenodeoxycholic acid, a primary bile acid, was shown to be effective for constipation predominant irritable bowel syndrome in improving stool consistency [137]. But to date, no studies on the use of bile acids in children with FC have been performed.

Cholinesterase inhibitors

Acetylcholinesterase inhibitors, such as pyridostigmine, increase gastrointestinal motility by increasing the availability of acetylcholine. One study, a case series of four children with gastrointestinal motility disorders using pyridostigmine, suggested a beneficial effect on defecation frequency in one patient with constipation [138].

TRANSANAL IRRIGATION

Transanal irrigation (TAI) involves infusion of fluids (usually tap water) into the rectum and colon in a retrograde fashion to mechanically clean out the intestine, typically used in children with FC who are unresponsive to oral laxative treatment [139]. TAI has been well established for the use in patients with neurogenic defecation disorders and anorectal malformations [139], but data on the effectiveness of TAI in children with FC are scarce. Pediatric cohort studies, in small populations of children with FC, have shown to be effective in the treatment of constipation with and without fecal incontinence and renders a high parental satisfaction [140-143]. Transanal irrigations are usually performed with a volume of 10–20 mL/kg of water and the frequency of irrigations depends on the patient's response [139]. In some patients, different irrigation fluids (saline, added laxatives) may be explored to optimize outcome.

BOTULINUM TOXIN

Intrasphincteric injections with botulinum toxin A (botox) have been used in the treatment of FC. By lowering the pressure of the anal sphincter, botox aims to facilitate an easier defecation

process. Botox injections have a temporary effect and repetitive injections may be necessary to maintain treatment effect. The injection of botulinum toxin A into the anal sphincter may lead to easier and more frequent passage of stools with less pain in children with intractable constipation, regardless of anal sphincter dynamics. But patients with fecal incontinence are less likely to respond [144]. The dose of botox administration in children ranges from approximately 75-200U, but 100U appears to be used most across studies [145]. However, since this method is rather invasive and other methods like electromotive drug administration (EMDA), in which the drug solution is delivered directly into the target site, are being explored. One recent study investigated the effect between regular botox injections and this EMDA botox method in 60 children with FC [146]. EMDA was as effective as an intrasphincteric botox injection of the treatment of FC but had several advantages, including less comorbidity, lower costs, and most importantly can be performed without general anesthesia [146]. Temporary side effects are fecal and urinary incontinence.

SURGERY

In patients with FC unresponsive to medical treatment, surgical treatment may be necessary. Guidance on how to choose the best procedure for a given patient may include antegrade colonic enema (ACE), pelvic floor surgery, botox injections and colorectal resection [84, 147]. However, the evidence is weak and more studies are needed to identify subgroups of patients who may benefit from surgical interventions in the treatment of FC and taking into account that conservative management for patients with FC is the best before moving to these dramatic surgical interventions [84].

Antegrade Continence Enemas (ACE)

ACE involves colonic irrigation in an antegrade direction through a surgically created access point into the colon, with as most commonly used procedures the Malone appendicocecostomy and the percutaneous cecostomy [2]. In the Malone appendicocecostomy the appendix is connected to the abdominal wall creating a valve. In the percutaneous cecostomy, a minimally invasive procedure, an artificial cecostomy tube connects the cecum with the abdominal wall. ACE surgery is considered minimally invasive and good clinical outcomes have been reported in children [84].

Colonic Resection

When minimally invasive surgical therapies fail in children such as in severe cases of intractable FC or when colonic manometry reveals a dysfunctional colonic segment, resection of the affected segment may be beneficial. This can be followed by subsequent colo-anal or ileo-anal anastomosis or creation of a diverting ileostomy or colostomy. In recent years several studies have been published investigating outcomes of colonic resection in idiopathic constipation in

children. One retrospective study in children who underwent ileostomy, colostomy or (sub)total colectomy found an improvement in symptoms and parent satisfaction of 91%, but also reported high rates of complications such as stoma problems or the need for stoma-revisions [148]. Another retrospective study found that, in the presence of a megarectum, a rectosigmoid resection via laparoscopic video-assisted low anterior resection of the colon was effective in children, and better than Soave pull-through operation [149]. Another retrospective study compared three different types of resection: pan-proctocolectomy with ileoanal pouch anastomosis, total colectomy with ileorectal anastomosis, and segmental resections and anastomosis. This study found no differences between these types of resection in terms of results or complications and concluded that there might be a role for colonic resection in constipated children. However, authors of this paper estimated that 2/5 will be left with a permanent stoma, of which children and parents should be aware [150]. A thorough review on surgical options available for the management of refractory constipation in children concluded that surgical options should be considered as they can lead to significant improvement in symptoms and quality of life [147]. However, due to the small study sizes, lack of prospective randomized studies, large differences in operation techniques and the high impact of surgical interventions, there is a great need for consensus guidelines on surgical decision-making.

ELECTRICAL STIMULATION/NEUROMODULATION

Electrical stimulation or neuromodulation involves the generation of currents that cross within the body or are used to stimulate a nerve. The exact mechanism of action is not yet understood, but the current may result in an alteration of neuronal function, and increase colonic motility by stimulating the interstitial cells of Cajal, the pacemaker cells of the gut, and/or enteric or extrinsic autonomic nerves [151].

Transcutaneous Electrical Stimulation (TES)

TES is a non-invasive, pain free form of electrical stimulation that uses interferential current via electrode pads applied across the skin of the abdomen and lower back. One RCT compared TES with sham stimulation in children with slow-transit constipation and found improvement in CTT and quality of life scores, but defecation frequency did not improve [152, 153]. In addition, a long-term follow-up of these studies found that 33% of children with slow-transit constipation had significant improvement in stool consistency and fecal incontinence two years after treatment with TES [154].

Percutaneous tibial nerve stimulation (PTNS)

PTNS involves (bilateral) stimulation of the posterior tibial nerve by inserting a needle electrode at the level of the medial malleolus and thereby indirectly stimulating the sacral nerves [2]. Findings in children are scarce, but preliminary results of a small study in children

with organic causes of constipation found that PTNS is effective for the treatment of fecal and urinary leakage [155]. Despite indications that PTNS or other forms of electroacupuncture may improve motility, such as described in a study in rodents in which they were able to enhance motility via stimulation of autonomic mechanisms, future studies in children with constipation are needed to determine the efficacy of such treatments.

Sacral Nerve Stimulation (SNS)

During SNS the anterior ramus of sacral spinal nerves S3 and S4 is stimulated via surgically positioned electrodes that are connected to an implanted pulse generator. Efficacy of SNS on fecal incontinence in pediatric patients is well established, but its mechanism of action and role in treatment of FC is less clear [2]. Small cohort studies in children with FC show promising effects of SNS on defecation frequency [156, 157]. Although considered minimally invasive, high rates of device-related adverse events have been reported such as pain, hematoma, infection and displacements of the leads [2]. However, SNS remains a specialized and expensive procedure and randomized-controlled studies with long-term follow-up are essential to gain more insights into the potential role of SNS in the management of FC in children.

PROGNOSIS

A large proportion of children with FC can be treated effectively with the therapeutic strategies that are currently available. A systematic review of prospective follow-up studies in the hospital setting concluded that within 6–12 months, approximately 50 % of the children recover and are taken off laxatives [158]. An additional 10 % of patients will be asymptomatic on treatment and the remaining 40 % remains symptomatic despite pharmacological treatment [158]. In children with intractable symptoms, unresponsive to medical treatment, symptoms may persist into adolescence or even adulthood despite laxative treatment [158-160].

Early adequate therapeutic interventions are of key importance; a delay between onset of symptoms and first presentation at a pediatric gastroenterologist is negatively related to recovery [159].

FUTURE PERSPECTIVES

The most significant advances in the management of FC in children are likely to result from more precise identification of the pathophysiology in order to select individualized treatment. Guidelines currently do not yet support the use of these treatments, however several of these might be promising to further investigate. These novel therapies might range from acupuncture, specific food exclusion diets, gut-microbiota directed interventions such as pre-, pro-, syn-, and postbiotics or fecal microbiota transplants, therapies influencing intestinal ion exchanges/ transporters and bile acid modulators.

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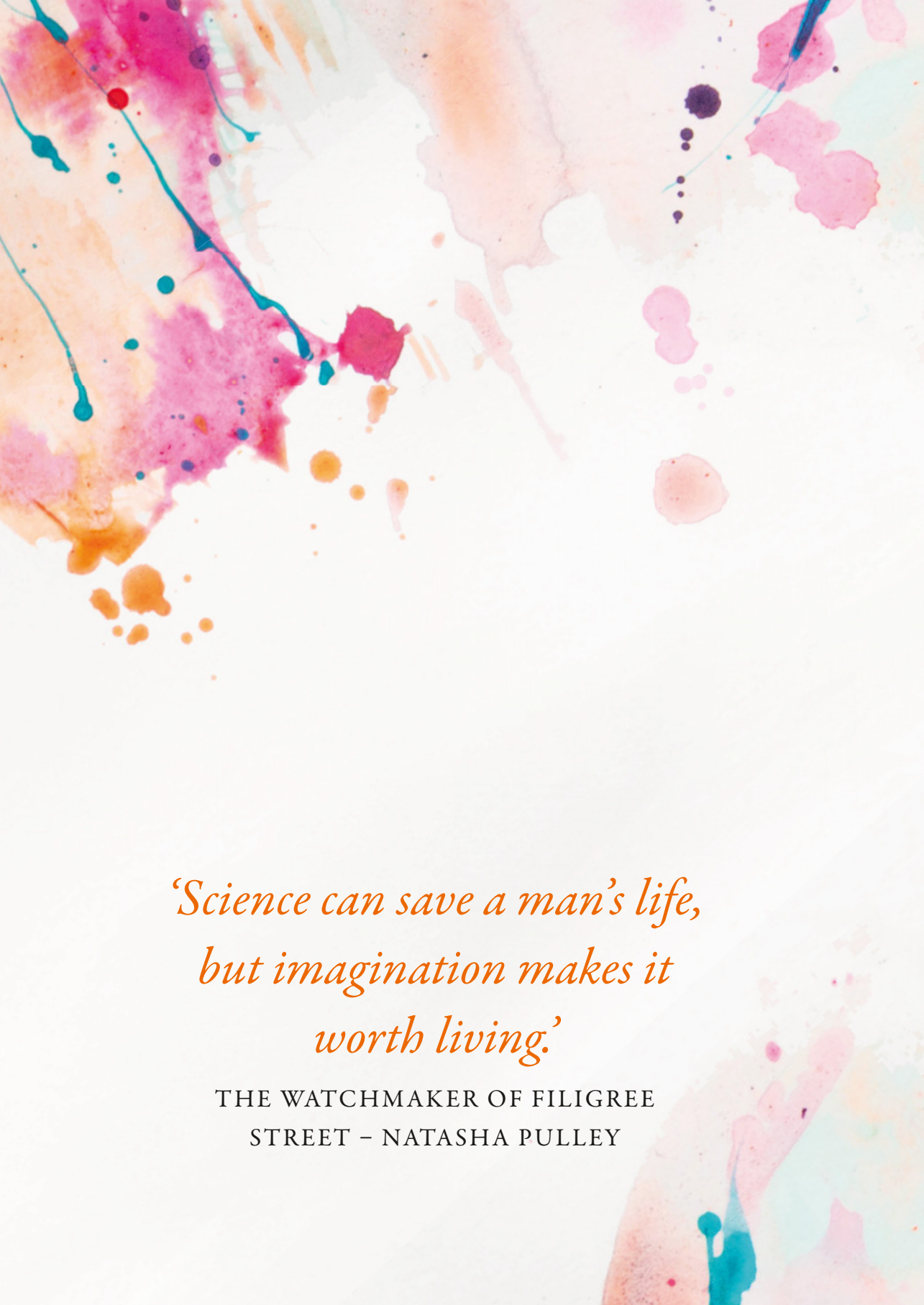

'Science can save a man's life, but imagination makes it worth living.'

THE WATCHMAKER OF FILIGREE STREET – NATASHA PULLEY

PART 2

Defining and measuring (healthy) defecation patterns

The modified BSFS: a reliable and valid tool to score stool consistency in Dutch (non) toilet trained toddlers

Carrie A.M. Wegh, Gerben D.A. Hermes, Margriet H.C. Schoterman, Elaine E. Vaughan, Hauke Smidt, Clara Belzer & Marc A. Benninga

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ABSTRACT

Objective: To assess whether the modified Bristol Stool Form Scale (m-BSFS) is reliable, valid and user-friendly to use by parents, grandparents and day childcare employees to evaluate stool consistency in toilet and non-toilet trained toddlers in the Netherlands.

Study design: Translation to Dutch and validity of the m-BSFS (scoring 32 general stool pictures) for 1-3 year old toddlers (n=89) was evaluated by parents, grandparents and day childcare employees. A subgroup of participants scored an additional seven pictures of stools in a diaper to validate the m-BSFS for non-toilet trained toddlers (n=16). To determine inter-rater reliability, two-way random effects single rater intraclass correlation coefficient (ICC)consistency was used. Intra-rater reliability was measured by Cohen's kappa (κ) by rating the same pictures in random order twice, with at least one week between the first and second scoring.

Results: Inter- and intra-rater reliability of the m-BSFS were above recommended minimal standards of 0.61 for the 32 general stool pictures as well as for the seven pictures of stools in a diaper. $ICC_{consistency}$ for the general stool pictures of the first and second ratings were 0.71 (n=89) and 0.79 (n=77), respectively, with a κ of 0.71 (n=77). $ICC_{consistency}$ for the stools in diaper pictures of the first and second ratings were 0.93 (n=16) and 0.93 (n=15), respectively, with a κ of 0.77 (n=15).

Conclusions: The m-BSFS is reliable, valid and user-friendly to use by Dutch-speaking parents, grandparents and day childcare workers to evaluate stool consistency in both toilet and non-toilet trained toddlers in the Netherlands.

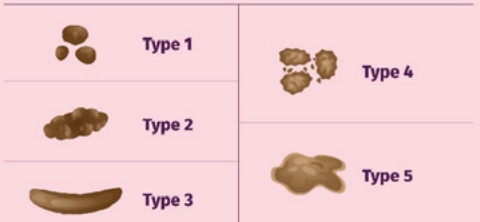
Keywords: Constipation; Bristol Stool Form Scale; modified Bristol Stool Form Scale for Children; BSFS; Functional GI Disorders.

Modified Bristol Stool Form Scale: A Reliable Scale to Assess Stool Consistency in Dutch Toddlers

Assessment of stool consistency in toddlers can help in:

- Evaluating defecation patterns
- Diagnosing gastrointestinal disorders

Bristol Stool Chart



Can the modified Bristol Stool Form Scale (m-BSFS) be used to evaluate stool consistency in toddlers in the Netherlands?

Translation and cultural adaptation of the Dutch m-BSFS

Evaluation of stool pictures by (grand)parents and day childcare employees

- General stool pictures (n = 89)
- Stools in a diaper (n = 16)

Inter-rater reliability

Intraclass correlation coefficient ($ICC_{consistency}$)

Intra-rater reliability

Cohen's Kappa (κ)

$ICC_{consistency}$ for general stool pictures

1st rating	0.71
2nd rating	0.79
κ	0.71

$ICC_{consistency}$ for diaper stool pictures

1st rating	0.93
2nd rating	0.93
κ	0.77

Reliability above the recommended minimal standard reliability of 0.61

The m-BSFS is a reliable scale for evaluation of stool consistency in both toilet and non-toilet trained toddlers in the Netherlands

Modified Bristol Stool Form Scale: a reliable and valid tool to score stool consistency in Dutch (non) toilet trained toddlers
Wegh et al. (2021)

JPGN Journal of Pediatric Gastroenterology and Nutrition

WHAT IS KNOWN

- Reliable and valid assessment of stool consistency is important for evaluating defecation patterns in toddlers and diagnosing gastrointestinal disorders.
- Reliability and validity has not been evaluated in current stool scales on defecation patterns in toddlers, that may include stools from a diaper as well as from a toilet/potty.

WHAT IS NEW

- The m-BSFS was reliable, valid and user-friendly to use to evaluate stool consistency in both toilet and non-toilet trained toddlers in the Netherlands.
- To the best of our knowledge, this is the first stool scale that has been validated to score stool consistency in both toilet/potty as well as in diapers. This will be of value for monitoring bowel habits in young children in clinical research for food ingredients, medicines or lifestyle changes that can impact this parameter at a critical age of toilet training.
- The reliability and validity of the modified Bristol Stool Form Scale has been assessed in caregivers.

INTRODUCTION

Alterations in stool frequency and stool consistency are associated with numerous organic and functional gastrointestinal (GI) disorders in children such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and functional constipation (FC). These diseases may be so serious that they affect quality of life of the children and their parents [2-4]. Medical professionals and scientists seek various approaches including dietary modifications and fiber-enriched foods/supplements to improve intestinal issues such as constipation [5, 6]. Stool scales are often used to diagnose and evaluate GI disorders in children or toddlers. Several stool scales can be used depending on the age of the target group. Examples of these scales include the Bristol Stool Form Scale (BSFS) and the modified-BSFS (m-BSFS), but also scales that allow for scaling stools in diapers, such as the Amsterdam infant stool scale (AISS) and Brussels infant and toddler stool scale (BITSS) [7-9]. However, these stool scales are either general scales often used for defecation on a toilet or scales specifically developed to evaluate stools in a diaper. This contrasts with the fact that research on defecation patterns in toddlers may include stools from a diaper as well as from a toilet/potty. The m-BSFS is a pediatric 5-point stool form rating scale developed to score stools of toilet trained children, and validated to be scored by pediatric gastroenterologists and children >8 years of age. The evaluation of stools of toddlers to manage defecation problems is often done by caregivers, such as parents, grandparents and day childcare employees. In addition, the m-BSFS is validated for the English language only, and in paper form, while currently there is a shift towards more online-based methods for scoring in clinical research. Therefore, the aim of this study was to assess whether the m-BSFS in paper form and online is reliable, valid and user-friendly to use by Dutch-speaking parents, grandparents and day childcare employees to evaluate stool consistency in both toilet and non-toilet trained toddlers in the Netherlands.

MATERIALS AND METHODS

TRANSLATION AND CULTURAL ADAPTATION OF THE DUTCH M-BSFS

Published guidelines were followed to translate and to culturally adapt the English version of the m-BSFS to Dutch [10-13]. To achieve linguistic equivalence to the original m-BSFS, two native English-speaking forward translators with excellent knowledge of Dutch were asked to individually translate the m-BSFS into Dutch. Both translators were then asked to discuss their results with each other until consensus was reached. This Dutch m-BSFS was then presented to two native Dutch backward translators with excellent knowledge of English but with no previous knowledge of the English m-BSFS. The Dutch backward translators were asked to discuss their results until consensus was reached. The original English m-BSFS was then compared to the English backward translation. Subsequently, the Dutch version was given to 12 pediatric gastroenterology fellows to check if the scale's language matched the language used in practice, and if necessary, additional

adaptations were made. The new Dutch m-BSFS was again sent to all four translators, who were asked whether they agreed with the new, culturally-adapted translation.

PARTICIPANTS

The Medical Ethical Reviewing Committee of Wageningen University (METC-WU) reviewed the research file and concluded that this research does not fall within the remit of the Dutch 'Medical Research Involving Human Subjects Act'. However, following International Conference on Harmonization – Good Clinical Practice (ICH-GCP) guidelines, informed consent was obtained. Participants were eligible when they were a parent of at least one child of 1-3 years old (12-36 months), day childcare employees working with toddlers of 1-3 years old, or grandparents of at least one grandchild of 1-3 years old. We sought to include approximately n=100 participants, based on published recommendations, for the 32 general stool pictures in the ratio 3:1:1 for parents, grandparents and day childcare employees, respectively [11]. More parents were included as parents would be looking after their children most of the time in a real-life situation. In order to validate the 7 stools in diaper pictures and assess user-friendliness, we aimed to include approximately n=20 participants. Flyers for the study were distributed over day childcare centers, public areas such as public libraries and spread online via multiple media. Questionnaires were then sent by e-mail to those willing to participate.

INTER- AND INTRA-RATER RELIABILITY

The same 32 color pictures of stools as were used to initially evaluate and re-evaluate the m-BSFS by pediatric gastroenterologists and children of 3 to 18 years of age by Chumpitazi et al. and Lane et al. [1, 14] were obtained from the authors. These pictures will be referred to throughout as 'general stool pictures'. These pictures comprised focused, close-ups of entire stools in a toilet or potty, but there were very few pictures of stools in diapers. To investigate whether it is possible to use only one scale for toddlers and avoid problems with comparisons between stool scales, and investigate if this scale can also be used for non-toilet trained toddlers, seven extra pictures were included in the validation. These additional pictures showed focused, close-ups of entire stools in diapers, as previously used by Huysentruyt et al. for their BITSS, that are referred to as 'stools in diaper pictures' [9]. Both general stool pictures and stools in diaper pictures depicted the full range of stool consistencies from type 1 to type 5 on the m-BSFS.

For the validation, interrater reliability was used as a measure for agreement between raters and intra-rater reliability as a measure for agreement within one person between the first and second time of scoring the pictures. To assess inter- and intra-rater reliability of the general stool pictures, participants were asked to complete a questionnaire that was built in the online platform Castor EDC [15]. The questionnaires were sent to parents, grandparents and day childcare employees as representatives for the people who most often take care of toddlers. Participants were asked to fill out the questionnaire twice with at least one week between the first and second scoring. The order of the pictures was different for both questionnaires to avoid bias.

For the stools in diaper pictures, inter- and intra-rater reliability was assessed in participants for seven focused, close-up color images of bowels in diapers. Participants were asked to fill out the questionnaire twice with at least one week between the first and second scoring and again the order of the pictures was different for both questionnaires to avoid bias.

PAPER VERSUS ONLINE USE AND USER-FRIENDLINESS IN A REAL-LIFE SITUATION

For the scoring of the stools in diaper pictures, the participants were randomly assigned to the scoring the stools in diapers either in a paper version or an online version to investigate whether this would impact the $ICC_{consistency}$. Participants were also asked to fill out a one-week diary, in which they scored all bowel movements of the child. Thus, the parent, grandparent or day childcare employee scored the bowel movements of the child(ren) for which they were present. Moreover, three statements were added to assess the user-friendliness, clarity of the instructions and feasibility to use the m-BSFS on fresh stool samples in real-life situations. Each category for user-friendliness was scored on a 5-point Likert scale ranging from strongly agree to strongly disagree [16]. Lastly, in case participants scored the questionnaire as unclear, user-unfriendly or demanding, they were asked to elaborate on this as an open question.

STATISTICAL ANALYSIS

Statistical analyses were performed with R software, version 3.6.1 using the 'irr' package version 0.84.1 and the 'ICC.Sample.Size' package version 1.0 [17-19]. A two-way random effects single rater model intraclass correlation coefficient for consistency (ICC)consistency was used for inter-rater reliability with the 'icc' function, whereas Cohen's κ (function 'kappa2' was used for intra-rater reliability [11, 20]. As there were no comparable studies in terms of type of scale or type of people to score them to use for a priori power calculation, a posteriori analyses were conducted to check if the sample size used provided enough power to draw valid conclusions (function 'calculateIccPower' from the 'ICC.Sample.Size' package) [19]. In addition, subject to item ratios were calculated for which many rules of thumb exist that range from a subject to item ratio of at least 2:1 to 20:1 [27].

RESULTS

TRANSLATION AND CULTURAL ADAPTATION OF THE DUTCH M-BSFS

The original English m-BSFS and the final translated Dutch version of the m-BSFS as used in the validation study are presented in **Figure 1**.

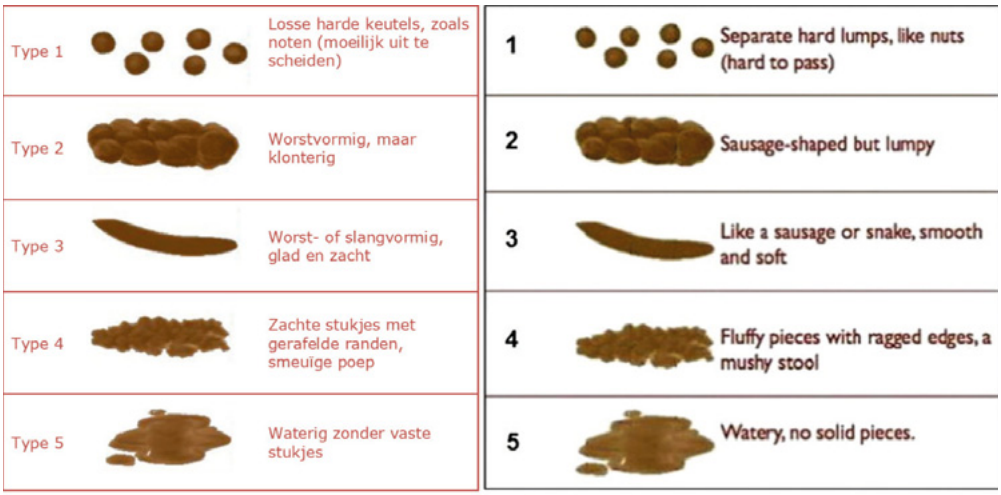


Figure 1 | Left: Dutch version of the modified Bristol Stool Form Scale (m-BSFS), right: the original English m-BSFS [1].

PARTICIPANTS

In total, 93 participants completed the questionnaire on the general stool pictures, of whom 69% were parents, 20% grandparents and 11% day childcare employees (**Figure 2**). A total of 16 participants, comprising 69% parents, 19% grandparents and 12% day childcare employees, completed the stools in diaper pictures and the user-friendliness questions in full. Three participants did not complete the questionnaire without giving reasons and one participant indicated that viewing and rating the pictures caused nausea. Participants reported to look after 1-2 year old (52%) and 2-3 year old toddlers (48%), 55% girls and 45% boys, of whom 15% were completely toilet-trained, 7% only during the day, 1% only for urine and 77% were non-toilet-trained.

INTER- AND INTRA-RATER RELIABILITY

Out of a total of 4505 ratings for both general stool pictures and stools in diaper pictures, 3505 (77.8%) were in agreement with the most commonly chosen rating, and 4272 (94.8%) were within one form type of the most commonly chosen rating for each stool picture. More specifically, for the general stool pictures, 3349 out of 4288 ratings (78.1%) were in agreement with the most commonly chosen rating, while this was the case for 156 out of 217 ratings (71.8%) for the stools in diaper pictures. For the general stool pictures, 4055 out of 4288 (94.6%) were within one rating from the most commonly chosen rating, compared to 217 out of 217 (100.0%) for the stools in diaper pictures.

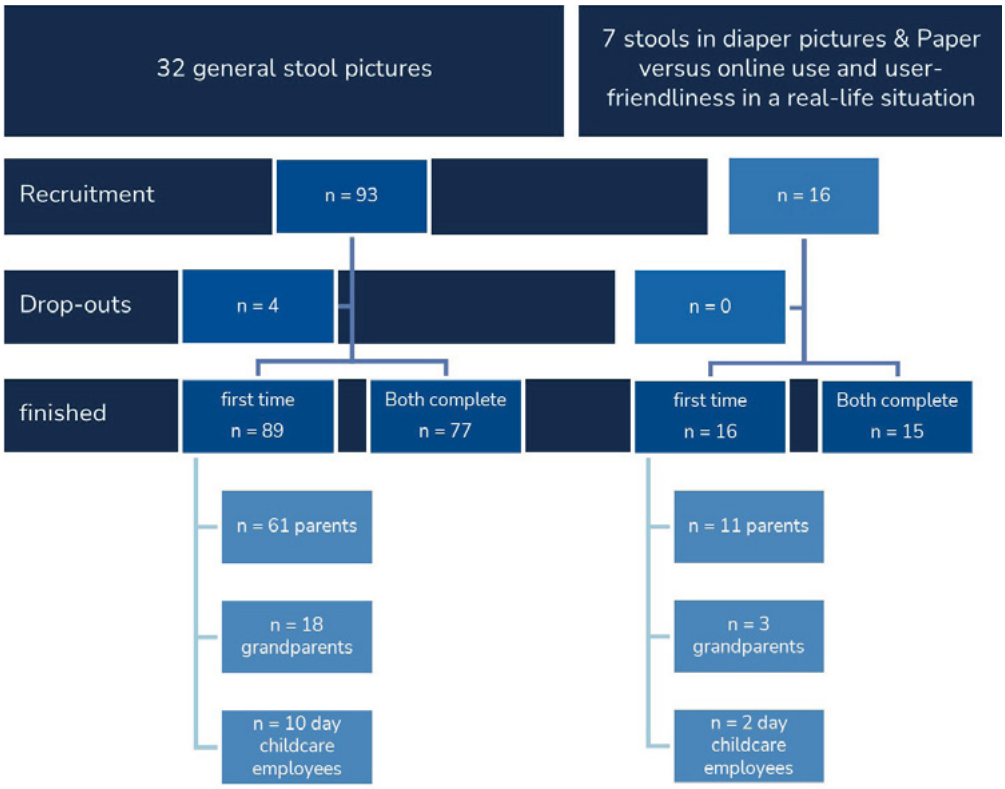


Figure 2 | Flow chart of participants in the validation study.

The proportions of exact agreement of each individual picture are presented in **Figure 3A** and **Figure 3B** for the general stool and stools in diaper pictures, respectively. Of the 32 general stool pictures, three pictures were most commonly scored as type 1, eight as type 2, eight as type 3, eight as type 4 and five as type 5 (**Figure 3**). For the seven stools in diaper pictures, one was most commonly scored as type 1, two as type 2, one as type 3, one as type 4 and two as type 5 (**Figure 3B**). Concerning the percentage of ratings that were in concordance with each other, the three weakest performing general stool pictures corresponded to m-BSFS type 4 (43%), type 5 (46%) and type 3 (47%). For the stools in diaper pictures the three weakest performing pictures were m-BSFS type 4 (48%), type 3 (52%) and type 1 (52%).

The a posteriori power calculation shows that, for a power of 0.8 and an α of 0.05, sample sizes for both general stool pictures and the stools in a diaper pictures numbers were above the sample size as used in this study (**Table 1**). To further support this, the subject to item ratio was calculated. For the general stool pictures this subject to item ratio was 2.8 for the first time and 2.4 for the second time and for the diaper specific pictures this was 2.3 for the first time and 2.1 for the second time.

Figure 3A

	picture 1	picture 2	picture 3	picture 4	picture 5	picture 6	picture 7	picture 8
m-BSFS type 1	99%	98%	91%	1%	1%	8%	3%	1%
m-BSFS type 2	1%	2%	9%	95%	89%	89%	86%	70%
m-BSFS type 3	0%	0%	0%	3%	6%	0%	4%	10%
m-BSFS type 4	0%	0%	0%	1%	4%	3%	7%	18%
m-BSFS type 5	0%	0%	0%	0%	0%	0%	1%	1%

	picture 9	picture 10	picture 11	picture 12	picture 13	picture 14	picture 15	picture 16
m-BSFS type 1	1%	0%	10%	0%	1%	0%	0%	0%
m-BSFS type 2	60%	57%	47%	0%	1%	2%	4%	9%
m-BSFS type 3	7%	42%	13%	99%	96%	95%	95%	78%
m-BSFS type 4	31%	1%	30%	1%	1%	3%	1%	13%
m-BSFS type 5	1%	0%	0%	0%	1%	0%	1%	0%

	picture 17	picture 18	picture 19	picture 20	picture 21	picture 22	picture 23	picture 24
m-BSFS type 1	1%	2%	1%	1%	1%	1%	1%	1%
m-BSFS type 2	39%	44%	17%	2%	4%	2%	4%	1%
m-BSFS type 3	60%	50%	47%	3%	2%	1%	2%	0%
m-BSFS type 4	0%	4%	32%	93%	90%	87%	87%	80%
m-BSFS type 5	0%	0%	3%	1%	1%	10%	6%	19%

	picture 25	picture 26	picture 27	picture 28	picture 29	picture 30	picture 31	picture 32
m-BSFS type 1	4%	7%	1%	10%	1%	1%	1%	1%
m-BSFS type 2	11%	6%	3%	2%	0%	1%	0%	0%
m-BSFS type 3	1%	0%	35%	0%	1%	0%	0%	0%
m-BSFS type 4	75%	60%	43%	42%	33%	21%	1%	1%
m-BSFS type 5	9%	27%	19%	46%	66%	77%	98%	99%

Figure 3B

	picture 1	picture 2	picture 3	picture 4	picture 5	picture 6	picture 7
m-BSFS type 1	52%	0%	48%	0%	0%	0%	0%
m-BSFS type 2	48%	100%	52%	10%	0%	0%	0%
m-BSFS type 3	0%	0%	0%	74%	3%	0%	0%
m-BSFS type 4	0%	0%	0%	16%	48%	0%	23%
m-BSFS type 5	0%	0%	0%	0%	48%	100%	77%

Figure 3 | Pictures are ordered to agreement on type. Participants received the pictures in random order. Colors of this figure are a gradient ranging from dark blue as highest values, light blue as lowest values.
A | Proportions of exact agreement for each of the 32 general stool picture.
B | Proportions of exact agreement for each of the seven stools in diaper pictures.
m-BSFS: modified Bristol Stool Form Scale

In conclusion, all inter- and intra-rater reliability scores were above the most commonly used thresholds of >0.61 or >0.7 (**Table 1**). Inter- and intra-rater reliabilities were found to be higher for the stools in diaper pictures, compared to the general stool pictures.

PAPER VERSUS ONLINE USE AND USER-FRIENDLINESS IN REAL-LIFE SITUATIONS

A comparison of the paper (n=10) versus the online (n=6) $ICC_{consistency}$ for the stools in diaper pictures revealed strong $ICC_{consistency}$ of 0.94 for both the online version (0.94) and paper version (0.93). To assess significance of the difference between these two ICCs a two-sided Fischer r-to-z transformation was used, resulting in a p-value of 0.90, indicating no statistical significance between the ICC of the paper and the online version.

In terms of user-friendliness by means of the 5-point Likert scale, all 16 participants, experienced the m-BSFS as user friendly based on the responses for clarity of instructions, which were 'neutral' for 10%, while 60% answered 'agree' and 30% 'strongly agree'. In response to the following translated statements, 'I think it is demanding to use the m-BSFS. For example, it takes effort to remember to use the m-BSFS or it was demanding to compare fresh stools to the m-BSFS', 20% of the participants answered with 'strongly disagree', 20% with 'disagree' and 60% with 'neutral'. In conclusion, both the paper and online version of the m-BSFS were largely considered user-friendly by the study participants.

DISCUSSION

The objective of this study was to assess whether the m-BSFS is reliable, valid and user-friendly to use by parents, grandparents and day childcare employees to evaluate stool consistency in both toilet and non-toilet trained toddlers in the Netherlands. Overall, the m-BSFS was successfully translated and culturally adapted to Dutch and showed to have a high degree of inter-rater reliability, intra-rater reliability and user-friendliness, regardless of whether this was on paper or online. This scale is the first that can be used for rating stools both from a diaper as well as from a potty or toilet.

We showed that both inter- and intra-rater reliability were above thresholds of >0.61 or >0.7, as recommended by published guidelines [11, 21-23]. These findings are consistent with previous studies that aimed to validate the m-BSFS by either pediatric gastroenterologists or children that found inter- and intra-rater reliabilities ranging from 0.72 (from 8-10 years of age and up) to 0.86 and 0.79 (from 8-10 years of age and up) to 0.87, respectively [1, 14]. Comparing our results to pediatric gastroenterologists' ratings on the same general stool pictures (in percentages) showed agreement with most commonly chosen ratings within one form type. ICCs were lower, 0.716 and 0.793 in our study compared to 0.85, but still well above recommended thresholds. Moreover, only four out of the 32 general stool pictures were scored differently by our participants compared to pediatric gastroenterologists, in which our participants scored three out of four pictures as a softer stool consistency and one out of four as a harder stool consistency [1]. These differences could be explained by the difference in training and familiarity with stool patterns of gastroenterologists compared to our study participants. When comparing our results to those of children aged 3-18 years, our results concerning the ICC were comparable to those

obtained with children aged 8-10 years and up, who only used ten out of 32 pictures for their final evaluation [14].

To the best of our knowledge, this is the first stool scale that has been validated for scoring stool consistency in stools in a potty/toilet and also for stools in a diaper. The added value of this validation is that data of potty-trained children can be directly compared to non-potty-trained children and can also be used in a period when children are being potty trained. Evaluation of the stools in diaper pictures resulted in a high ICC. Related to these findings concerning our seven stools in diaper pictures and the findings by Lane et al. who also showed a high ICC in children aged 8-10 and up, with their ten selected pictures, we noticed that the approach for calculating the ICC can lead to misleading results when comparing results for small numbers of items (pictures in this case) to those obtained with higher numbers of items [14]. More specifically, the lower the number of items to be rated, the higher the ICC without having actual higher agreement [24]. For example, when computing a random set of seven items, n=7, an ICC of 0.760 was calculated while four times the exact same seven items, n=28, gave an ICC of 0.738, without an actual difference in agreement percentages. Therefore, we recommend critically considering the type of ICC being used, how this is reported and checking if the number of items that were rated are comparable in order to directly compare ICCs to each other [20, 24]. Therefore, in order to compare ICC's one-on-one, it is recommended to use the same number of items. For this study this does not only hold for comparing the data by Lane et al. to our data, but also for comparing the ICCs of the general stool pictures to the stools in diaper pictures. However, as the ICCs for our stools in diaper pictures are very high, ranging from 0.925 to 0.934, we are confident that, even with the possible bias described above, our questionnaire is valid and well above the thresholds of 0.61 or 0.7 as the computations did not show a bigger difference than 0.06 [11, 21-23]. Altogether, we can conclude that we not only confirmed previous findings in a different target group, but with the extra stools in diaper pictures, we furthermore confirmed that the m-BSFS can be used for non-toilet trained toddlers. To the best of our knowledge, this is the first stool scale that is validated for both stools in a toilet/potty and for stools in a diaper. The advantages are that results of older and younger children can be more easily compared, and research on stool consistency in toddlers, especially during potty training, can be done with only one stool scale instead of switching between validated scales. Moreover, despite the widespread use of stool scales in the research and management of GI diseases and functional GI disorders, this is one of the few stool scales that has been validated in a target age group.

Moreover, it has been suggested that stool form, as measured for example by the m-BSFS, is a proxy for colonic transit rate [7, 25]. However, in order to confirm the validity of this statement, and use stool form as proxy for colonic transit rate, it becomes even more important to not only validate all different stool scales in the respective target group but also validate if this statement remains valid for other stool scales and different target groups.

In addition, most stool scales have previously been completed on paper while currently there is a shift towards more online-based methods [26]. This shift towards online-based methods can have multiple reasons, of which the most obvious probably is the all-round presence of mobile

devices. This comes with several challenges such as data protection, validation of online tools and questionnaires, difficulties with reaching certain respondent groups such as elderly and residents of remote areas and survey fraud. Online-questionnaires also come with many advantages, including automation in data input handling, quick inclusion and response of participants, real-time data collection and anonymity that may lead to more honest responses as there is no social consequence to participation. Moreover, other important advantages include data validation and collection of all raw data in one database without losing or incorrectly entering data from paper files [26]. To our best knowledge this is the first study to compare a paper and online stool scale and we show that both questionnaires give comparable results in terms of $ICC_{consistency}$. In short, when the m-BSFS is used in practice, caregivers can use the paper or online version, or even mix and match during research or management of GI diseases or functional GI disorders to the method that suits best at that moment. Furthermore, in general participants indicated that the m-BSFS is user friendly, with clear user instructions and not very demanding to use.

Strengths of this study includes that parents, grandparents and day childcare employees were divided as proposed on forehand (3:1:1, respectively). Moreover, division of the toddlers in terms of gender and age ranges was close to 50:50, indicating a good representation of the population in which this stool scale might be used. Moreover, we did an a posteriori sample size calculation to check whether the number of participants was sufficient. By using our own data, i.e. the actual ICCs found in this study, the power calculation is even more reliable as it is not an estimate based on comparable studies or study populations. We used the function 'calculateIccPower' from the ICC.Sample.Size package in R, which calculates a post-hoc power from an ICC. This function demonstrates the additional power gained by increasing the number of subjects or the number of subjects needed to increase power. In addition, it determines the number of participants needed for a specific power. Consequently, we can conclude, based on the results from the 'calculateIccPower' function, that the sample size boundary for a power of 0.8 would be 6 participants, as shown in **Table 1**. Our conclusion is therefore valid based on the a posteriori power calculation as well as confidence intervals of which even the lowest boundary is well above the most commonly used thresholds of 0.61 or 0.7. In addition, the subject to item ratios were between 2.8 and 2.1 in this study, which is according to Anthoine *et al*. in line with the most commonly found number (92% of all studies are in the range of 2:1 to 20:1) in scale validation studies [27].

Considering limitations of this study, the 32 pictures generously provided by Chumpitazi et al. are at least 10 years old and a few of which are relatively low in resolution and quality. The extra stools in diaper photographs helped to address this. This potentially leads to a lower $ICC_{consistency}$ or Cohen's κ, not because of a true difficulty to discriminate the type of stool but because of the resolution and quality of the images. Possibly in line with this, we observed a higher $ICC_{consistency}$ and Cohen's κ for the seven stools in diaper samples. The latter pictures were more comparable to each other in terms of size, lighting and photographic composition but were also of higher resolution and quality, which might be a factor influencing $ICC_{consistency}$ and Cohen's κ. Demographic data and socioeconomic status of the participants in our study are lacking and

it is therefore unknown if this sample is representative of the general population. However, we do not expect that differences in demographics will significantly change the results of this study.

In conclusion the modified m-BSFS, as paper or online version, is reliable, valid and user-friendly to use for Dutch-speaking parents, grandparents and day childcare employees to evaluate defecation parameters in toddlers whether in diapers or toilet trained. This validated m-BSFS is likely to prove useful in both clinical and research settings as a validated measure to record stool form from both diapers and toilet or potty. The m-BSFS can be used in clinical practice and clinical trials as tool for diagnosis, management and evaluation of bowel patterns in healthy toddlers as well as in several disorders such as functional constipation (FC), functional diarrhea (FD) or irritable bowel syndrome (IBS).

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What are normal defecation patterns in healthy 0-4 years old children? A systematic review and meta-analysis

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ABSTRACT

Objective: To summarize available data on defecation frequency and stool consistency of healthy young children in order to estimate normal references values for children 0-4 years old.

Data sources: We searched the Cochrane Library, EMBASE, and MEDLINE.

Study selection: Cross-sectional, observational, and intervention studies which reported on defecation frequency and/or stool consistency in healthy children aged 0-4 years, published in English.

Data extraction: PRISMA guidelines for extracting data were followed. Primary outcomes were defecation frequency and stool consistency.

Results: Seventy-five studies were included with a total of 16,393 children and 40,033 measurements of defecation frequency and/or stool consistency. Infants 0 to 14 weeks old had a mean defecation frequency of 21.8 per week (RI 3.9 to 35.2) compared to 10.9 (RI 5.7-16.7) in 15 weeks to 4 year old children. Within the group of infants, human milk-fed (HMF) children had the highest mean defecation frequency of 23.2 per week (RI 8.8-38.1), followed by mixed-fed (MF) children (20.7 per week [RI 7.0-30.2]) and formula-fed (FF) children (13.7 per week [RI 5.4-23.9]). Very few healthy infants were reported to have the hardest stool consistency (1.5%) compared to around one in ten young children (10.5%). Vice versa, many infants were reported to have the softest stools (27.0%) compared to 6.2% of young children. Based on visual inspection of the data, an increase in stool consistency with age was observed. Moreover, HMF children have softer stools than FF children. No differences were found between countries or between world regions for defecation frequency and/or stool consistency.

Conclusions: This study provides an estimation of normal reference values for defecation patterns in infants and young children. Infants of 0-14 weeks old have a higher defecation frequency compared to young children of 15 weeks – 4 years old with HMF infants having the highest defecation frequency. Stool consistency becomes harder with age and soft stools are more common in HMF and MF infants compared to FF infants. Hard stools in infants of 0-14 weeks old should be considered abnormal and requires additional attention, since very few infants were reported to have a hard stool consistency.

INTRODUCTION

Defecation disorders are common among children worldwide [1]. In order to adequately diagnose, treat, and monitor children with defecation disorders, it is crucial to obtain normal reference values regarding defecation patterns in healthy children. According to the pediatric Rome IV criteria for functional constipation (FC), a defecation frequency of less than three times per week is abnormal, as is a history of painful or hard stools, and the occurrence of large-diameter stools [2,3]. The Rome IV criterion of infrequent defecation defined as a defecation frequency of less than three times per week is based on adult studies, which consider a defecation frequency between three times per week and three times per day as normal [4,-6]. However, it is unclear if this range also applies to young children. Toddlers and young children may exhibit a different defecation pattern compared to adults due to the ongoing development of their digestive system and gut microbiota, and differences in nutritional intake [7.8]. In addition, infant feeding practices and the acquisition of toilet training skills may affect defecation patterns. Since normal reference values for defecation patterns in young children are lacking, there is a risk of either underdiagnosis or overdiagnosis of disorders related to defecation such as FC and functional diarrhea resulting in delaying appropriate management [9-12].

Therefore, the objective of this systematic review and meta-analysis was to summarize all available data on normal defecation frequency and stool consistency in healthy children aged 0-4 years old in order to provide normal reference values.

METHODS

This systematic review was registered at the international prospective register of systematic reviews PROSPERO with registration number CRD4021220453 and is reported in accordance with the PRISMA statement [13].

SEARCH STRATEGY AND STUDY SELECTION

The Cochrane Library, EMBASE, and MEDLINE were searched from inception to the 4th of January 2022, with the help of a clinical librarian. Search terms included, but were not limited to, 'defecation patterns', 'defecation frequency', 'stool consistency', 'children', 'infants', 'toddlers', 'preschoolers', and synonyms. To identify additional studies, reference lists of included studies and (systematic) reviews were searched manually. The full search strategy can be found in the supplementary material (**Supplementary Table 1**). We included cross-sectional, observational, and intervention studies in healthy children that were published in English. Studies were eligible for inclusion if they met the following criteria: (1) the study population consisted of healthy children 0-4 years of age; (2) data on defecation frequency and/or stool consistency were reported. Studies were excluded if they included children (1) who were reported to use

any medication; (2) with a known (intercurrent or chronic) illness or disease which may affect their defecation pattern; and (3) with a reported gestational age <37 weeks. Title and abstracts were screened independently by three authors (C.A.M.W., D.F.B., and T.J.M.L.) with the use of Rayyan, a web application for systematic reviews.[14] Full-text review and data extraction were executed independently by two authors (C.A.M.W. and D.F.B.). Any disagreements were resolved upon mutual agreement.

DATA EXTRACTION

Extracted data included general study details (author, year, country), study design, primary aim, population information (inclusion and exclusion criteria, sample size, age, sex distribution, type of feeding), methods used to assess defecation frequency and stool consistency, and results. If data were reported for children in a certain age range, the reported mean or median age of that group, or the median age of that range was used. For instance, a defecation frequency of infants 0-2 months old was considered to occur at an age of 1 month. If data were reported in both a wide range as well as smaller ranges, we included data of the smallest age ranges. For instance, if a defecation frequency of children 0-12 months old was given, but also the specified frequency of the children 0-2 months old, 3-4 months old etc., we included the latter ones. If multiple data-points were available, all were extracted. This resulted in children being in the dataset more than once, therefore we reported the number of children included in each study and the total number of measurements of those children. For intervention studies, we collected baseline data of intervention groups and all prospective data of control groups. If the intervention concerned a commercially available formula, all prospective data of the intervention group were also taken into account. If it was unclear if the baseline data of intervention groups were collected before onset of the interventions, the data were not used. If it was unclear how many participants completed each follow-up, the lowest number was used. If data were only reported graphically, we used WebPlotDigitizer, a web-based plot digitizing tool to extract data from figures with an accuracy of 1 decimal point [15, 16].

DEFECATION FREQUENCY

Defecation frequency was collected as number of bowel movements per week. If studies reported defecation frequencies per day, these data were transformed to obtain a defecation frequency per week using equations 1, 2, and 3 (**Supplementary File 1**) [17, 18].

STOOL CONSISTENCY

Stool consistency was collected as a percentage of children with a specified stool consistency on the stool consistency scale used in a given study. For toilet-trained children the modified Bristol Stool Form Scale (m-BSFS) has shown to be a valid and reliable tool to measure stool consistency [19]. The m-BSFS is also validated to assess stools in diapers, as are the Amsterdam infant stool scale (AISS), and Brussels infant and toddler stool scale (BITSS) [20-22]. In this

study we also included studies with non-validated stool scales. We included data from studies if data were reported as the percentage or number of subjects with their most common stool consistency. Data were not included in the analysis if data were provided as percentage of a stool consistency in all measured stools. These data were excluded because children with a higher defecation frequency would have more influence on the reported average stool consistency than children with a lower defecation frequency. In order to limit heterogeneity, we planned to only pool results of studies using a validated stool consistency scale. In case only few studies used validated stool consistency scales, we pooled the data of the softest stool consistency and data of the hardest stool consistency together. We composed a visual overview of the data in accordance with the description of the scale used in the original studies. Missing or incomplete stool consistency data are shown as 'not reported' in order to prevent relative bias. In order to identify changes in stool consistency with age, all stool consistency data were summarized in a graph sorted by age. In addition, an exploratory analysis was performed with continuous stool consistency data. Continuous data were transformed to a 0-1 range by min-max normalization, where 0 indicates the hardest consistency and 1 the softest consistency.

DEFINITION OF SUBGROUPS

In order to identify differences in defecation frequencies in 0-4 years old children with different ages, all defecation frequency data were visualized in a scatterplot combining defecation frequencies per week and age (**Supplementary Figure 1**). Instead of using traditional age categories, we decided to divide our population in subgroups based on our collected data. After visual inspection of defecation frequency data, we divided the population, both for defecation frequency and for stool consistency, in two subgroups: infants (0-14 weeks old), and young children (15 weeks-4 years old).

QUALITY ASSESSMENT

Per outcome, the quality of the study was assessed individually by two authors (C.A.M.W. and D.F.B). The method of Agarwal *et al.* designed for assessing risk of bias in cross-sectional surveys was adjusted to include only the relevant criteria for our study [23]. Factors taken into consideration included the inclusion and exclusion criteria used for subject selection, the validity and reliability of the outcome assessment, and the accuracy of data reporting. The quality of the study outcome was assessed as good, fair, or poor. A detailed description of the quality assessment is available in **Supplementary File 2**. A third author (I.J.N.K.) was consulted in case of disagreement.

DATA SYNTHESIS

If continuous data were reported as medians with interquartile ranges (IQRs), the assumption was made that the median was equal to the mean. Moreover, the standard deviation (SD) was

calculated from the IQR according to equation 1 (**Supplementary File 1**). For both subgroups (infants and young children) we determined normal reference values for defecation frequency.

To determine the effect of feeding type in infants of 0 to 14 weeks old, we compared data of infants who were human milk-fed (HMF), formula-fed (FF), and mixed-fed (MF). This was not done for the young children (aged 15 weeks to 4 years) due to the introduction of solid foods in this age category. In addition, subgroup analyses were performed based on the geographical location of the study, to investigate if differences exist between countries and/or world regions. For this purpose, six world regions were defined in accordance with the World Health Organization (WHO) (Africa, the Americas, Europe, Eastern Mediterranean, Southeast Asia, and Western Pacific) [24]. If case studies were executed in more than one country, this study was taken into account for the world region only (if they were within one world region), but not as separate countries. Hence, to assess differences between countries, studies were only used if they were executed within one country.

STATISTICAL ANALYSES

RStudio version 1.4.1106 and Microsoft Excel version 1908 (Microsoft corporation, Redmond, Washington, United States of America) were used to perform statistical tests and to visually represent data [25]. The distribution of continuous variables was assessed by visual inspection of the Q-Q plot. For normally distributed data, a (weighted) Students t-test and ANOVA were used. For non-normally distributed data, Mann-Whitney U test and the Kruskal-Wallis test were used. The post-hoc Tukey HSD test for pairwise comparisons was used to specify statically significant differences between groups in case a significant difference was detected during multiple comparisons. Reference intervals (RI) for defecation frequency were estimated by the R package 'referenceIntervals', either parametric or non-parametric. Outliers were determined using Horn's method in a Box-Cox transformed dataset using Tukey's IQRs; a data point was regarded an outlier when it lied outside 1.5*IQR from the 1st or 3rd quartile point. A p-value of <0.05 was considered statistically significant.

EXPLORATIVE ANALYSIS OF THE EFFECT OF CHANGES IN INFANT FORMULA OVER TIME ON DEFECATION PATTERNS

An explorative analysis was performed to obtain insight into the potential effect of differences in formula milk composition on defecation frequency in infants (0-14 weeks old) over time. We hypothesized that over time, the defecation frequency in FF infants would more closely resemble the defecation frequency of HMF infants. We took into account the introduction of compounds known to influence defecation frequency or stool consistency such as prebiotics (such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS)), other dietary fibers, long-chain poly-unsaturated fatty acids (LC-PUFAs), and β-palmitate [26,27]. Moreover, stool consistency of FF infants was visualized in a bar graph over time.

RESULTS

A total of 3,756 studies were identified, of which 75 were eligible for inclusion. **Figure 1** depicts the PRISMA flow chart including reasons for exclusion. Altogether these 75 studies included 16,393 children 0-4 years old, with 1 to 25 data points resulting in 40,033 measurements on defecation frequency and/or stool consistency. Taking into account the 47 studies which reported on sex distribution, there was an even distribution with 50.0% (5,176/10,358) of the children being female. Studies were conducted in well-child clinics, hospitals, day care centers, or at home (**Table 1,**

https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/). Studies were conducted in 43 different countries spread across all six world regions as defined by WHO [28].

Study designs of included studies included cross-sectional studies, prospective cohort studies, and clinical trials in healthy children (**Table 1,** https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/). Nineteen studies primarily aimed to assess defecation patterns of healthy children [11, 12, 29-45]. Thirty-eight studies primarily aimed to investigate safety, effects and/or tolerability of infant/young-child formulas or supplements [46-83]. Six case-control studies were included with various aims [84-89]. Three studies primarily aimed to evaluate differences in feeding regimens and/or diets [90-92]. Two studies primarily aimed to validate stool scales,[93, 94] and five studies had other primary aims [95-99].

QUALITY ASSESSMENT

A summary of the overall quality assessment per outcome measure is shown in **Figure 2**. More details on the quality judgement per study can be found in **Supplementary Table 2**. The majority of studies had clear inclusion and exclusion criteria to select a healthy population. Most studies evaluated defecation frequency or stool consistency as reported by parents, either with a diary or by recall. In three studies, stool samples were collected or examined by investigators [44, 63, 92]. Concerning defecation frequency, 17 studies were rated as good quality [32-34, 37, 46, 49, 51, 52, 55, 57, 62, 66, 69, 80, 84, 85, 98]. Concerning defecation consistency, none of the studies

were rated as good quality. Only a few studies used validated stool consistency scales without adapting them [36, 68, 72, 73, 78, 94, 100, 101]. However, those studies did not collected data via a 2-day dairy, or reported data only in graphs, or as a continuous outcome.

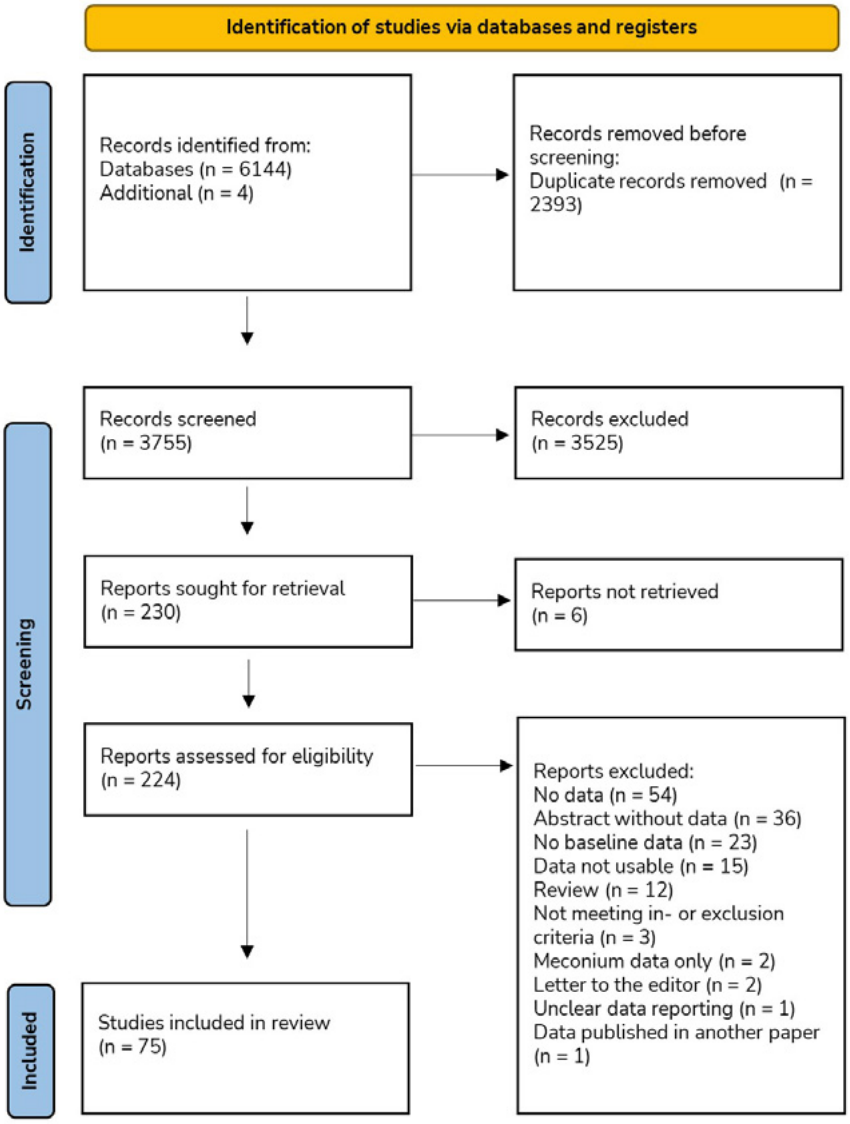


Figure 1 | PRISMA flow diagram of the selected studies [124].

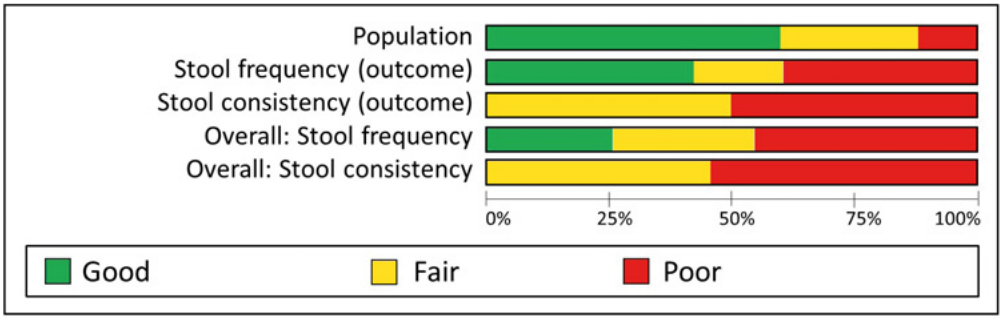


Figure 2 | Quality assessment summary of all included studies.

NORMAL REFERENCE VALUES FOR DEFECATION PATTERNS IN INFANTS AND YOUNG CHILDREN

Defecation frequency

When pooling data of 51 studies with 9,875 infants (0-14 weeks old) including 21,668 measurements, we found a RI for defecation frequency per week ranging from 3.9 to 35.2, with a weighted mean of 21.8 compared to a significantly lower ($p<0.001$) defecation frequency in 5,747 young children (14 weeks to 4 years old), including 7,455 measurements of a weighted mean of 10.9 with a range from 5.7 to 16.7 (**Table 2**).

The majority of studies (n=45 [88%]) measured defecation frequency with the use of a diary. The range in mean defecation frequency was wide. In infants, this ranged from a minimum mean of 7.0 to a maximum mean of 44.9 stools per week with two outliers: 4.9, and 62.6 stools per week [31, 76]. In young children, the mean defecation frequency ranged from a minimum mean of 6.2 to a maximum mean of 17.5 per week, with no outliers.

Stool consistency

We were able to include data from 19 studies in infants, combining data of 4,142 infants (0-14 weeks old) including 7,296 measurements on stool consistency [11, 30, 32, 34, 42, 45, 47, 57, 58, 61, 73, 76, 78, 79, 83, 87, 89, 93, 101]. Stool consistency was measured via a validated stool consistency scale in nine studies [46, 52, 53, 67, 68, 72, 73, 78, 82] For young children, we were able to include data from 20 studies, combining data of 2,919 children, including 7,773 measurements [11, 29, 30, 32, 37, 41, 42, 45, 47, 58, 59, 61, 73, 78, 83, 89, 93, 94, 101, 102]. Based on visual inspection, categorical stool consistency data of infants and young children show an increase in hard stools with age and a decrease of soft stools with age (**Figure 3A**). This is in line with the pooled percentages of hardest and softest stools per age group (**Table 2**), where 1.5% of infants reported the hardest stools and 27.0% of infants reported the softest stools. In young children, 10.5% reported to have the hardest stools, compared to 6.2% having the softest stools.

Some of the included studies provided stool consistency data on a continuous scale ranging from hard to soft. These data were transformed to a normalized stool consistency scale with 0

Table 2 | Weighted mean stool defecation frequency and percentages of children with hardest and softest stool consistencies

	Defecation frequency				Stool consistency			
	Number of children	**Number of measurements**	**Weekly defecation frequency (range)**	**Daily defecation frequency (range)**	**Number of children**	**Number of measurements**	**Children with hardest stools (%)**	**Children with softest stools (%)**
All infants 0-14 weeks old	9875	21668	21.8 (3.9-35.2) [a]	3.1 (0.6-5.0) [a]	4142	7296	1.5%	27.0%
human milk-fed children	4109	7327	23.2 (8.8-38.1)	3.3 (1.3-5.4)	1094	2979	0.3%	47.7%
Formula-fed children	3477	6801	13.7 (5.4-23.9) [b]	2.0 (0.8-3.4) [b]	1172	3739	1.8%	10.4%
Mixed-fed children	690	972	20.7 (7.0-30.2) [c]	3.0 (1.0- 4.3) [c]	78	189	1.2%	53.4%
Young children 14 weeks to 4 years old	5747	8257	10.9 (6.7- 16.7) [c]	1.6 (0.8- 2.4) [c]	2919	7773	10.5%	6.2%

[a] this group also includes all children of which no information was given regarding feeding type; $p<0.001$ compared to young children
[b] $p<0.001$ compared to human milk-fed infants
[c] data not normally distributed, non-parametric method used to determine range

representing the hardest stools and 1 representing the softest stools (equation 4, **Supplementary File 1**) . An explorative analysis including data of 4,399 infants, including 6,699 measurements, and 237 young children, including 735 measurements, revealed a weighted mean stool consistency of 0.609 in infants and 0.541 in young children on the transformed scale of 0 to 1 (**Supplementary Figure 2**).

ASSOCIATION BETWEEN SEX AND DEFECATION PATTERNS

The majority of studies did not provide data for boys and girls separately. Four studies, including 1,636 children between 0 and 60 months of age, compared defecation frequency or stool consistency between boys and girls. No significant differences in defecation frequency or defecation consistency were found [12, 29, 39, 97].

ASSOCIATION BETWEEN INFANT FEEDING APPROACH/TYPE AND DEFECATION PATTERNS

Differences between children receiving different types of feeding were only investigated in the infant group (0 – 14 weeks), due to the introduction of solid foods in the young children group (14 weeks – 4 years). From 9,875 infants and their 21,668 measurements, 65% of infants could be grouped into HMF, FF or MF children. In total 4,109 infants, with 7,327 measurements, in the HMF group were compared to 3,477 infants, with 6,801 measurements, in the FF group and 690 infants, with 972 measurements, in the MF group. Weighted mean weekly defecation frequency was highest in the HMF group (23.2; RI 8.8 to 38.1) followed by the MF group (20.7; RI 7.0 to 30.2) and the FF group (13.7; RI 5.4 to 23.9) and the In the HMF group one outlier of 4.9 was removed [76]. HMF infants had a significant higher defecation frequency compared to FF infants ($p<0.001$). There were no significant differences in mean defecation frequency between MF and FF or HMF infants (**Table 2**). Since age was found to influence defecation frequency, we evaluated if there was a difference in mean age between children in different feeding groups. The HMF group had a weighted mean age of 6.3 weeks, the FF group had a weighted mean age of 7.6 weeks, and the MF group a weighted mean age of 5.8 weeks. There were no significant differences in age between groups.

Concerning stool consistency, data of 4,142 infants, including 7,296 measurements, were included. Of those infants, 41% were HMF (2,979 measurements), 51% were FF (3,739 measurements), and 3% were MF (189 measurements). For 5% of the infants, no information was available on feeding type. For categorical data of stool consistency we found that hardest stools were infrequently reported in all feeding groups (0.3-1.8%), see **Table 2**. Softest stools were reported most in the small group of MF (53.4%) and HMF (47.7%) groups and less frequent in the FF group (10.4%). This is also visualized in **Figure 3B.**

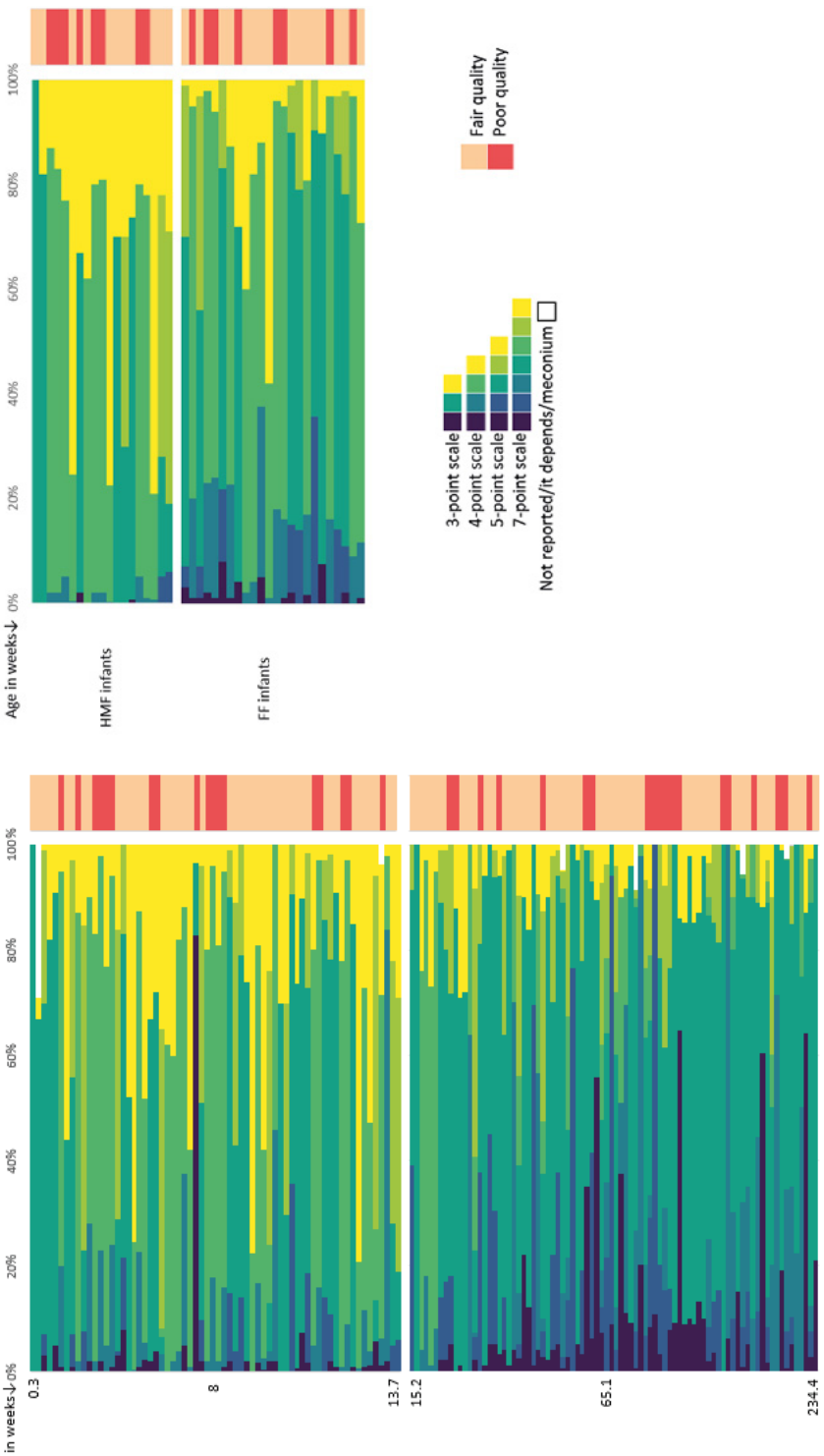


Figure 3 | Vertical bar charts of stool consistency by age **A |** Stool consistency for infants and young children. **B |** Stool consistency human milk fed infants (HMF, top) compared to formula fed infants (FF, bottom). Darkest blue colour represents the hardest stool consistency while yellow represents the softest stool consistency. Additionally, the quality of the outcome has been indicated per row in the bar on the right side of the bar graph.

ASSOCIATION BETWEEN GEOGRAPHICAL LOCATION AND DEFECATION PATTERN

Data on geographical location, for studies performed in a single country, were available for 6,561infants, including 17,457 measurements, and data were available for 4,748 young children, including 5,872 measurements (see **Supplemental Table 3**). For the world regions, data of one study performed in multiple countries within one world region were taken into account as well.

Defecation frequency in both infants and young children was found to vary slightly between regions and countries without any significant differences (all p-values >0.05). Weighted means for infants and young children per country can be found in **Supplemental Table 3**.

Stool consistency data per country is visualized in **Figure 4A&B**. Concerning young children, one study from India stands out, as they report almost exclusively children with hard (1 on a 5-point scale; 9.8%) or semi-hard (2 on a 5-point scale; 87.2%) stools and no children with the softest stools (4 and 5).[42] This study did not use a validated stool consistency scale. The authors noted that parents received a verbal explanation about different stool consistencies with the help of colored photographs of different types of stools.

EXPLORATIVE ANALYSIS OF THE EFFECT OF CHANGES IN INFANT FORMULA OVER TIME ON DEFECATION PATTERNS

For the explorative analysis, 24 studies were included which reported defecation frequency in FF children (n=7,888 infants 0-14 weeks old). A graphical representation of defecation frequency over time can be found in **Supplementary Figure 3**. The year of data collection or, if not available, the year of publication were used to plot the defecation frequency. Overall, we observed an increase of the range and a slight increase of mean defecation frequencies between and across studies in the years after the introduction of prebiotics such as GOS, FOS and inulin, dietary fiber and β-palmitate. Until 2000, the weighted mean defecation frequency was 14.15 per week, compared to a weighted mean defecation frequency of 14.79 per week after the introduction of the above mentioned substances from 2008. No obvious pattern was observed in defecation consistency over time, see **Supplementary Figure 4**.

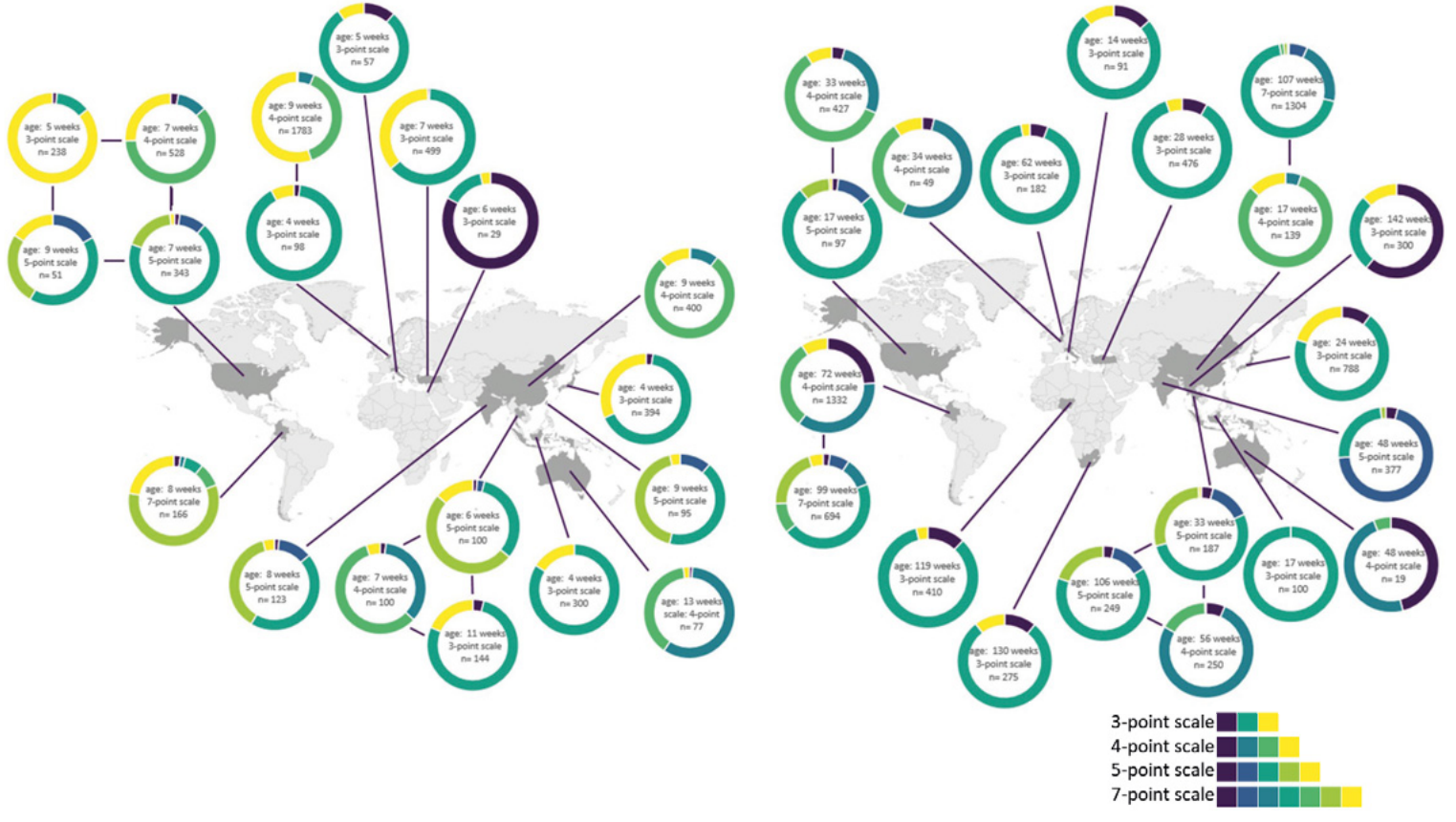


Figure 4 | Donut charts for stool consistency per country; the outer circle representing the stool consistency as measured for the scale used per study with information on age of the children, stool scale and sample size indicated within the donut chart. **A |** is the stool consistency worldwide for infants (0-14 weeks old) **B |** is the stool consistency worldwide for young children (15 weeks – 4 years old).

DISCUSSION

This systematic review provides normal reference values for defecation patterns in healthy children aged 0-4 years old. The mean weekly defecation frequency in infants (0-14 weeks old) is 21.8 (RI 3.9 to 35.2), whereas in young children (15 weeks – 4 years old) the reported mean weekly defecation frequency was 10.9 (RI 6.7 to 16.7). With respect to stool consistency, we found an increase in stool consistency with age. Only few healthy infants were reported to have the hardest stool consistency (1.5%) compared to one in ten young children (10.5%).

In this study we found a decrease in defecation frequency and an increase in stool consistency with age. These changes in defecation frequency and consistency are likely related to differences in frequency of feeding, feeding content, gastric emptying, and possibly transit time.[103, 104] The frequency of feeding may have an effect on the frequency of the gastrocolic reflex. A more frequent feeding regime may lead to a more frequent gastrocolic reflex resulting in a higher defecation frequency.[104] Regarding feeding content, the introduction of solid foods around 16 weeks of age may explain the stabilization in defecation frequency observed at this age and therefore, the cut-off in our dataset at 14 weeks of age [31, 32, 42, 45]. As demonstrated in gastric emptying studies in healthy adults and children, solids are digested more slowly than liquids which may affect transit time and thereby defecation frequency [105, 106]. Moreover, faster gastric emptying was found in HMF infants compared to FF infants as measured by a ^{13}C-octanoic acid breath test [107]. Unfortunately, we were unable to find any studies on total transit time in infants 0-14 weeks old to support or contradict this hypothesis. Whether knowledge about this change in defecation frequency at the age of 14 weeks could be used to prevent children from developing FC is unclear, as studies evaluating if an early introduction of solid foods may increase the risk of infants to develop FC describe conflicting findings [108, 109].

We found that HMF children have a higher and more variable defecation frequency compared to FF children whereas stool consistency was similar in HMF and MF but softer compared to FF infants. The difference in defecation frequency may be explained by differences in feeding pattern (on-demand versus scheduled feeding), sucking effort, milk content and its microbiota composition and its effect on gastric emptying [110-112]. Indeed, potential shifts in feeding from HMF to FF are known to influence defecation characteristics [12, 113]. Over the years, infant formulas have undergone changes in order to better resemble HM. These changes range from macronutrient composition changes to the addition of specific additives such as prebiotics and HM oligosaccharides [114, 115]. In an explorative analysis, we found that since the introduction of these additives, the mean defecation frequency of FF infants slightly increased from 14,15 to 14,79 times per week, suggesting that the composition of the milk likely attributes to the defecation pattern of infants.

We found no association between sex or geographical location and defecation patterns. We expect to find differences in defecation patterns between countries based on differences in their diets, e.g. their intake of dietary fiber and processed foods [116-118]. However, our current analysis was limited because we did not have any data on individual dietary intake to match

the data on defecation patterns per country. In addition, dietary habits in countries may change over time and may be different in rural or urban areas. Therefore, it would be of interest if future studies studying defecation patterns take into account individual dietary intake by means of food diaries or food frequency questionnaires.

With the data collected in this SR, it is uncertain whether toilet-training affects defecation frequency or stool consistency since most of our data included children who were younger than the mean age of toilet-training (around 2.5 years of age) [99, 119] In addition, none of the included studies evaluated a potential association between toilet-training and defecation patterns. Such data would, however, be of interest since a previous study has reported an association between toilet training before 24 months and constipation [120]. However, the latter finding may more likely be secondary to the early initiation of toilet training by parents, as opposed to the effect of the toilet training itself. When a child may not be interested in toilet-training yet, pushing him or her to do so may lead to withholding behavior, increasing the risk to develop FC [121].

Strengths of our study include the extensive collection of data of not only studies designed to investigate defecation patterns of healthy young children, but also data from studies with other primary aims. This resulted in a large amount of data from all regions of the world. In addition, we performed an extensive quality assessment including both the definitions of healthy children used in the studies, as well as how studies measured and reported defecation frequency and stool consistency.

Our study has several limitations. Firstly, we only had access to the original datasets of one out of 75 studies [93]. Hence, the RIs described in the current systematic review are based on the means of the reported studies. Therefore, the ranges reported in this review should be interpreted with caution and cannot be considered a normal range for 95% of the representative population. We want to encourage authors of future studies to share anonymized original datasets to enable pooling of data in the future. This may also aid in investigating whether statistical outliers were caused by a total group with very low or very high defecation frequencies, or whether this was caused by errors in reporting of a defecation frequency per week instead of per day or vice versa. Moreover, since the original datasets were not available except for one and since not all studies used the same effect size measures, we had to make assumptions as described in **Supplementary File 1**. Any assumption can introduce bias, even if they would be statistically reasonable. Therefore results should be interpreted with caution. Secondly, the quality of the majority of the studies included in our review was fair or poor, limiting the reliability and validity of the results reported here. Stool consistency was usually not measured via a validated stool scale, which resulted in high levels of heterogeneity among stool consistency data. Even studies using a validated stool scale might not have results which can be pooled, as one of our included studies reported significant differences between intra-individual outcomes of two validated stool scales [94]. To overcome this, we encourage authors of future studies to use validated stool scales, and to consider the use of photographic assessment, limiting the inter-rater variability [102, 103]. In addition, stool consistency was often reported as a continuous outcome which, in our opinion, is not preferable. To illustrate, a reported stool consistency of 2.5 is not interpretable, as visual stool

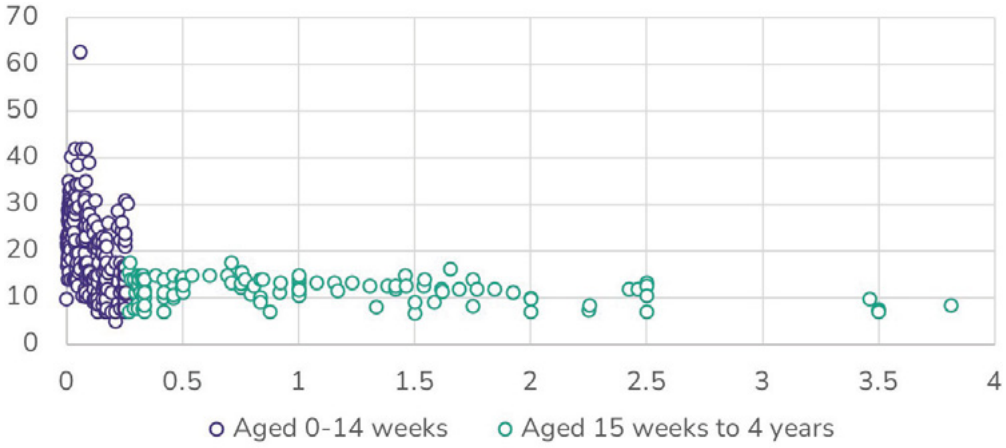
consistency scales are integer and there is no such thing as a consistency of 2.5. In addition, by reporting stool consistency as continuous data, the extreme values of hard or soft stools, which are clinically most relevant, are averaged out. For example, stool consistency data of three children with a hard (1), soft (4), and watery (7) consistency would result in the same average consistency (4) as three children with a soft (4) stool consistency. Moreover, we excluded stool consistency data reported as percentage of all stools. This choice was made because children with a higher defecation frequency would have more influence on the summarized data compared to children with a lower defecation frequency. Therefore, we encourage authors to report stool consistency as categorical data per individual to allow researchers to identify the differences between these integer outcomes. Thirdly, we included all data of each study, which resulted in some children providing only one measurement in our dataset, while others provided more measurements at different points in time. Although this introduced bias, we wanted to take into account the intra-individual variability over time and we wanted to avoid the bias which would be introduced when we averaged multiple data points occurring over months or years in time.

Defecation frequency and stool consistency are part of the current diagnostic Rome IV criteria for FC and functional diarrhea. Concerning functional diarrhea, our found results including an upper limit of 2.4 defecation per day for young children and 6.2% of young children having the softest stools, are in line with the criterion of daily passage of 4 or more unformed stools [2]. Concerning FC, our found results on lower limits on defecation frequencies (3.9 per week for infants and 6.7 per week for young children) are in line with the FC criterion for infrequent defecation of fewer than 3 defecations per week [2]. If anything, one could argue that for young children a defecation frequency of 4 or 5 times per week may already be considered abnormal. When looking at our data on hard stools, we found that hard stools were very uncommon in infants, but relatively common in young children. The occurrence of hard stools in around 1 in 10 children may represent children who may have undiagnosed FC. However, in terms of defining of a disorder, looking at what might be considered normal is only one element to take into consideration. When defining a disorder, the most important factor to take into account is the suffering which may be accompanied by the symptoms of the disorder [122]. To illustrate, a recently developed and validated pediatric bowel management scoring tool found no relation between defecation frequency and subjectively impaired quality of life in children with FC [123]. Unfortunately this study did not measure quality of life by a validated questionnaire. Still, defecation frequency, although considered one of two pillars of treatment success of children with FC and of course closely related to other defecation symptoms, may not be the most important factor to define a functional defecation disorder [124]. Future studies may evaluate specifically which defecatory symptoms may result in an impaired quality of life in children and therewith better establish and validate the current diagnostic criteria for functional diarrhea and FC.

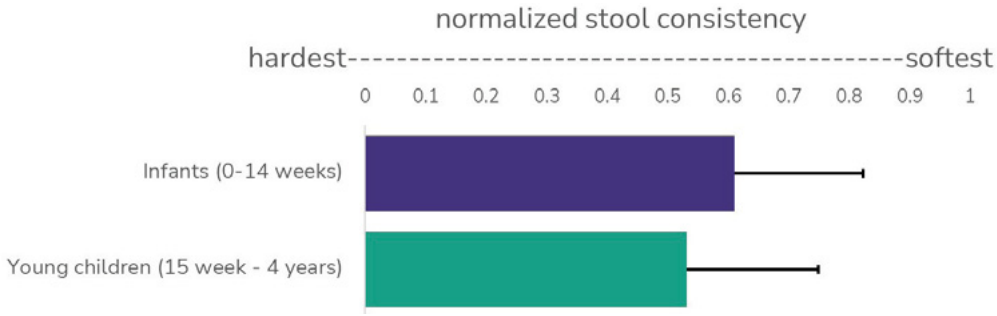
In conclusion, infants (0-14 weeks) have a higher defecation frequency compared to young children (15 weeks - 4 years), with HMF infants having the highest defecation frequency. Stool consistency becomes harder with age, and both HMF and MF infants have softer stools

compared to FF infants. Hard stools in infants should be considered abnormal and require additional attention, since very few infants were reported to have a hard stool consistency. These data may be used to serve as a guide for defining normal defecation behavior in infants and young children. In children approaching the lower or upper boundaries of the RI, attention should be paid to detect signs of developing defecation disorders such as FC.

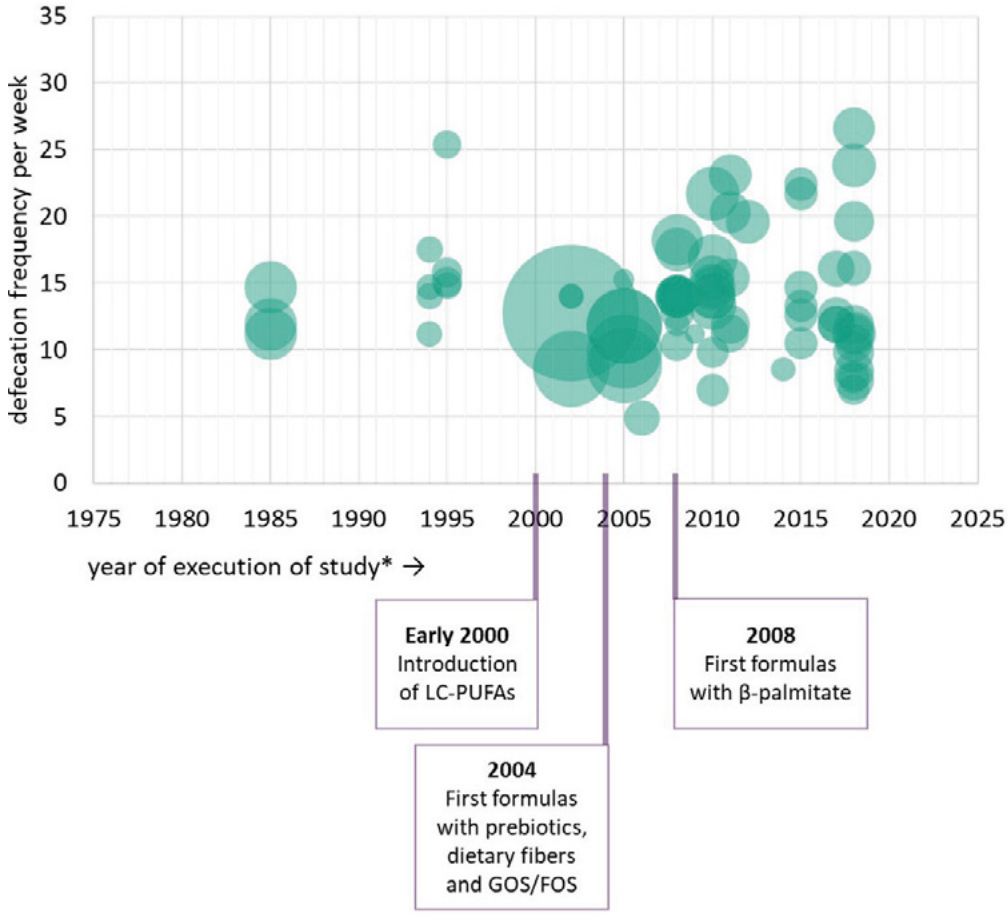
SUPPLEMENTARY MATERIAL



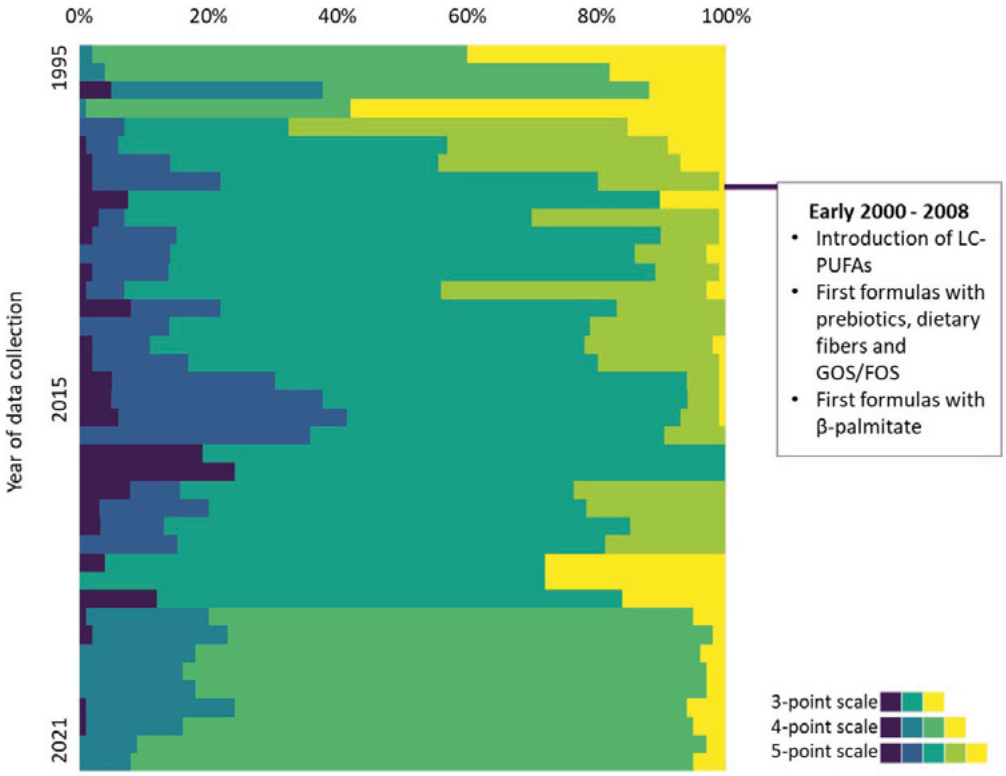
Supplementary Figure 1 | Scatter plot defecation frequency by age



Supplementary Figure 2 | Normalized stool consistency; adjusted values based on equation 4 of **Supplementary File 1** where 0 represents the hardest stools and 1 represents the softest stools.



Supplementary Figure 3 | Scatter plot formula over time in data-collection or year of publication. The size of the dot represents the sample size of that datapoint. *when the year of execution of the study was not provided, data were plotted on the year of publication. Abbreviations: LC-PUFAs: long chain poly-unsaturated fatty acids, GOS: galacto-oligosaccharides, FOS: fructo-oligosaccharides.



Supplementary Figure 4 | Bar chart of stool consistency in formula-fed infants over time.

Supplementary File 1 | Assumptions and equations used during data analysis

If a median was provided, it was assumed that the median was equal to the mean.

If an interquartile range (IQR) was provided, it was assumed that the width of the IQR was approximately 1.35 standard deviations (SD). The SD was then obtained via:

$$SD=IQR/1.35 \quad \text{(equation 1)}$$

If a mean defecation frequency per day was provided, the mean defecation frequency per week was obtained by:

$$Mean_{week}=Mean_{day}*7 \quad \text{(equation 2)}$$

If an SD of the mean defecation frequency per day was provided, the SD of the mean defecation frequency per week was obtained by:

$$SD_{week}=\sqrt{Var_{week}}=\sqrt{(Var_{day1}+Var_{day2}+...+Var_{day7})}=\sqrt{(SD_{day}^{2}*7)} \quad \text{(equation 3)}$$

Normalization of mean stool consistencies

$$Mean_{normalized}=Mean*\frac{1}{max} \quad \text{(equation 4)}$$

Supplementary File 2 | Quality assessment

1. **Is the method of subject selection described and appropriate?**
   Good: Clear and appropriate inclusion and exclusion criteria.
   Fair: Limited inclusin and exclusion criteria, or inappropriate inclusion (e.g. children with constipation and/or using laxatives).
   Poor: No inclusion or exclusion criteria described.

2. **Is the survey instrument reliable and valid?**

2.1 Defecation frequency:
   Good: diary of at least 2 days, reported as frequency per day/week with SD/IQR
   Fair: diary of one day or recall of max 3 days, reported as frequency per day/week
   Poor: recall longer than 3 days, no details on data collection, or data only in figures

2.2 Stool consistency:

   Good: diary of at least 2 days with the use of a validated stool scale (BSFS/mBSFS/AISS/BITSS), reported as most common stool in the number of participants or in the percentage of participants
   Fair: diary of one day or recall of max 3 days, description of the items of a non-validated stool scale, reported as categorical data or as mean/median consistency with SD/IQR
   Poor: recall longer than 3 days, no details on stool scale or data collection, or data only in figures

If a study used different methods simultaneously, the score was given according to the lowest scoring method (e.g. a study using a one day diary for baseline and three day diary for follow-up data would get a 'fair' score based on the one day diary). A final decision of the quality of a study was determined per outcome by combining the quality of the method of subject selection and the reliability and validity of the outcome assessment. The final quality score was determined by the lowest score on either one of the pillars. Since some studies only report on either defecation frequency or stool consistency, these were only included in the quality assessment of that respective outcome. For example, study A reports both on defecation frequency and stool consistency and will therefore receive two final quality outcomes. Study A receives the following scores: subject selection: fair, defecation frequency: good, stool consistency: poor. The final quality score would then be: study A defecation frequency: fair (combination of fair & good), study A stool consistency: poor (combination of fair & poor)

Supplementary Table 1 | Search strategies for a. MEDLINE b. EMBASE c. Cochrane Central Register of Controlled Trials.

a. Search strategy for MEDLINE

#	Searches	Results
1	*Defecation/	2712
2	((defecat* or defaecat* or bowel or stool) adj3 (pattern* or habit*)).ti,ab,kf.	3218
3	(stool adj3 (frequen* or consisten* or color or weight)).ti,ab,kw.	3884
4	1 or 2 or 3	9123
5	Pediatrics/ or exp child/ or exp infant/	2523160
6	(child* or pediatr* or paediatr* or peadiatr* or infan* or baby or babies or newborn* or toddler* or preschool* or pre-school* or minors or juvenile* or boy or boys or girl* or kid or kids or prematur* or preterm* or underag* or under ag*).ti,ab,kw.	2334667
7	5 or 6	3355109
8	4 and 7	1962
9	comment/ or editorial/ or letter/ or (letter or comment* or editorial).ti.	2000711
10	8 not 9	1933
11	(exp Animals/ or exp Animal Experimentation/ or exp models, animal/ or exp Veterinary Medicine/ or (animal* or monkey* or sheep or ovine or lamb or lambs or goat* or pig or pigs or swine or porcine or pup or pups or dog or dogs or canine or bitch* or beagle or feline or rodent* or rabbit* or rat or rats or mice or mouse or murine or cow or cows or horse or horses or ape or apes or gorilla or gorillas).ti,ab,kw.) not (humans/ or human*.ti,ab,kw.)	4814453
12	10 not 11	1909

b. Search strategy for EMBASE

#	Searches	Results
1	*defecation/ or *defecation habit/	2699
2	((defecat* or defaecat* or bowel or stool) adj3 (pattern* or habit*)).ti,ab,kw.	6116
3	(stool adj3 (frequen* or consisten* or color or weight)).ti,ab,kw.	6921
4	1 or 2 or 3	14646
5	pediatrics/ or child/ or preschool child/ or toddler/ or juvenile/ or boy/ or girl/ or exp infant/ or juvenile/ or exp childhood/	3094490
6	(child* or pediatr* or paediatr* or peadiatr* or infan* or baby or babies or newborn* or toddler* or preschool* or pre-school* or minors or juvenile* or boy or boys or girl* or kid or kids or prematur* or preterm* or underag* or under ag*).ti,ab,kw.	3188760
7	5 or 6	4168872

Supplementary Table 1 | Continued

#	Searches	Results
8	4 and 7	2698
9	letter/ or editorial/ or note/ or (letter or comment* or editorial).ti.	2627017
10	8 not 9	2671
11	(exp animal/ or exp animal experiment/ or exp animal model/ or exp veterinary medicine/ or (animal* or monkey* or sheep or ovine or lamb or lambs or goat* or pig or pigs or swine or porcine or pup or pups or dog or dogs or canine or bitch* or beagle or feline or rodent* or rabbit* or rat or rats or mice or mouse or murine or cow or cows or horse or horses or ape or apes or gorilla or gorillas).ti,ab,kw.) not (human/ or human*.ti,ab,kw.)	5906323
12	10 not 11	2592

c. Search strategy for Cochrane Central Register of Controlled Trials.

ID	Search	Hits
#1	MeSH descriptor: [Defecation] explode all trees	826
#2	((defecat* or defaecat* or bowel or stool) near/3 (pattern* or habit*)):ti,ab,kw	1031
#3	(stool near/3 (frequen* or consisten* or color or weight)):ti,ab,kw	2740
#4	#1 or #2 or #3	4052
#5	(child* or pediatr* or paediatr* or peadiatr* or infan* or baby or babies or newborn* or toddler* or preschool* or pre-school* or minors or juvenile* or boy or boys or girl* or kid or kids or prematur* or preterm* or underag* or under ag*):ti,ab,kw	286697
#6	#4 and #5 in Trials	1089

Supplementary Table 2 | Quality assessment per study. Descriptions of the criteria as used to evaluate studies as poor, fair or good can be found in **Supplementary File 2.**

Study	ref	Population	Outcome: Stool frequency	Outcome: Stool consistency	Overall Stool frequency	Overall: Stool consistency
Akinbami 1995	29	Fair	Poor	Poor	Poor	Poor
Alarcon 2002	90	Good	Fair	Poor	Fair	Poor
Aloisio 2018	46	Good	Good	Fair	Good	Fair
Ashley 2012	47	Good	Fair	Fair	Fair	Fair
Bekkali 2010	89	Fair	Good	Good	Fair	Fair
Belson 2003	87	Fair	Poor	Poor	Poor	Poor
Ben 2008	48	Poor	n.a.	Poor	n.a.	Poor
Benjasuwantep 2009	30	Fair	Good	Fair	Fair	Fair
Bhatia 1986	96	Fair	Good	n.a.	Fair	n.a.
Bhatnagar 2018	125	Good	Fair	Poor	Fair	Poor
Björmsjö 2020	49	Good	Good	n.a.	Good	n.a.
Bloom 1993	99	Good	Poor	n.a.	Poor	n.a.
Borgo 2009	43	Good	Poor	Poor	Poor	Poor
Bradley 1993	50	Poor	Good	Poor	Poor	Poor
Çamurdan 2014	32	Good	Good	Fair	Good	Fair
Chen 2002	51	Good	Good	Fair	Good	Fair
Chen 2011	84	Good	Good	n.a.	Good	n.a.
Closa-Monasterolo 2013	52	Good	Good	Fair	Good	Fair
Corazziari 2005	33	Good	Good	n.a.	Good	n.a.
Dalili 2016	85	Good	Good	n.a.	Good	n.a.
Den Hartog 2012	34	Good	Good	Fair	Good	Fair
Escribano 2018	53	Fair	Good	Fair	Fair	Fair
Estorninos 2021	54	Good	n.a.	Poor	n.a.	Poor
Fontana 1989	35	Poor	Good	n.a.	Poor	n.a.
Gianni 2018	55	Good	Good	n.a.	Good	n.a.
Gounaris 1998	86	Fair	Fair	n.a.	Fair	n.a.
Holscher 2012	56	Good	Fair	n.a.	Fair	n.a.
Hyams 1995	57	Good	Good	Fair	Good	Fair
Jinno 2020	83	Good	n.a.	Poor	n.a.	Poor
Johnston 2015	58	Good	Fair	Fair	Fair	Fair
Khunovich 2021	44	Poor	Fair	n.a.	Poor	n.a.

Supplementary Table 2 | Continued

Study	ref	Population	Outcome: Stool frequency	Outcome: Stool consistency	Overall Stool frequency	Overall: Stool consistency
Kondolot 2009	91	Fair	Poor	n.a.	Poor	n.a.
Koppen 2016	93	Fair	Poor	n.a.	Poor	n.a.
Kosuwon 2018	59	Good	n.a.	Fair	n.a.	Fair
Lemoh 1979	92	Fair	Good	n.a.	Fair	n.a.
Litmanovitz 2014	60	Fair	Good	n.a.	Fair	n.a.
Lloyd 1997	61	Fair	Good	Fair	Fair	Fair
Lungu 2021	100	Fair	Poor	n.a.	Poor	n.a.
Marriage 2015	62	Good	Good	n.a.	Good	n.a.
Moretti 2019	36	Fair	Poor	Poor	Poor	Poor
Moro 2002	63	Good	Poor	Poor	Poor	Poor
Myo-khin 1994	37	Poor	Good	Fair	Good	Poor
Nakamura 2009	64	Fair	Good	Fair	Fair	Fair
Neumer 2021	65	Good	Poor	Poor	Poor	Poor
Newell 1976	97	Fair	Poor	n.a.	Poor	n.a.
Nowacki 2014	66	Good	Good	Fair	Good	Fair
Nyhan 1952	38	Fair	Good	n.a.	Fair	n.a.
Osatakul 1995	45	Good	Poor	Poor	Poor	Fair
Oswari 2019	67	Good	n.a.	Fair	n.a.	Fair
Paese 1985	39	Fair	Poor	n.a.	Poor	n.a.
Parschat 2021	68	Good	Poor	n.a.	Poor	n.a.
Piemontese 2011	69	Good	Good	Poor	Good	Poor
Rodriguez-herrera 2019	70	Good	Poor	Poor	Poor	Poor
Savino 2010	95	Good	Fair	n.a.	Fair	n.a.
Shen 2021	71	Good	Poor	n.a.	Poor	n.a.
Shrago 2006	98	Good	Good	n.a.	Good	n.a.
Smilowitz 2017	72	Good	Poor	Poor	Poor	Poor
Tehuteru 2004	11	Fair	Fair	Fair	Fair	Fair
Ten Haaf 2021	101	Poor	n.a.	Fair	n.a.	Poor
Tham 1996	40	Good	Poor	Poor	Poor	Poor
Tunc 2008	12	Poor	Poor	n.a.	Poor	n.a.
Vandenplas 2017	74	Good	Poor	Poor	Poor	Poor
vandenPlas 2020	73	Good	Poor	Poor	Poor	Poor

Supplementary Table 2 | Continued

Study	ref	Population	Outcome: Stool frequency	Outcome: Stool consistency	Overall Stool frequency	Overall: Stool consistency
Veereman (2011)	75	Good	Poor	Poor	Poor	Poor
Velasco-benitez 2020	94	Poor	n.a.	Fair	n.a.	Fair
Vivatvakin 2010	76	Good	Fair	Fair	Fair	Fair
Walker 1985	41	Poor	Fair	Fair	Poor	Poor
Wang 2021	78	Good	n.a.	Poor	n.a.	Poor
Wang 2021/2	77	Good	Poor	Poor	Poor	Poor
Wernimont 2015	79	Good	Fair	Fair	Fair	Fair
Williams 1999	80	Good	Good	n.a.	Good	n.a.
Williams 2014	81	Good	n.a.	Poor	n.a.	Poor
Wu 2017	82	Good	Poor	Poor	Poor	Poor
Yadav 2014	42	Fair	Poor	Fair	Poor	Fair
Yonezawa 2014	88	Fair	Poor	n.a.	Poor	n.a.

Supplemental Table 3 | Weighted mean defecation frequency per week per country, grouped by region

Country	Defecation frequency per week (mean)	Number of children	Number of measurements
Infants (0-14 weeks old)			
United States of America	17.3	2023	8393
Belgium	21.6	152	152
France	21.0	40	159
Greece	14.0	15	15
Israel	20.2	136	136
Italy	19.3.	832	1017
Spain	18.2	286	932
The Netherlands	15.5	760	1881
Turkey	23.3	523	1219
United Kingdom	22.9	27	27
China	16.5	1140	1140
Japan	26.5	106	266
Taiwan	11.7	109	603
India	26.5	187	667
Indonesia	13.0	100	600
Thailand	20.4	100	150
Iran	18.7	25	100
Young children (15 weeks – 4 years old)			
Brazil	11.3	57	57
Colombia	12.9	173	519
United States of America	11.6	550	863
Italy	8.11	1050	1050
Spain	12.4	125	125
The Netherlands	11.9	29	80
Turkey	10.1	765	958

Supplemental Table 3 | Continued

Country	Defecation frequency per week (mean)	Number of children	Number of measurements
United Kingdom	12.2	28	28
China	9.8	139	143
India	11.1	657	737
Indonesia	8.4	100	100
Myanmar	6.9	261	261
Thailand	11.8	50	187
Malawi	13.0	79	79
Nigeria	13.2	410	410
South-Africa	12.2	275	275

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'Als alles in één richting stroomt, dan is het zaak om zoveel mogelijk opstoppingen in die stroom te verwijderen.'

BASTAARDSUIKER – ARJEN LUBACH

PART 3

Non-pharmacological and intestinal microbiota directed interventions in functional gastrointestinal disorders and health

Effectiveness of probiotics in children with functional abdominal pain disorders and functional constipation: a systematic review

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ABSTRACT

Objective: The objective of this study was to investigate the effect of probiotics on functional abdominal pain disorders (FAPD) and functional constipation (FC).

Methods: A systematic review was conducted, searching PubMed and Cochrane databases from inception to January 2018 for randomized controlled trials (RCTs) investigating the efficacy of probiotics in children aged 4 to 18 years with FAPD or children aged 0 to 18 years with FC.

Results: A total of 657 citations were identified. Finally, 11 RCTs for FAPD and 6 RCTs for FC were included. Some evidence exists for *Lactobacillus rhamnosus* GG (n=3) in reducing frequency and intensity of abdominal pain in children with irritable bowel syndrome. There is no evidence to recommend *L. reuteri* DSM 17938 (n=5), a mix of *Bifidobacterium infantis*, *Bifidobacterium breve* and *Bifidobacterium longum* (n=1), *Bifidobacterium lactis* (n=1) or VSL#3 (n=1) for children with FAPD. No evidence exists to support the use of *Lactobacillus casei rhamnosus* LCR35 (n=1), *B. lactis* DN173 010 (n=1), *B. longum* (n=1), *L. reuteri* DSM 17938 (n=1), a mix of *B. infantis*, *B. breve* and *B. longum* (n=1), or Protexin mix (n=1) for children with FC. In general, studies had an unclear or high risk of bias.

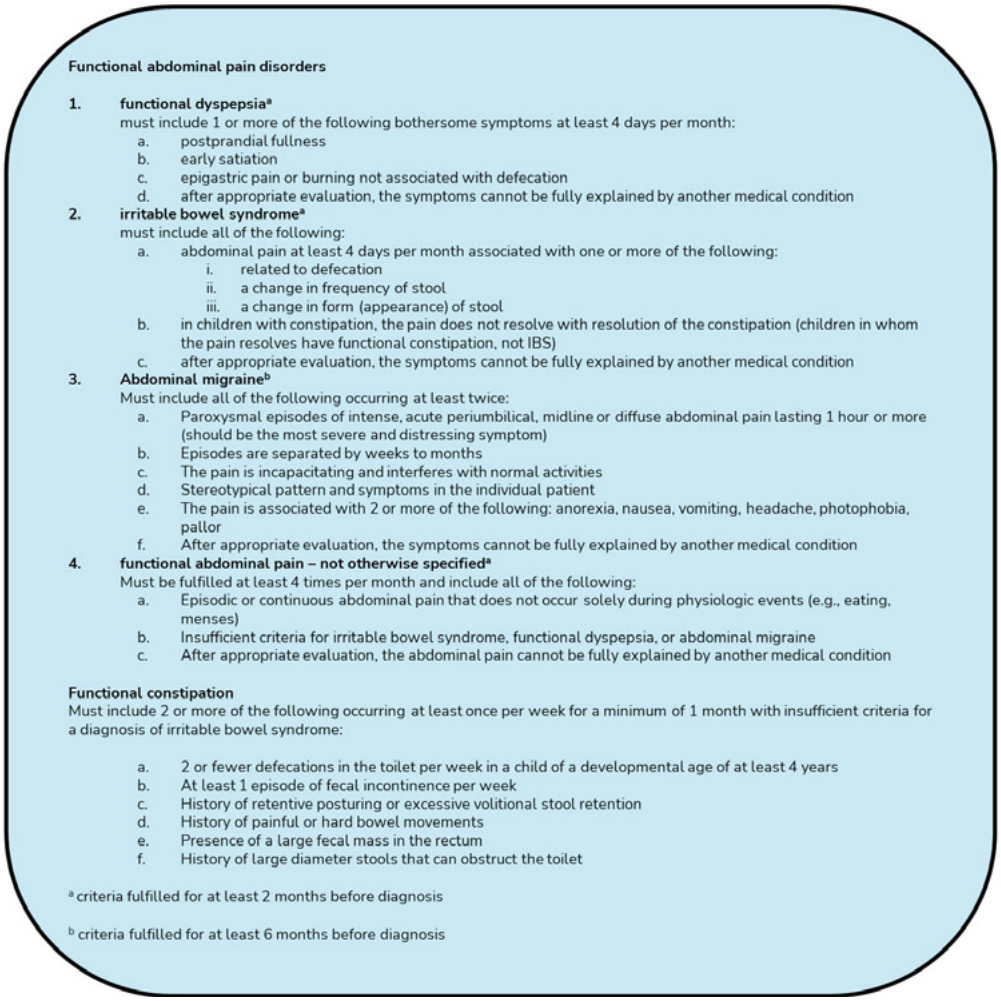
Conclusions: Insufficient evidence exists for the use of probiotics in FAPD and FC, only *L. rhamnosus* GG seems to reduce frequency and intensity of abdominal pain but only in children with irritable bowel syndrome. A better understanding of differences in intestinal microbiota in health and disease might lead to better probiotic strategies to treat disease.

Keywords: Probiotics; Intestinal Microbiota; Children; Functional Gastrointestinal Disorders.

INTRODUCTION

Childhood functional gastrointestinal disorders (FGIDs) are an umbrella term for multiple disorders. They include a variable combination of often age-dependent, chronic or recurrent symptoms, including colic, regurgitation, abdominal pain and defecation related disorders. Importantly these symptoms cannot be attributed to another medical condition after appropriate medical evaluation [1]. The recently published and revised Rome IV criteria describe different FGIDs including functional abdominal pain disorders (FAPD): (1) functional dyspepsia (FD), (2) irritable bowel syndrome (IBS), (3) abdominal migraine (AM) and (4) functional abdominal pain – not otherwise specified (FAP-NOS), and functional constipation (FC) (**Figure 1**) [2, 3]. FAPD are one of the most common clinical entities encountered in pediatric practice with a prevalence ranging from 0.2% to 23%, whereas the prevalence of FC varies from 0.7% to 29.6% [4-6]. The etiology underlying abdominal pain and constipation is not well understood but many risk factors are associated with the onset of both disorders [7, 8].

In the last decade, it has been suggested that the intestinal microbiota may play an essential role in the development of these functional disorders [9]. The human gut is colonized by a complex microbial community. This complex ecosystem, the intestinal microbiome, is an integral part of the gastrointestinal tract (GI-tract) and changes are associated with a wide variety of diseases and disorders [10]. Aberrations in the intestinal microbiota composition have not only been correlated to gastrointestinal complaints, but also to diseases like obesity, diabetes and autism [11]. Due to the use of culture-independent techniques to study the intestinal microbiota, our understanding of the role of the intestinal microbiota in health and disease has increased. Not only is the characterization of great interest, but also how we might influence our intestinal microbiota to correct for aberrations. One way to do so is by probiotics, this term was originally introduced as the opposite of antibiotics. Many definitions exist for probiotics, but all include that it should contain living microorganisms which, upon ingestion of adequate amounts, exert health benefits for the host [12]. The working mechanism of probiotics is based on the fact that they can interfere with pathogens, can improve barrier function, and have a role in immunomodulation and neurotransmitter production [12]. Important in the context of FAPD and FC, probiotics such as *Bifidobacterium* and *Lactobacillus* species produce Short-Chain Fatty Acids (SCFA's) that lower the intestinal pH, thereby enhancing peristalsis of the colon [13]. Moreover, the intestinal microbiota can also modulate intestinal pain by the same mechanisms of influencing neural, immune and endocrine activity of the host, and secretion of bacterial metabolites that can influence the neural pathway [14, 15]. Because of these properties probiotics have been suggested as potential treatment for children with FAPD and FC. Conventional treatment such as the use of antispasmodics or amitriptyline in FAPD and laxatives for FC turns out to be insufficient in a substantial number of children, and many parents look for alternatives [1, 16]. This review provides an update on current literature on the efficacy of probiotics in the treatment of FAPD and FC in children.



Functional abdominal pain disorders

1. **functional dyspepsia**[a]
   must include 1 or more of the following bothersome symptoms at least 4 days per month:
   a. postprandial fullness
   b. early satiation
   c. epigastric pain or burning not associated with defecation
   d. after appropriate evaluation, the symptoms cannot be fully explained by another medical condition
2. **irritable bowel syndrome**[a]
   must include all of the following:
   a. abdominal pain at least 4 days per month associated with one or more of the following:
      i. related to defecation
      ii. a change in frequency of stool
      iii. a change in form (appearance) of stool
   b. in children with constipation, the pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not IBS)
   c. after appropriate evaluation, the symptoms cannot be fully explained by another medical condition
3. **Abdominal migraine**[b]
   Must include all of the following occurring at least twice:
   a. Paroxysmal episodes of intense, acute periumbilical, midline or diffuse abdominal pain lasting 1 hour or more (should be the most severe and distressing symptom)
   b. Episodes are separated by weeks to months
   c. The pain is incapacitating and interferes with normal activities
   d. Stereotypical pattern and symptoms in the individual patient
   e. The pain is associated with 2 or more of the following: anorexia, nausea, vomiting, headache, photophobia, pallor
   f. After appropriate evaluation, the symptoms cannot be fully explained by another medical condition
4. **functional abdominal pain – not otherwise specified**[a]
   Must be fulfilled at least 4 times per month and include all of the following:
   a. Episodic or continuous abdominal pain that does not occur solely during physiologic events (e.g., eating, menses)
   b. Insufficient criteria for irritable bowel syndrome, functional dyspepsia, or abdominal migraine
   c. After appropriate evaluation, the abdominal pain cannot be fully explained by another medical condition

Functional constipation
Must include 2 or more of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome:

a. 2 or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years
b. At least 1 episode of fecal incontinence per week
c. History of retentive posturing or excessive volitional stool retention
d. History of painful or hard bowel movements
e. Presence of a large fecal mass in the rectum
f. History of large diameter stools that can obstruct the toilet

[a] criteria fulfilled for at least 2 months before diagnosis

[b] criteria fulfilled for at least 6 months before diagnosis

Figure 1 | Diagnostic criteria for functional abdominal pain disorders and functional constipation according to Hyams et al. [1]

MATERIALS AND METHODS

Cochrane Library and the PubMed database were searched from inception to January 2018. Search terms related to FAPD, FC and probiotics in children were used. The full search strategy is available from the authors. Studies were eligible for inclusion if they were: (1) (systematic reviews of) randomized controlled trials (RCTs) in which a probiotic was compared to placebo, no treatment or another treatment; (2) written in English; (3) children aged 4-18 years for functional abdominal pain by the Rome II, III or IV criteria; (4) children aged 0 to 18 years for functional constipation by the Rome II, III or IV criteria. Reasons for exclusion were 1) treatment arms with <10 patients.

Screening of the eligibility was done independently by two authors (C.A.M.W. and M.T.). In case of disagreement, consensus was reached through discussion. In addition, citation searching was done using key papers which met the inclusion criteria for the review. Risk of bias was assessed using the Cochrane Collaboration's tool for assessing risk of bias [17].

RESULTS

The search strategy identified 657 citations (n=421 FAPD and n=236 FC), of which 67 for FAPD and 32 for FC appeared to be relevant. Full texts were further evaluated. As depicted in **Figure 2**, 11 RCTs for FAPD and 6 RCTs for FC were eligible for inclusion. Of the included studies, three were crossover trials [19-21]. Data from 880 children with FAPD and 411 children with FC were included. For one study, children were recruited from a public school [21], two studies recruited children from tertiary hospitals [19, 20], one study included children from a primary care site [22] and all other were outpatient studies. Studies were conducted in Asia, Europe, North America and South America. We decided not to perform a meta-analysis due to heterogeneity of studies and the difference in types and dosages of probiotic strains.

FUNCTIONAL ABDOMINAL PAIN DISORDERS

Five systematic reviews (SRs) [23-27] and a total of 11 RCTs were identified. Studies used different probiotic strains, therefore they will be discussed per strain, a summary is given in **Table 1**

(https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/). Results of the risk of bias are given in **Figure 3** [17].

Lactobacillus reuteri DSM 17938

Maragkoudaki et al. compared *Lactobacillus reuteri* DSM 17938 to a placebo in 54 children, aged 5-16 years, with FAP [28]. In this study both this strain and placebo showed a significant reduction in pain frequency and intensity when compared to baseline at week 4 and week 8

(all p<0.001). However, no significant differences were found between groups during the treatment period in pain frequency (week 4: probiotic 2.9±4.5 vs placebo 3.1±4.1, p=0.68, week 8: probiotic 4.8±9.9 vs placebo 2.8±3.3, p=0.59) or pain intensity (week 4: probiotic 4.3±8.5 vs placebo 4.0±5.6, p=0.72, week 8: probiotic 7.2±17.7 vs placebo 2.5±3.4, p=0.42). Other outcomes such as the use of pain-relieving drugs and school or adult work absence did not differ significantly between groups. The authors reported that the probiotic, but not the placebo, showed a significant decrease in the average number of school absences and the average loss of workdays of parents (for both, probiotic p<0.025, placebo p>0.025 at both week 4 and week 8).

Jadresin et al. investigated the effect of *Lactobacillus reuteri* DSM 17938 versus a placebo in 55 children, aged 4-18 years, with FAP or IBS [29]. A significant difference was found between groups in the number of days without pain during the trial period (probiotics median 89.5, range 5-108, placebo median 51, range 0-107, p=0.029). Differences in severity of pain between the first and fourth month were not significantly different between groups (p=0.481), neither the difference in duration of pain between the first two months and the last two months (p=0.143). However, abdominal pain was less severe in the probiotic group in the second (p=0.049) and fourth month (p=0.007). Both groups showed a significant reduction in abdominal pain from baseline to month 4 (probiotic p<0.001, placebo p=0.004). No differences were found in stool consistency or absence from school or activities.

Weizman et al. compared the *Lactobacillus reuteri* DSM 17938 to a placebo in 101 children, aged 6-15 years, with FAP [30]. Significant differences were found between the probiotic and placebo after 4 weeks in pain frequency (probiotic 1.9±0.8 vs placebo 3.6±1.7, p<0.02) and pain intensity (probiotic 4.3±2.7 vs placebo 7.2±3.1, p<0.02). After follow-up, only a reduction in pain intensity remained significant between groups (p<0.02). No significant differences between the two groups were found with respect to the secondary outcomes including school absenteeism, GI-symptoms and adverse effects.

Eftekhari et al. also compared the *Lactobacillus reuteri* DSM 17938 to a placebo in 80 children, aged 4-16 years, with FAP [31]. No primary or secondary objectives were reported. No significant differences were found in pain episodes after 4 weeks intervention and after follow-up at 8 weeks (p-values between 0.16 and 0.44) or abdominal pain intensity between groups after 4 weeks intervention and after follow-up at 8 weeks (p-values between 0.16 and 0.44). The authors reported a significant difference between the groups at baseline and the first month after treatment for average pain episodes and abdominal pain intensity (both p=0.0001).

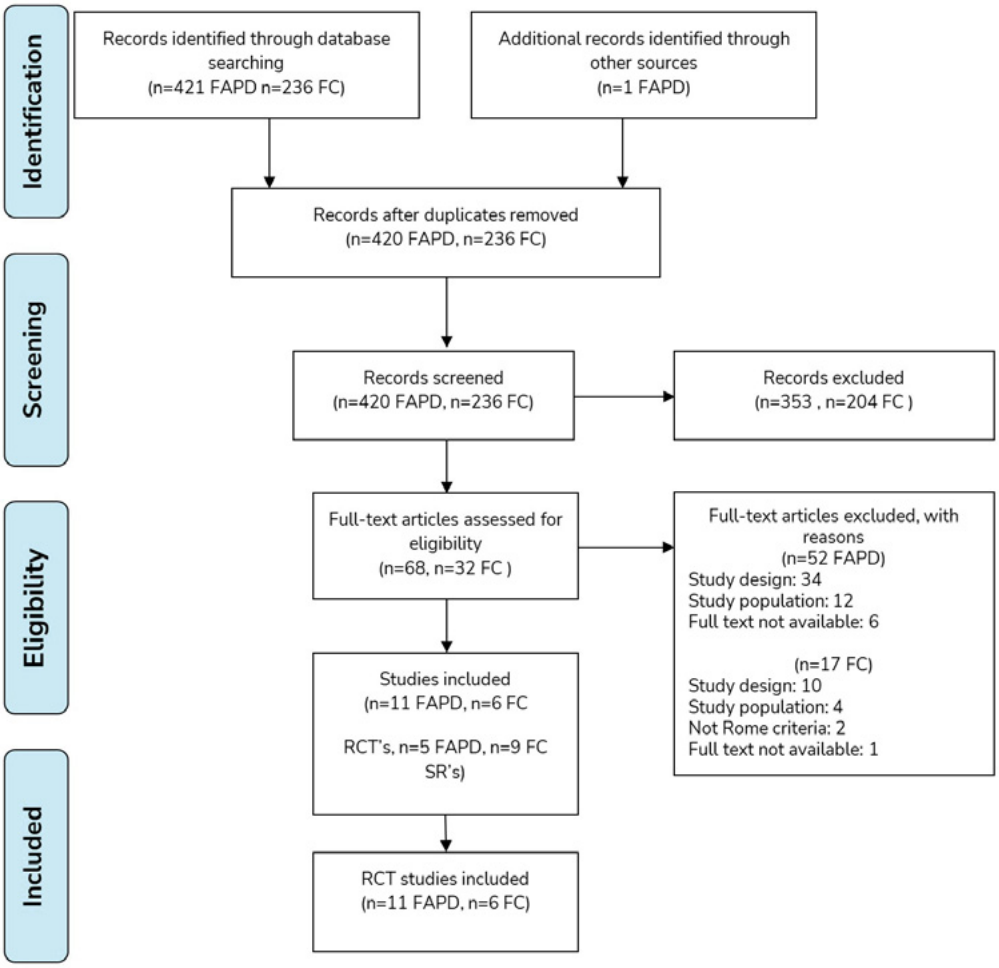


Figure 2 | PRISMA flow diagram of the in- and exclusion of studies [18]. FAPD indicates functional abdominal pain disorders; FC, functional constipation; RCT randomized controlled trial; SRs, systematic reviews.

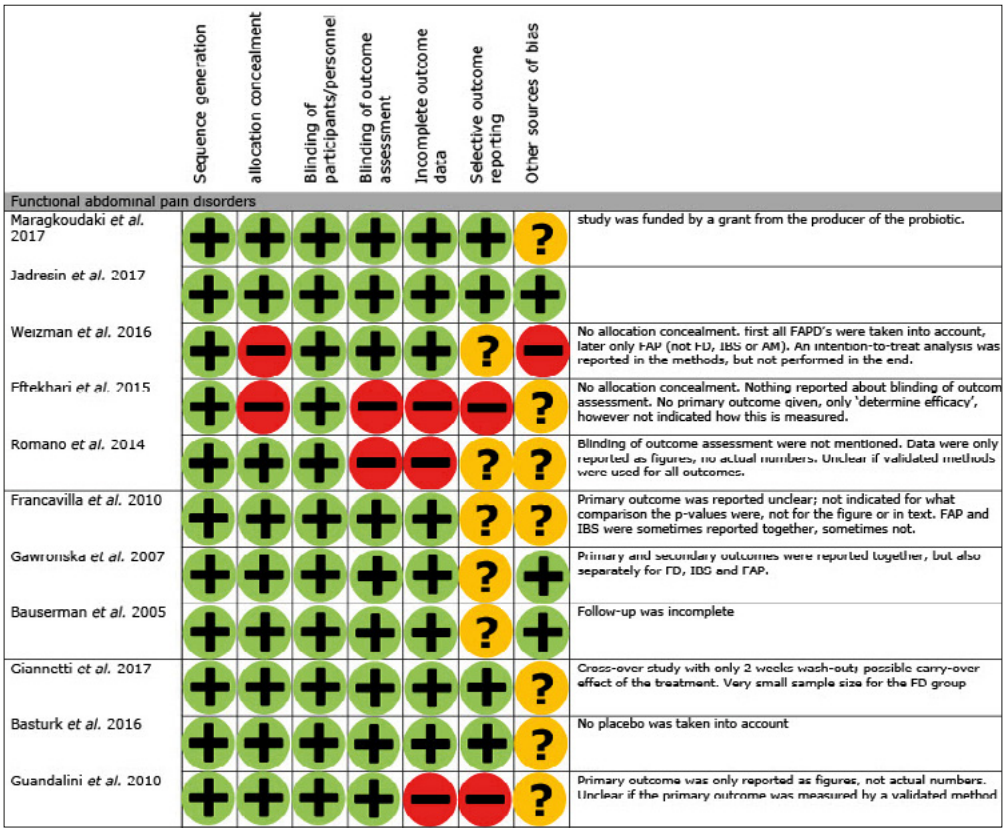


Figure 3 | Cochrane Risk of Bias Tool for the assessment of methodological quality and potential risks of bias for studies in children with FAPD [36].

Romano et al. investigated the effect of *Lactobacillus reuteri* DSM 17938 compared to a placebo in 60 children, aged 6-16 years, with FAP [32]. The authors reported a significant reduction in pain frequency, which decreased significantly with time for both groups ($p<0.05$ in both groups). However, data were only graphically shown and apart from p-values numeric data are missing to support these statements. The same holds for pain intensity which was significantly different for the probiotic group at week 4 and week 8 compared to the placebo group (probiotic $p=<0.001$, placebo $p>0.05$).

Lactobacillus rhamnosus GG (LGG)

Francavilla et al. investigated if the LGG could relief symptoms compared to a placebo in 141 children, aged 5-14 years, with IBS or FAP [22]. The authors reported a significant decrease in episodes of pain per week from baseline to week 12 in the probiotic, but not in the placebo group (probiotic from 3.7±2.5 to 1.1±0.8 vs placebo from 3.5±2.4 to 2.2±1.2, $p<0.01$) which remained after follow-up at 20 weeks (probiotic 0.9±0.5 vs placebo 1.5±1.0, $p<0.02$). Severity of

pain decreased significantly in the probiotic group but not in the placebo group (probiotic from 4.3±1.8 to 2.3±1.3 vs placebo 4.3±1.8 to 3.4±2.1, p<0.01) which also persisted after follow-up (probiotic 0.9±0.5 vs placebo 1.5±1.0, p<0.001). Treatment success, defined as a decrease of at least 50% in the number of episodes and intensity of pain, was significantly higher in the probiotic group (probiotic 72% vs placebo 53%, p<0.03). Parents rated global improvement higher in the probiotic group compared to the placebo group after intervention (probiotic 54% vs placebo 33%, p<0.02) and after follow-up (probiotic 70% vs placebo 55%, P<0.04). Intestinal permeability, measured by the lactulose-to-mannitol ratio, decreased significantly in the probiotic group from baseline to the end of intervention (from 0.036±0.01 to 0.026±0.005, p=0.002) but not in the placebo group (from 0.038±0.01 to 0.034±0.01, p=0.6). The authors indicated that the change in intestinal permeability was mainly observed in the group of children with IBS.

Gawronska et al. compared LGG to a placebo in 104 children, aged 6-16, with FD (n=20), IBS (n=37) or FAP (n=47) [33]. Results were reported for the whole group, but also in subgroups; FD, IBS and FAP. Overall, treatment success, defined as no pain at the end of the intervention, was not statistically significantly different between both groups (probiotic 25% vs placebo 9,6%, p=0.08). No differences were found between groups for frequency of pain (p>0.15), severity of pain (p>0.23), improvement of symptoms (p=0.76), use of medication (p>0.77), or school absenteeism (p>0.07). However, results per subgroup showed that only in IBS patients treatment success was statistically significantly higher compared to placebo (probiotic 33,3% vs placebo 5.3%, p=0.04).

Bausserman et al., investigated the effect of LGG versus placebo in 64 children, aged 6-17 years with IBS [34]. The change in abdominal pain severity from baseline to endpoint was not significantly different (probiotic change -1.7±0.6 vs placebo -1.3±0.3, p=0.175). The number of responders did not differ between groups (p=0.774), and improvement in GI symptoms did not differ between groups either, except for perceived abdominal distention at 6 weeks (probiotic 0% vs placebo 24%, p=0.022).

Other strains

In a cross-over study by Giannetti et al. the effect of a mix of *Bifidobacterium infantis* M-63, *Bifidobacterium breve* M-16V and *Bifidobacterium longum* BB536 versus placebo was investigated in 50 children, aged 8-17 years, with IBS and 28 children, aged 8-17 years, with FD [19]. In the per-protocol analysis for IBS, abdominal pain completely disappeared in a significantly higher proportion in the children receiving probiotics (probiotic 42% vs placebo 14,5%, p=0.006), but not in FD (probiotic 20% vs placebo 36%, p=0.3). Similar results were found for the intention-to-treat analysis (p=0.003 for IBS and p=0.5 for FD). For abdominal pain in IBS, improvement was found in the probiotic (p=0.02), not the placebo (p=0.1), but not for FD (probiotic p=0.06, placebo p=0.09). Quality of life improved for IBS (probiotic 42% vs placebo 17%, p=0.002), but not for FD (28% vs 24%, p=1). No significant differences were found for constipation (p>0.6), or other GI symptoms.

Basturk et al. investigated the effect of *Bifidobacterium lactis* B94 versus inulin, a prebiotic, versus a combination of both, a synbiotic, in 76 children, aged 4-16 years, with IBS [35]. Complete benefit,

defined as resolution of all present complaints, was observed with no difference between the synbiotic and probiotic group (synbiotic 39,1% vs probiotic 29,2%, prebiotic 12.5%, comparison between synbiotic and probiotic p=0.471). Improvement from baseline to week 4 in the probiotic group was found for bloating after meals (p=0.016), belching-abdominal fullness (p<0.001) and difficulty with defecation (p=0.031). The authors report that overall, the use of the synbiotic and probiotic, but not the prebiotic, resulted in improvements in initial complaints.

A cross-over study by Guandalini et al. investigated the effect of a probiotic mixture, VSL#3 versus placebo in 67 children, aged 4-18 years, with IBS [20]. Grouped data for global assessment of relief showed improvement over time in both probiotic and placebo groups. Changes from baseline were significant for the probiotic (week 2 p<0.05, week 4 p<0.01, week 6 p<0.001) but were only significant at week 4 (p<0.05) and week 6 (p<0.05) for the placebo. However, data were only graphically shown and apart from these p-values numeric data are missing. The authors reported a significant improvement in abdominal pain in both groups, but more significant in the probiotic group (probiotic p=<0.001, placebo p<0.05). Abdominal bloating decreased significantly in both groups as well, and a significant difference in favor of the probiotic was found between groups for week 4 and week 6 (p<005). No differences between groups were found for the number or characteristics of stools, as measured by using their own created 5-point scale (p=0.06) [20]. For the assessment of family life disruption, a significant difference in favor of the probiotic was found between groups, but only for week 6 (p<0.01).

Adverse effects or side effects

None of the studies reported adverse effects or side effects of the different strains.

FUNCTIONAL CONSTIPATION

A total of 9 SRs [25, 37-44] and 6 RCTs were identified regarding probiotic use in children with FC. All studies used different probiotic strains. However, a distinction could be made between studies that allowed laxatives during the study and those that only used probiotics

(**Table 2**, https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/). Results of the risk of bias are given in **Figure 4**.

Probiotic studies without the use of laxatives

Wojtyniak et al. evaluated the effect of *Lactobacillus casei rhamnosus* Lcr35 compared to a placebo in 94 children, aged <5 years, with FC [45]. Treatment success, defined as ≥ 3 spontaneous stools per week, without episodes of fecal soiling (in toilet-trained children), in the last week of the intervention (week 4), did not differ between groups (probiotic 70% vs placebo 58,8%, p=0.4). Stool frequency was significantly lower in the probiotic group compared to the placebo group throughout the study period (week 4 probiotic 4.0 [3.0, 5.0] vs placebo 6.0 [4.0, 9.0], p=0.005). Except for stool frequency, all other outcomes such as stool consistency, frequency of fecal soiling, frequency of pain during defecation, frequency of abdominal pain or flatulence and the need for intake of additional laxative treatment were not significantly different between groups.

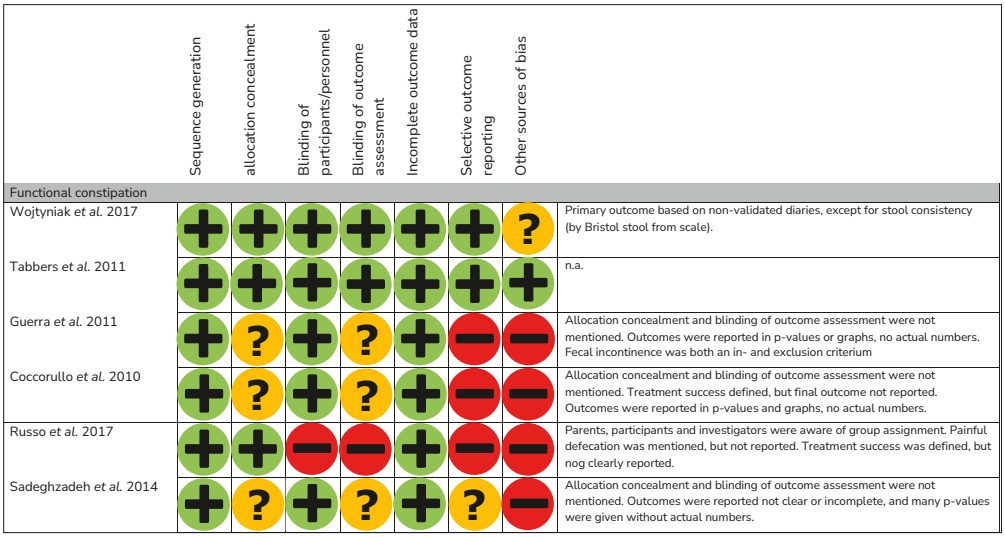


Figure 4 | Cochrane Risk of Bias Tool for the assessment of methodological quality and potential risks of bias for studies in children with FC [36].

Tabbers et al. assessed the effect of a fermented milk containing *Bifidobacterium lactis* DN-173 010 compared to a control product consisting of a milk-based, non-fermented dairy product without probiotics in 159 children with FC [46]. The change in stool frequency from baseline to after 3 weeks was not significantly different (increase probiotic 2.9±3.2 vs placebo 2.6±2.6, p=0.35). No significant differences were found for stool consistency (probiotic mean 3.3 vs placebo 3.5, p=0.07), rate of success (probiotic 38% vs probiotic 24%, p=0.06), rate of responders (probiotic 72% vs placebo 64%, p=0.31), fecal incontinence (p=0.19), pain during defecation (p=0.14), abdominal pain (p=0.92) or bisacodyl use (p=0.12). The authors reported an overall difference between groups in flatulence over the 3-week intervention period in favor of the probiotic (p=0.02).

Guerra et al. investigated the effect of a goat yoghurt containing *Bifidobacterium longum* versus goat yoghurt only in a cross-over design in 59 children, aged 5-15 years, with FC [21]. No primary or secondary objectives were mentioned. Data were only graphically shown and apart from p-values numeric data are missing. However, the authors reported an improvement compared to baseline in both groups for stool consistency. When all data of the cross-over were analyzed, significant differences between groups were observed for defecation frequency (p=0.012), defecation pain (p=0.046) and abdominal pain (p=0.015), but the authors did not mention if it was in favor of the probiotic or the control.

Coccorullo et al. evaluated the effects of *Lactobacillus reuteri* (DSM 17938) versus placebo in 44 infants, aged> 6 months, with FC [47]. Stool frequency improved in the probiotic group compared to baseline. Moreover, between groups stool frequency significantly increased compared to placebo after week 2 (p=0.042), week 4 (p=0.008) and week 8 (p=0.027). Stool consistency was not significantly different between groups at week 2 (p=0.63), week 4 (p=0.38) or week 8 (p=0.48). A significant increase in inconsolable crying episodes was found for the probiotic group over time (p=0.02), but not in the placebo group (p=0.08). However, between groups at the different time points, no differences were found (week 2, p=0.64, week 4 p=0.50, week 8 p=0.66).

Probiotic studies with laxatives

Russo et al. investigated the efficacy of a probiotic mixture of *Bifidobacterium breve* M-16 V, *Bifidobacterium infantis* M-63, and *Bifidobacterium longum* BB536 along with PEG 4000 versus PEG 4000 only in 55 children, aged 4-12 years, with FC [48]. Compared to baseline both stool frequency and consistency improved in both groups (p<0.05). However, no significant differences were found between groups for stool frequency (week 2 p=0.168, week 4 p=0.659, week 8 p=0.924) or stool consistency (week 2 p=0.271, week 4 p=0.267, week 8 p=0.857). No significant differences were found between groups for abdominal pain (p>0.369), fecal incontinence (p>351) and rectal bleeding (p>0.505). Treatment success was only significantly different in favor of PEG only at week 2 (p=0.02), but not at week 4 (p=0.27) or week 8 (p=0.24). In terms of acceptability, in total 3 children refused to consume PEG (2/28) or PEG with the probiotic (1/27).

Sadeghzadeh et al. assessed the effectiveness of lactulose and a multispecies probiotic containing *Lactobacillus casei* PXN 37, *Lactobacillus rhamnosus* PXN 54, *Streptococcus thermophilus* PXN 66, *Bifidobacterium breve* PXN 25, *Lactobacillus acidophilus* PXN 35, *Bifidobacterium infantis* PXN 27, and *Lactobacillus bulgaricus* PXN 39 versus lactulose and a placebo in 56 children, aged 4-12 years, with FC [49]. No primary or secondary outcomes were given, only a list of outcome measures. The authors reported an improvement in stool frequency from baseline to the end of intervention (probiotic from 1.67±0.82 to 2.08±0.65, placebo from 0.79±0.83 to 1.54±0.98, p=0.042) and stool consistency (probiotic from 0.42±0.50 to 0.88±0.45, placebo from 0.21±0.41 to 0.63±0.50, p=0.049). Moreover, a significant improvement was found in the probiotic group versus placebo in fecal incontinence (p=0.30) and abdominal pain (p=0.017) after week 1, but not after week 4 (p=0.125 and p=0.161, respectively).

Adverse effects or side effects

Two studies reported adverse effects [45, 46]. However, for one study the adverse effects (n=3) were only reported in the placebo group and included change in stool odor (n=1), abdominal pain and flatulence (n=1) and loss of appetite (n=1) [45]. The other study reported adverse events that might be related to consumption of the study product; gastroenteritis (intervention group n=1, control group n=3), nausea/vomiting (intervention group n=3, control group n=2), and candida-infection of the anorectal region (control group n=1).

DISCUSSION

This systematic review shows that all studies for both FAPD and FC are heterogeneous with respect to study design, study population, types of probiotics used, duration of the study and follow-up, and outcome measures. Moreover, a relatively high risk of bias was found across studies. Based on these findings it is hard to draw any firm conclusions and therefore results should be interpreted with caution.

In accordance with previous SRs, this current systematic review is unable to show a significant clinical effect of a single probiotic strain or a mixture of different probiotic strains in children with FAPD or FC [23-29, 37-44, 48]. Only the *Lactobacillus rhamnosus* GG showed efficacy in two studies in reducing the frequency and intensity of abdominal pain but only in children with IBS, but not for the other FAPD. However, one of these two studies had a very small sample size of IBS patients in which treatment success was only statistically significant for the IBS group, not for FD or FAP. Therefore, larger trials are needed to confirm these findings. Likewise, in adult literature, many studies do not report the benefits of probiotics in functional gastrointestinal disorders, except for abdominal pain reduction in IBS [50].

In adult literature on FC, some evidence can be found for the use of probiotic strains for reducing symptoms of constipation in adults. However, results are strain specific and not all probiotics improved all outcomes, so more adequately powered RCTs with the use of standardized outcome measures are needed to determine which strains, doses and duration are efficacious in the adult population [51]. Not surprisingly, a large proportion of children included in this SR responded to placebo. Indeed, a recent systematic review reported that approximately 41% of children with abdominal-pain-related FGIDs improve on placebo [52]. This placebo effect can be caused by multiple factors; by the 'true placebo effect', but also by symptom fluctuations, the natural cause of the disease, or a regression to the mean. Therefore, detecting the true difference between, in this case, the probiotic and the placebo, is difficult. Additionally, a few studies have been published that reported on the intestinal microbiota composition in children with FC [53, 54]. These studies show conflicting results, which can be due to differences in analysis methods, reporting of the level of taxonomy rank and high inter-individual variability. In contrast, adult literature shows consistently deceased bifidobacteria and lactobacilli and increased *Bacteroidetes* in patients with constipation compared to healthy controls [55]. The bifidobacteria and lactobacilli

are well known for the production of the SCFAs acetate and lactate [56]. Interestingly, one of the prevailing theories on the mechanistic actions of probiotics in constipation is that probiotics increase SCFA concentration, thereby normalizing gut motility [55]. So reduced abundances of these bacteria may have influence on gut motility via the SCFAs.

The prevailing idea is that the pathophysiology in children with FGIDs involves the interrelationship between changes in visceral sensation or hypersensitivity and altered gut motility. Several factors have been linked to this hypersensitivity and altered gut motility [7]. It is clear that the intestinal microbiota also plays an essential role in children with FGIDs [57, 58]. However, it remains a challenge to determine whether there is a causal link between the intestinal microbiota and the disease state and how a disease state of the microbiota can be adapted. This will require large prospective cohort studies to investigate the development of the intestinal microbiome in healthy children compared to those who develop e.g. FGIDs. Although increasing, the number of studies that investigate the complex and dynamic interaction between the intestinal microbiota and host is not sufficient to bridge the gap between pathogenesis in the host, the intestinal microbiota, and alterations in the microbial metabolism and function. Understanding the complex microbial communities and the possible gaps in the intestinal microbiome contributing to disease are essential to reach the next step; develop individualized probiotics for one specific patient, so called 'personalized probiotic treatment'.

This systematic review has several strengths. We carried out a comprehensive and contemporaneous literature search and we reported the full search strategy. The evaluation of study eligibility was done by two investigators to decrease reviewer error and bias. Moreover, the risk of bias of included studies was assessed. However, we are aware of some limitations. We refrained from pooling data because of the study heterogeneity and the use of different study outcomes. A previous SR investigating the effect of probiotics in FGIDs in children did pool the data of all different probiotics [25]. They concluded that probiotics are more effective than placebo in the treatment of patients with abdominal-pain-related FGIDs, especially for IBS. In this current SR, we investigated the effect per probiotic strain instead of treating them as a group, since it is essential to investigate the effects per strain as health-promoting properties of probiotics are strain specific.

The main limitations of this SR arise from the nature of the included studies. In general, the majority of studies included in this SR have an unclear or high risk of bias. Many studies only compared their outcomes between groups at certain time points but did not compare their results to baseline. Moreover, in general, the included studies had heterogeneous outcome measures and varying definitions of these outcomes. Heterogeneity across studies can be reduced by making use of recommendations for conducting clinical trials in children with FC or FAPD [59, 60]. This was also found by Rashid et al. who concluded that a core outcome set (COS) should be developed for each pediatric FGID, taking the patient's function into account as FGIDs have a substantial influence on the quality of life of both the patient as the parents [61]. For FC a COS was published recently to provide a basis for comparing outcomes of different trials [62]. Another limitation is that we only included studies in English. It is possible that studies were not included in this systematic review because they were published in a different

language. Furthermore, studies were only included if children were diagnosed according to the Rome criteria in order to have a homogenous patient population. Another limitation was that cross-over studies were included, while this may not be the optimal study design due to the many pitfalls in analyzing data but also due to the chance of a carry-over effect [63]. The studies that were included in this SR had a wash-out period of 2 weeks only. It is questionable if a two-week washout period is enough in probiotic research. A clear answer is however not possible as no data on intestinal microbiota composition were reported in these studies. Therefore, carry-over effects cannot be excluded.

For FC, studies with and without laxatives were taken into account. In theory, the non-fermentable laxative PEG should not affect intestinal microbiota composition, while the fermentable lactulose might be able to affect the intestinal microbiota composition. However, it has been shown that both PEG and lactulose are associated with an increase in Bifidobacteria rRNA and changes in the intestinal microbiota, interfering with the actual effect of the probiotic [64].

CONCLUSION

In conclusion, the use of probiotics in children with FAPD or FC is safe but current evidence does not support the use of probiotics in the treatment of FAPD or FC in children. However, it is likely that *Lactobacillus rhamnosus* GG can reduce the frequency and intensity of abdominal pain but only in children with IBS. A better understanding of the differences in microbiota in children with FAPD or FC compared to healthy children is required to bridge the gap between pathogenesis in the host, individual microbes, and alterations in the gut microbial metabolism and function. In addition, this systematic review stresses the need for well-designed, adequately powered RCTs using a COS to determine which strains, doses, and duration are effective for the treatment of FAPD and FC in children.

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Nonpharmacologic treatment for children with functional constipation: a systematic review and meta-analysis

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ABSTRACT

Objective: To evaluate the effectiveness and safety of nonpharmacologic interventions for the treatment of childhood functional constipation.

Study design: Randomized controlled trials (RCTs) evaluating nonpharmacologic treatments in children with functional constipation which reported at least 1 outcome of the core outcome set for children with functional constipation.

Results: We included 52 RCTs with 4668 children, aged between 2 weeks and 18 years, of whom 47% were females. Studied interventions included intestinal microbiome-directed interventions, other dietary interventions, oral supplements, pelvic floor-directed interventions, electrical stimulation, dry cupping, and massage therapy. An overall high risk of bias was found across the majority of studies. Meta-analyses for treatment success and/or defecation frequency, including 20 RCTs, showed abdominal electrical stimulation (n=3), *Cassia Fistula* emulsion (n=2), and a cow's milk exclusion diet (n=2 in a subpopulation with constipation as a possible manifestation of cow's milk allergy) may be effective. Evidence from RCTs not included in the meta-analyses, indicated that some prebiotic and fiber mixtures, Chinese herbal medicine (Xiao'er Biantong granules), and abdominal massage are promising therapies. In contrast, studies showed no benefit for the use of probiotics, synbiotics, an increase in water intake, dry cupping, or additional biofeedback or behavioral therapy. We found no RCTs on physical movement or acupuncture.

Conclusions: More well-designed high quality RCTs concerning nonpharmacologic treatments for children with functional constipation are needed before changes in current guidelines are indicated.

Keywords: Functional Constipation; Traditional Medicine; Probiotics; Prebiotics; Alternative Medicine

INTRODUCTION

Functional constipation is a common disorder in children and adolescents worldwide [1]. It is characterized by infrequent, painful, and hard stools and may be accompanied by fecal incontinence and abdominal pain [2]. Functional constipation is a clinical diagnosis based on history and physical examination and is defined according to the Rome IV criteria (**Table 1**) [3,4]. According to international guidelines, the first steps in the treatment of children with functional constipation include demystification, education, toilet training, and laxative treatment with polyethylene glycol (PEG) [5,6]. In addition, guidelines advise a normal fiber and fluid intake, and regular physical activity, but do not recommend the use of probiotics, prebiotics, or behavioral therapy owing to a lack of evidence [5,6]. Laxatives are safe, but adherence to laxatives is low, and except for the use of PEG, little is known about the long-term effects of chronic laxative use [7,8]. This factor may explain why 36.4% of parents of children with functional constipation seek help in the form of complementary or alternative medicine [9].

A systematic review on the nonpharmacologic treatment of childhood functional constipation reported that fiber supplements were more effective than placebo, but no evidence was found regarding the effect of fluid supplements, probiotics, prebiotics, physical movement, or behavioral interventions [10]. Our objective was to review the currently available evidence on the effectiveness and safety outcomes of the core outcome set (COS) [11] of nonpharmacologic treatments for children with functional constipation compared with any other, or no treatment, as studied in randomized controlled trials (RCTs).

Table 1 | Rome IV criteria for functional constipation

Patients <4 years of age	Developmental age of >4 years4
Must include 1 month of ≥2 of the following in infants ≤4 years of age: 1. ≤2 defecations per week 2. History of excessive stool retention 3. History of painful or hard bowel movements 4. History of large-diameter stools 5. Presence of a large fecal mass in the rectum In toilet-trained children, the following additional criteria may be used: 6. ≥1 episode/week of incontinence after the acquisition of toileting skills 7. History of large-diameter stools that may obstruct the toilet	Must include ≥2 of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome 1. ≤2 defecations in the toilet per week in a child of a developmental age of ≥4 years 2. ≥1 episode of fecal incontinence per week 3. History of retentive posturing or excessive volitional stool retention 4. History of painful or hard bowel movements 5. Presence of a large fecal mass in the rectum 6. History of large diameter stools that can obstruct the toilet After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

METHODS

This systematic review, including the protocol, was registered at the international prospective register of systematic reviews, with registration number CRD42020193119 and is reported in accordance with the PRISMA Statement [12].

SEARCH STRATEGY AND STUDY SELECTION

The Cochrane Library, PubMed, and EMBASE databases were searched by a clinical librarian from inception to August 2020. The search protocol with the full search strategy can be obtained from the authors. Keywords used were, including synonyms, "constipation", "child" combined with nonpharmacologic treatments such as, but not limited to, "probiotics", "prebiotics", "nutrition therapy", "physical therapy", "alternative medicine", and "biofeedback." To identify additional studies, reference lists of included studies and (systematic) review articles were searched manually. No language restrictions were applied. Studies were eligible for inclusion if they met the following criteria: (1) The study was a (systematic review of) RCT(s) in which a nonpharmacologic treatment was compared with any other treatment, placebo, or no treatment; (2) the study population consisted of children 0-18 years old with functional constipation; (3) the diagnosis of functional constipation was clearly defined by the authors or by the use of internationally recognized criteria, such as the Rome III [13,14] or Rome IV criteria [3,4]; (4) the study used at least 1 outcome of the COS for clinical trials in constipation, namely, defecation frequency, stool consistency, painful defecation, quality of life of parents and patients, side effects of treatment, fecal incontinence, abdominal pain, and school attendance [11]. Studies were excluded if they included children with an organic cause of constipation (e.g., Hirschsprung disease, anorectal malformations, or cerebral palsy) or if the study was a pilot study. Titles and abstracts of the papers identified by the initial search were independently screened by 2 reviewers for eligibility with the use of Rayyan, a web application for systematic reviews [15]. Full-text manuscripts were obtained of all potentially relevant articles and evaluated more in detail. Foreign language articles were translated if necessary, with the help of native speakers.

OUTCOME ASSESSMENT

The primary outcome measures for this systematic review and meta-analysis were treatment success and defecation frequency. Treatment success and defecation frequency were chosen because they are recommended outcomes for clinical trials in children with functional constipation [16]. Treatment success was collected as dichotomous outcome as defined by authors when it consisted of at least 2 outcomes, of which at least 1 was part of the COS. If treatment success was categorized, the highest level of treatment success was used as a cutoff point (e.g., if subcategories included patients who were not cured, 50% cured, and 90% cured; the latter was collected as dichotomous outcome). Defecation frequency was collected as continuous outcome: the number of bowel movements per week after treatment completion, or if not available, at first follow-up. Secondary outcomes included all other

outcomes of the COS: stool consistency, painful defecation, quality of life of parents and patients, side effects of treatment, fecal incontinence, abdominal pain, and school attendance [11].

DATA EXTRACTION

Data were extracted from each selected study by 2 authors, including general information of the study (author, year, country), study design, criteria for functional constipation diagnosis, population information (age, sex distribution, previous treatment), intervention (comparison[s] and duration), and reported outcomes of the COS including results. When extraction was completed, data were checked by the other author and the disputes were solved by consensus. Data were extracted according to the intention-to-treat principle, where all dropouts were assumed to be treatment failures. When studies had a cross-over design, only the first period was taken into account owing to insufficient run-out periods, especially for microbiome-directed interventions. Fibers and prebiotics were labeled as 1 type of intervention, because the term prebiotic is strictly spoken a health claim, so not all substrates that possess prebiotic properties might be labeled as such, and some studies used a mixture of fibers and prebiotics [17-19].

RISK OF BIAS ASSESSMENT

The risk of bias of each included study was measured independently by 2 authors according to the Cochrane risk of bias tool version 2 [20]. Assessment of the domain "bias owing to deviations from intended interventions" was based on the intention-to-treat principle and evaluated the outcome of treatment success after treatment or at first follow-up of the study, or if not available defecation frequency, or if not available the primary outcome of the study. Any disagreement between reviewers was resolved by consensus.

DATA SYNTHESIS AND STATISTICAL ANALYSES

If possible, data were pooled using a random effects model. Data that could not be pooled were reported per type of intervention. The effect of the interventions of interest on treatment success was expressed as risk difference accompanied by 95% CI by the Mantel-Haenszel method [21]. The effect of interventions of interest on defecation frequency was examined using a standardized mean difference with a 95% CI [21]. If medians were provided, we estimated the mean and SD from the median, range, and sample size with the aid of the formula as proposed by Hozo et al [22]. Moreover, in case defecation frequencies were given per day, data per week were estimated by $Mean_{week}=Mean_{day}\times 7$ and $SD_{week}=SD_{day}\times\sqrt{7}$ or $SD_{week}=\sqrt{(Var_{week}=Var_{day1} + Var_{day2} + ... + Var_{day7})}$ [22, 23]. Heterogeneity across individual trials included in our meta-analysis was assessed with I^2 ranging from 0% to 100%, with higher values indicating higher levels of heterogeneity. An I^2 of less than 25% was arbitrarily chosen to correspond with low levels of heterogeneity [24]. The "meta," "metafor," "robvis," and "dmetar" packages, a hands-on guide, and RevMan5 (The Cochrane Collaboration) were used to generate Forest plots of pooled standardized mean differences for outcomes with 95% CIs [25-29].

RESULTS

A total of 4.240 studies were identified, of which 52 studies were eligible for inclusion, 49 were RCTs and 3 were long-term follow-ups of already included RCTs. **Figure 1** depicts the PRISMA flow chart, including reasons for exclusion. These studies included 4668 children aged between 2 weeks and 18 years, of whom 47% were female. The included RCTs were carried out in Asia (n=21; 43%), Europe (n=19; 39%), South America (n=5; 10%), North America (n=4; 8%), and Oceania (n=1; 2%); 37 studies (71%) were conducted in tertiary care, 11 (21%) in secondary care, 3 (6%) in primary care, and 2 (4%) did not report on the setting. Thirty-seven studies (71%) used the Rome criteria for functional constipation and 15 (29%) used author-defined criteria. Besides the interventions of interest, 28 (57%) studies reported to give advice on toilet training, and 19 (39%) gave dietary advice to all their participants.

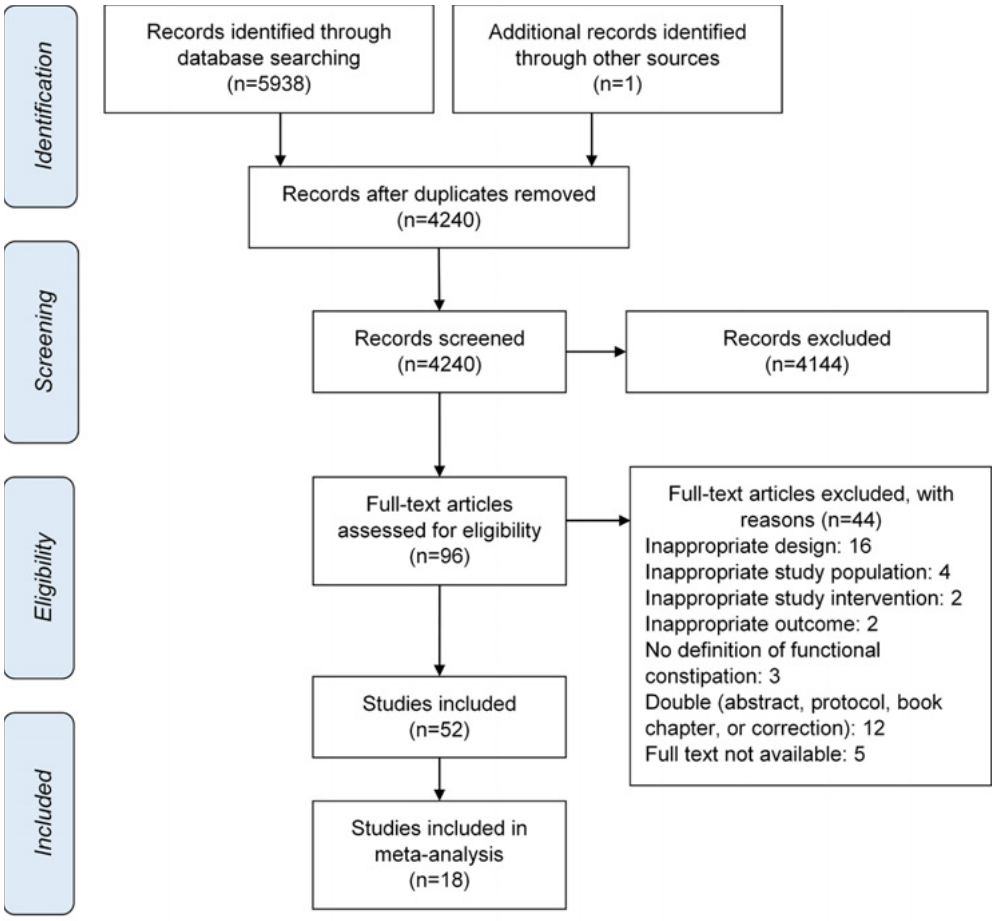


Figure 1 | PRISMA Flow chart [12].

Interventions of the studies included probiotics (n=15), prebiotics/fiber/infant formulas (n=11), synbiotics (n=2), a cow's milk exclusion diet (n=2), (additional) water (n=1), oral supplements (*Cassia fistula* emulsion, Sophia seeds, Xiao'er Biantong granules, green banana biomass, or black strap molasses) (n=6), biofeedback (n=4), electrical therapy (1 with cryotherapy) (n=4), massage therapy (n=3), pelvic physiotherapy (n=1), behavioral therapy (n=1), dry cupping (n=1), and a combination of abdominal muscle training, breathing exercises, and abdominal massage (n=1). The hypotheses on the mode of action of the interventions, accompanied by a summary of the evidence found in this review, are shown in **Table 2**. A summary of study characteristics of all included studies (including results of outcomes not discussed in this section) is available in the **Supplementary Table 1** (https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/).

An overview of which COS outcomes are reported by which studies is available in **Table 3** (https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/). A summary of the risk of bias of all included studies can be found in **Figure 2**, and more details on the risk of bias judgement per domain can be found in **Figure 3, A-D**.

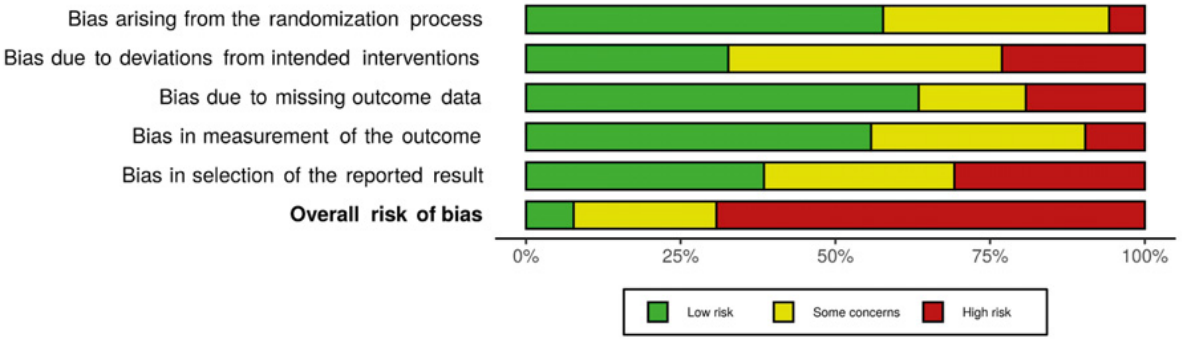


Figure 2 | Risk of bias summary of all included studies.

Table 2 | Summary of interventions with their potential mode of action on FC and findings of this systematic review

Interventions	Mode of action	Findings
Probiotics	Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host [30]." Associations have been found between gut motility and several probiotic strains [31]. Moreover, several genera and community compositions have been associated with a harder stool consistency and others with softer stool consistencies [32]. *Bifidobacteria* and *Lactobacilli* are well-known for the production of acetate and lactate, which might increase gut motility [33]. Therefore, directing the intestinal microbiota composition towards compositions associated with softer stools may be obtained with the use of probiotics.	RoB: low/some concerns/high Two studies were found to be as effective as laxative treatment [34,35] and 3 were more effective than placebo [36-38]; in contrast, several studies reported not to be effective in the treatment of FC [39-43].
Fiber	Fibers can be divided in several ways, one of which by properties of solubility, viscosity and fermentation. Those that are fermentable are often but not exclusively regarded as prebiotics [44]. The mode of action for soluble viscous fibers is by forming a gel-like consistency with water resulting in an improvement consistency of stools (both hard and loose stools). Insoluble fibers can exert a laxative effect by stool bulking, irritation, and stimulation of the gut mucosa to increase peristalsis.	RoB: Some concerns/high Some evidence that specific fibers or prebiotic supplements may be more effective than placebo, [45,46] or as effective as laxative treatment [47-49].
Prebiotics	In addition to the effect of soluble, fermentable fibers as mentioned, prebiotics are defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit."[17] Mode of action of prebiotics in FC may include increasing microbial biomass and SCFA production which may increase stool consistency and gastrointestinal motility [50], and several specific bacterial species have been reported to promote gastro-intestinal motility including genera that are stimulated by prebiotics such as *Lactobacilli* and *Bifidobacteria* [31].	
Synbiotics	Synbiotics are defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" [51] and are thought to have a synergetic effect of both prebiotics and probiotics.	RoB: Some concerns/high Minimal evidence was found for the use of synbiotics [52].

Table 2 | Continued

Interventions	Mode of action	Findings
Water	Sufficient water intake is of importance for normal defecation patterns and is therefore often advised [54]. It is based on the assumption that additional oral intake of fluid leads to an increase in colonic fluid, which would promote increased stool output or a softer consistency. However, this seems contrary to physiologic expectation given the large adaptive absorptive capacity of the gut in response to acute or chronic challenges [55].	RoB: High. No evidence was found for the increase of water or hyperosmolar liquid intake [56].
Cow's milk-free diet	Symptoms of cow's milk allergy might be very unspecific and resemble symptoms of FC [57]. Therefore, it has been suggested that in children, whose onset of constipation symptoms occurred with the introduction of dairy, a cow's milk-free diet challenge can be considered to evaluate if these children may have an underlying cow's milk allergy [58.59]. The hypothetic pathogenic mechanism lies in increased anal pressure at rest, probably caused by allergic inflammation of the internal sphincter area owing to mucosal eosinophil and mast cell infiltration [59].	RoB: High. Some evidence that suggests it may be useful in children with constipation as manifestation of an underlying cow's milk allergy [60,61].
Cassia Fistula	*Cassia Fistula* emulsion is an extract from the plant *Cassia Fistula leguminosae*, which belongs to the same Genus (*Cassia*) as *Cassia Officinalis*, more known as *Senna alexandrina*, from which the laxative senna is made. The precise mechanism of action of senna is unknown, but both senna and *Cassia Fistula* seem to act as stimulant laxatives via anthraquinone type derivates that are naturally occurring in plants as glycosides [62,63].	RoB: High. Minimal evidence that suggests it may be more effective than treatment with mineral oil [64], and just as effective as PEG [65].
Flixweed seeds	The exact working mechanism of flixweed seeds (*Descurainia Sophia*) is unknown. The seeds may produce a mucilage that can absorb water from bowel lumen thereby softening stools. One of the compounds in the seeds, allyl disulfide, may have a relaxing effect on smooth muscles and facilitate defecation [66].	RoB: High. Minimal evidence that suggests it may be just as effective as PEG treatment, but with worse taste [67].
Xiao'er Biantong granules	Xiao'er Biantong granules are a Chinese patent medicine composed of 7 herbs. Traditional Chinese medicine considers the spleen and stomach as the most important organs for digestion. Improper feeding increases the burden of the stomach and intestine, leading to food stagnation. This disturbs qi movement so that the weakened qi cannot push the chime to move powerfully and quickly in the intestine. Based on these mechanisms, the principle of treatment is to remove food retention (Houpo, LaiFuZi), promote defecation (XingRen, LuHui, and JueMingZi,), regulate qi movement (HouPo, ZhiQiao), and strengthen and nourish the spleen and stomach (BaiZhu) [68].	RoB: High. Minimal evidence that suggests it may be more effective than placebo treatment [68].

Table 2 | Continued

Interventions	Mode of action	Findings
Green banana biomass	Green banana biomass has a high content of dietary fiber and resistant starch, which may result in the effects describes in the fiber section [69]. Important to note is that resistant starch is a wide category of substances that differ in their effects on intestinal microbiota and thereby in their effect on constipation symptoms [44,70].	RoB: High. Inconclusive evidence to use on its own, may be effective as addition to PEG or sodium phosphate treatment [69].
Black strap molasses	Black strap molasses syrup is a black and viscous product resulting from sugarcane after 3 stages of sugar extraction. It contains several minerals and a small amount of polysaccharides and other compounds, including polyphenols [58]. The exact mechanism of action in unknown, but several types of polysaccharides and polyphenols might exhibit laxative effects [71].	RoB: some concerns Minimal evidence that suggests it may be just as effective as PEG treatment [58].
Biofeedback	Biofeedback training entails teaching children how to coordinate muscle relaxation with the use of anorectal monitoring instruments to make physiological information accessible to the child's consciousness. It is thought to improve the dyssynergic defecation often seen during anorectal manometry in children with FC. Dyssynergic defecation refers to dysfunction of the pelvic floor muscles which contract instead of relax during a bowel movement. It is thought to be secondary to, or the manometric equivalent of, stool withholding which is considered the major cause for the development and persistence of childhood constipation [72,73].	RoB: High. Evidence suggests no additional benefit for the use of biofeedback over conventional treatment in all children with FC, inconclusive evidence for its use in children with dyssynergic defecation [74-76].
Transabdominal interferential electrical therapy	Transabdominal (interferential) electrical stimulation involves the generation of 2 sinusoidal currents that cross within the body with the use of 4 electrode pads applied on the skin of the abdomen and lower back. The exact mechanism of action is not yet understood, the current may result in an alteration of neuronal function, and increase colonic motility by stimulating the interstitial cells of Cajal, the pacemaker cells of the gut, and/or enteric or extrinsic autonomic nerves [77].	RoB: High. Minimal evidence that suggests benefit as addition to conventional treatment when combined with pelvic floor muscles exercises [78-81].
Cryotherapy	Scientific evidence for the use of cryotherapy and its role in pathophysiology of FC is lacking. It is thought that cryotherapy might influence local blood circulation and normalize vascular tone and motility [81].	RoB: High. Minimal evidence suggests it may be beneficial as addition to therapy with electrical stimulation and pelvic floor muscles exercises [81].

Table 2 | Continued

Interventions	Mode of action	Findings
Abdominal massage	The mechanisms behind abdominal massage's constipation-reducing are most likely a combination of local stimulation and relaxation, and by stimulation of the parasympathetic nervous system. Direct pressure over the abdominal wall alternately compresses and then releases sections of the digestive tract, briefly distorting lumen size and activating stretch receptors that can reinforce the gastrocolic reflex and trigger intestinal and rectal contraction [82,83].	RoB: High. Minimal evidence that suggests benefit as additional to Chinese herbal treatment [84,85], or as part of a combination therapy [86].
Foot reflexology	The science of reflexology is based on the premise that there are zones and reflex areas (e.g., the feet) that correspond with all glands, organs, parts, and systems of the body. Pressure applied to these specific areas by applying specific techniques assists in potentiating the normal function of the corresponding body part and activates the body's innate healing power, reduces stress, and promotes physiologic changes in the body.	RoB: High. Minimal evidence that suggests no additional benefit over regular advice [87].
Pelvic physiotherapy	Pelvic physiotherapy consists of exercises, practicing a stabilized posture on the toilet, teaching effective straining to defecate, increasing awareness of sensations, and exercising adequate pelvic floor muscle functions.	RoB: High/low. Minimal evidence that suggests benefit as addition to conventional treatment [88].
Behavioral therapy	Withholding behavior may be the result of fear and avoidance of defecation. The phobic reactions related to withholding defecation may be decreased and adequate toileting behavior and appropriate defecation straining may be (re)acquired by teaching parents behavioral procedures and by behavioral play therapy with the child.	RoB: High. Minimal evidence that suggests no benefit as additional to conventional treatment [89].
Dry cupping	Cupping therapy is based on applying negative pressure suction on the skin. During dry cupping, a glass cup is placed on the skin and a vacuum is created inside it for a few minutes to congest the skin. The underlying treatment mechanism is not yet understood, it possibly induces muscle relaxation, and may decrease pain [90].	RoB: High. Minimal evidence that suggests it may be less effective as PEG treatment [91].

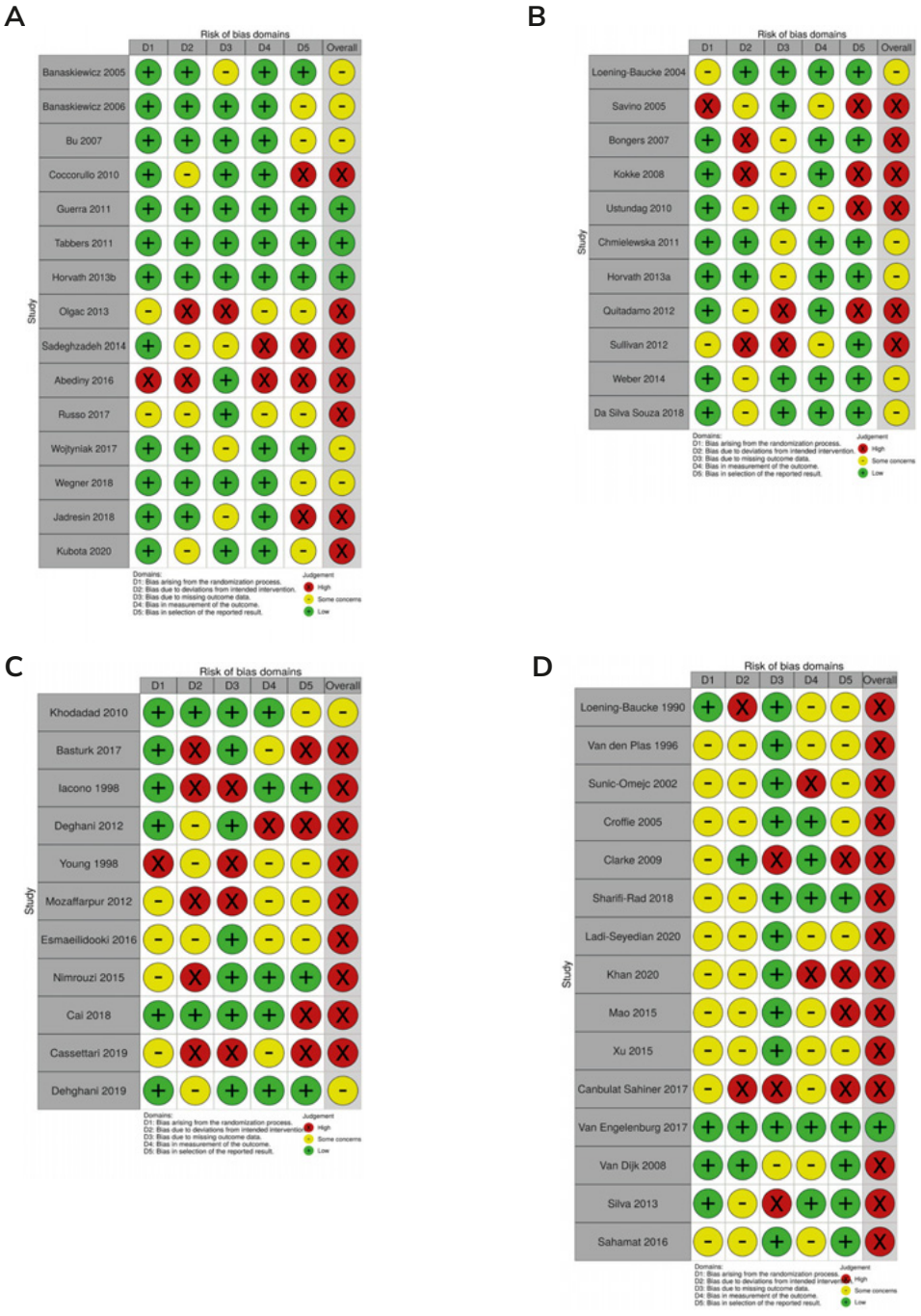


Figure 3 | A | Risk of Bias traffic light plot for probiotics. **B |** Risk of bias traffic light plot for prebiotics and/or fiber. **C |** Risk of bias traffic light plot for synbiotics, dietary interventions, and oral supplements. **D |** Risk of bias traffic light plot for biofeedback, electrical stimulation and cryotherapy, massage therapy, and other and combined treatments.

PROBIOTICS

Thirteen studies, including 965 children [34-43, 92-94] and 2 follow-up studies, including 166 children [95,96], investigated the effect of (or the addition of) probiotics versus placebo or laxative treatment (**Supplementary Table 1,** https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/).

A low risk of bias was found in 2 of 13, some concerns of bias in 4 of 13, and a high risk of bias in 7 of 13 studies and some concerns of bias for both follow-ups (**Figure 3A**).

Meta-analysis

The meta-analysis of 2 studies evaluating *Lacticaseibacillus rhamnosus* (previously *Lactobacillus rhamnosus*) (Lcr 35) versus placebo, with considerable levels of heterogeneity, showed no significant effect on treatment success or defecation frequency (**Figures 4 and 5**) [36, 41].

Treatment Success

Treatment success was reported in 5 of 15 studies, of which 1 (with 3 *Bifidobacterium* spp. strains) was found to be as effective as laxative treatment [34], 1 more effective than placebo [36], and 3 (*L. rhamnosus* GG, *B. lactis* DN-173 010, *L. rhamnosus* Lcr35) not more effective than placebo or control [39-41]. Both follow-up studies reported no difference in treatment success rates between groups [95,96]. The authors, who did not define treatment success, concluded that their probiotic was more effective than placebo on stool consistency (goat yoghurt with *Bifidobacterium longum*) [37] or on fecal incontinence and abdominal pain (7-strain multispecies mix) [38], and 2 concluded that probiotics were not successful as additional treatment on any reported outcomes (both *Limosilactobacillus* [previously *Lactobacillus*] *reuteri* DSM 17938) [42,43]. The authors of 1 study did not compare outcomes between treatment groups (*L. reuteri* DSM 17938) [35].

Defecation Frequency

Defecation frequency was reported in 10 of 15 studies and was comparable with laxative treatment in 2 studies (*L. rhamnosus* Lcr35 and a 3-strain *Bifidobacterium* spp. mix)

[34,36], higher than placebo or control in 3 studies (*L. rhamnosus* Lcr35, *L. reuteri* DSM 17938, and a 7-strain multispecies mix) [36,38,92], and similar to placebo or control in 6 studies (*L. rhamnosus* GG, *B lactis* DN-173 010, *L. rhamnosus* Lcr35, and 3 studies with *L. reuteri* DSM 17938) [35,39-43]. The follow-up studies (*L. rhamnosus* GG and *B. lactis* DN-173 010) found no significant difference in defecation frequency between groups, after 2 years [96] and 3 years of follow-up [95], respectively.

Adverse Events

Adverse events were reported in 12 of 15 studies. Of these studies, 6 of 12 (50%) observed no adverse events. One study observed abdominal pain (n=3) and vomiting (n=1) in children receiving treatment with *L. rhamnosus* GG [39]. One study reported gastroenteritis (n=1) and nausea/vomiting (n=3) in children receiving *B. lactis* DN-173 010 [40]. One study reported transient diarrhea, which disappeared after dose reduction (3-strain *Bifidobacterium* spp. mix and PEG [34]), and another study reported abdominal pain (n=2) (*L. reuteri* DSM 17938) [42].

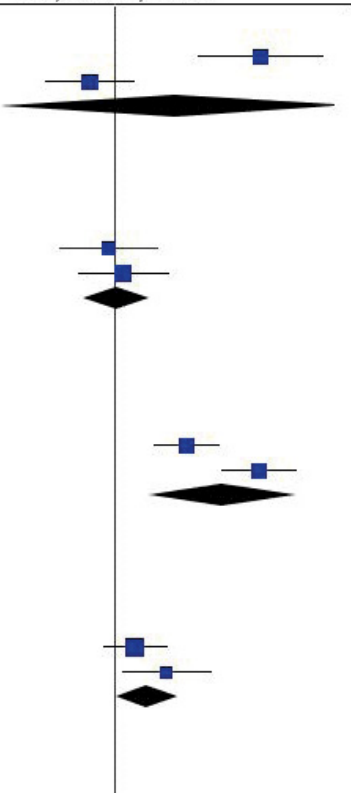
(MIXTURES OF) FIBERS AND/OR PREBIOTICS

Ten studies, including 728 children [45-49, 97-101], and 1 follow-up study including 80 children [95] investigated the effect of (or the addition of) 7 different (mixtures of) fibers and/or prebiotics and/or infant formulas (designed to support bowel habit problems) compared with placebo or control treatment (**Supplementary Table 1,** https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/). Some concerns of bias were found in 4 of 10 studies, a high risk of bias in 6 of 10 studies, and some concerns of bias in the follow-up study (**Figure 3B**).

Meta-analysis

The meta-analysis of the 2 studies evaluating glucomannan vs placebo showed no significant effect on treatment success or defecation frequency (**Figures 4 and 5**) [45,97]. The meta-analysis of the 2 studies evaluating an infant formula with added β-palmitate, prebiotics, and hydrolyzed whey protein (Omneo/Conformil) vs regular formula showed no evidence for an effect on defecation frequency (**Figure 5**) [46,98].

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Risk Difference M-H, Random, 95% CI
1.1.1 Lcr 35 (intervention vs placebo)						
Bu 2007	14	18	1	9	49.2%	0.67 [0.39, 0.95]
Wojtyniak 2017	24	48	28	46	50.8%	-0.11 [-0.31, 0.09]
Subtotal (95% CI)		**66**		**55**	**100.0%**	**0.27 [-0.52, 1.06]**
Total events	38		29			
Heterogeneity: Tau² = 0.31; Chi² = 20.93, df = 1 (P < 0.00001); I² = 95%						
Test for overall effect: Z = 0.68 (P = 0.50)						
1.1.2 Glucomannan (intervention vs placebo)						
Chmielewska 2011	20	40	21	40	45.7%	-0.03 [-0.24, 0.19]
Loening-Baucke 2004	4	26	2	18	54.3%	0.04 [-0.16, 0.24]
Subtotal (95% CI)		**66**		**58**	**100.0%**	**0.01 [-0.14, 0.16]**
Total events	24		23			
Heterogeneity: Tau² = 0.00; Chi² = 0.23, df = 1 (P = 0.63); I² = 0%						
Test for overall effect: Z = 0.16 (P = 0.88)						
1.1.3 Cow's milk exclusion diet (intervention vs diet containing cow's milk))						
Dehghani 2012	56	70	33	70	50.6%	0.33 [0.18, 0.48]
Iacono 1998	21	32	0	33	49.4%	0.66 [0.49, 0.82]
Subtotal (95% CI)		**102**		**103**	**100.0%**	**0.49 [0.16, 0.82]**
Total events	77		33			
Heterogeneity: Tau² = 0.05; Chi² = 8.70, df = 1 (P = 0.003); I² = 89%						
Test for overall effect: Z = 2.90 (P = 0.004)						
1.1.4 Cassia fistula (intervention vs control PEG (Esmaelidooki) or mineral oil (Mozaffarpur)						
Esmaeilidooki 2016	45	52	44	57	61.7%	0.09 [-0.05, 0.24]
Mozaffarpur 2012	19	41	9	40	38.3%	0.24 [0.04, 0.44]
Subtotal (95% CI)		**93**		**97**	**100.0%**	**0.15 [0.01, 0.29]**
Total events	64		53			
Heterogeneity: Tau² = 0.00; Chi² = 1.38, df = 1 (P = 0.24); I² = 28%						
Test for overall effect: Z = 2.08 (P = 0.04)						



1.1.5 biofeedback (intervention as addition vs control)

Loening-Baucke 1990	12	23	1	20	32.3%	0.47 [0.25, 0.70]
Sunic-Omejc 2002	21	25	14	24	31.4%	0.26 [0.01, 0.50]
Van der Plas 1996	31	98	31	94	36.4%	-0.01 [-0.15, 0.12]
Subtotal (95% CI)		**146**		**138**	**100.0%**	**0.23 [-0.08, 0.54]**
Total events	64		46			

Heterogeneity: Tau² = 0.06; Chi² = 14.70, df = 2 (P = 0.0006); I² = 86%
Test for overall effect: Z = 1.44 (P = 0.15)

1.1.6 Interferential electrical stimulation (intervention as addition vs control (Khan and Ladi-Seyedian) or vs sham (Sharifi-Rad)

Khan 2020	18	20	10	20	26.8%	0.40 [0.14, 0.66]
Ladi-Seyedian 2020	14	17	9	17	19.6%	0.29 [-0.00, 0.59]
Sharifi-Rad 2018	38	45	19	44	53.6%	0.41 [0.23, 0.59]
Subtotal (95% CI)		**82**		**81**	**100.0%**	**0.39 [0.25, 0.52]**
Total events	70		38			

Heterogeneity: Tau² = 0.00; Chi² = 0.46, df = 2 (P = 0.79); I² = 0%
Test for overall effect: Z = 5.72 (P < 0.00001)

1.1.7 Abdominal massage (intervention as addition vs control)

Mao 2015	7	47	4	47	52.5%	0.06 [-0.07, 0.19]
Xu 2015	13	61	9	61	47.5%	0.07 [-0.07, 0.20]
Subtotal (95% CI)		**108**		**108**	**100.0%**	**0.06 [-0.03, 0.16]**
Total events	20		13			

Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.99); I² = 0%
Test for overall effect: Z = 1.35 (P = 0.18)

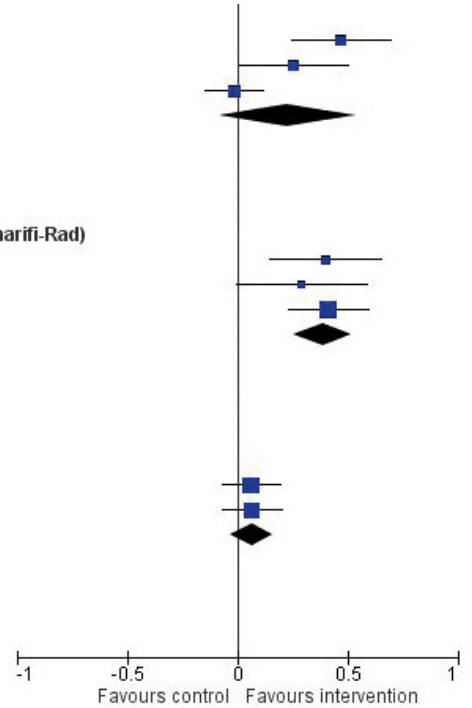


Figure 4 | Forest plot of trials on treatment success.

Treatment Success

A definition of treatment success was reported in 5 of 10 studies, of which 1 (a mixture of acacia fiber, psyllium fiber, and fructose) was as effective as laxative treatment [47], 1 (glucomannan) was more effective than placebo [45], and 3 (glucomannan, fiber/prebiotic mixture [fructo-oligosaccharides [FOS], inulin, gum arabic, resistant starch, soy polysaccharide, and cellulose], FOS) were not more effective than placebo [97,99,100]. The authors of 3 of the remaining 5 studies did not define treatment success. However, they reported that the studied treatment was as effective as lactulose on defecation frequency, fecal incontinence, and abdominal pain (yogurt drink with dietary fiber/prebiotic mixtures of transgalacto-oligosaccharides, inulin, soy fiber, and resistant starch) [48], or on defecation frequency, consistency of stools, and abdominal pain (partially hydrolyzed guar gum) [49]. The third remaining study reported that an infant formula containing modified vegetable oil with β-palmitate, prebiotics and hydrolyzed whey protein (Omneo/Conformil) was not more effective than standard infant formula on any outcomes at endpoint (day 14), although an increase in stool frequency was seen at day 7 [46].

Defecation Frequency

Defecation frequency was reported in all 10 studies, of which 3 found no difference in improvement of defecation compared with laxative treatment [47-49] and 7 found no difference in improvement of defecation compared with placebo or control treatment [45,46,97-101].

Adverse Events

Adverse events were reported by 8 of the 10 studies: 4 observed mild side effects in the experimental group, such as diarrhea, abdominal distention, flatulence, and vomiting [47,48,98,100].

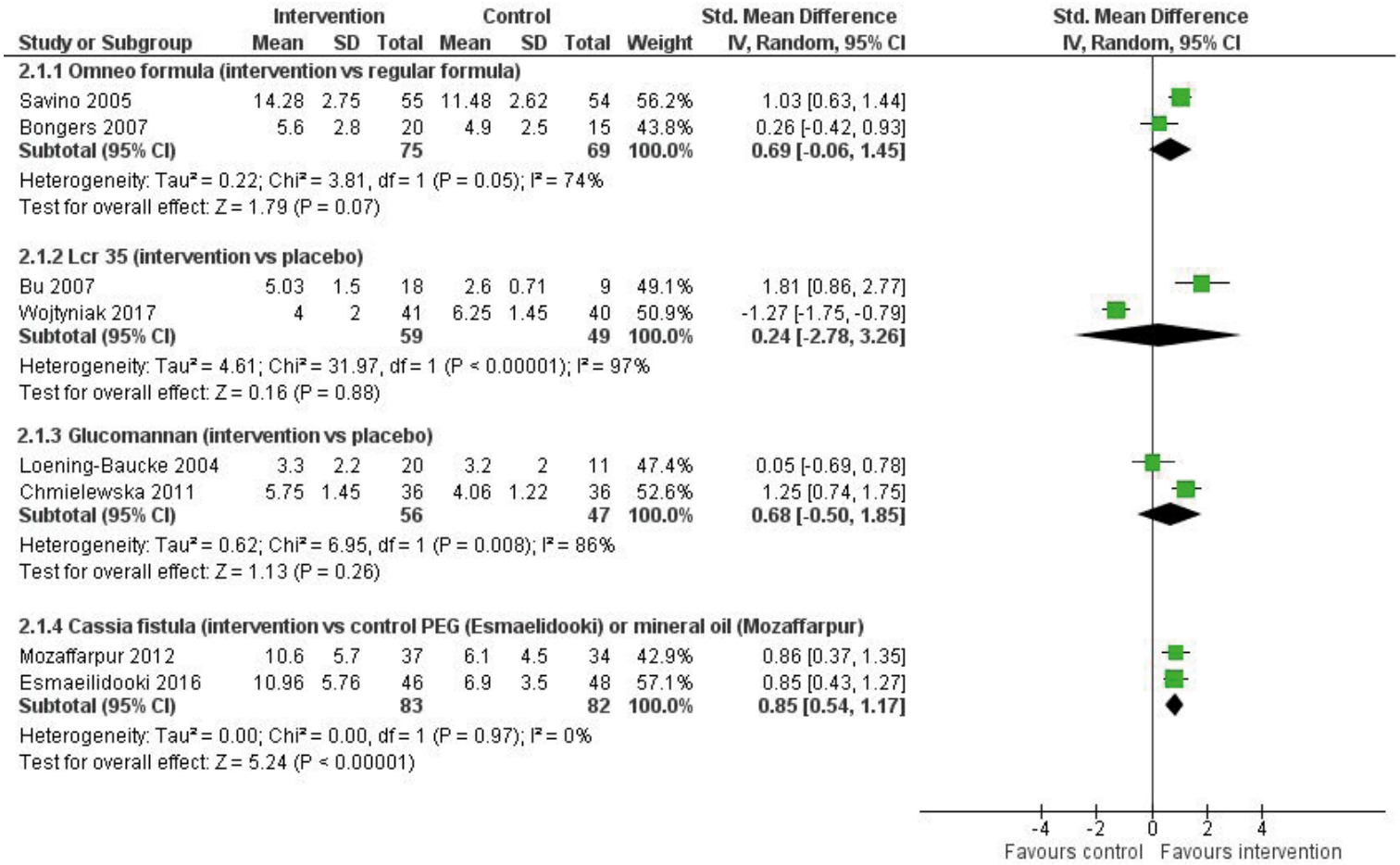


Figure 5 | Forest plot of trials for defecation frequency per week.

SYNBIOTICS

Two studies, including 252 children, investigated the effect of 2 different synbiotics on constipation symptoms (a combination *of L. casei, L. rhamnosus, Streptococcus thermophilus, B. breve, Lacidophilus, B. infantis*, and FOS), and the other study a combination of *L. casei, L. rhamnosus, L. plantarum, B. lactis*, fiber, polydextrose, FOS, and GOS, respectively [52,53]. A high risk of bias was found in both studies (**Figure 3C**). A meta-analysis was not possible owing to the use of different intervention products.

Treatment Success

Treatment success was reported in both studies, 1 of which found similar success rates in all groups (multispecies probiotic with FOS, multispecies probiotic with FOS plus oral liquid paraffin, or oral liquid paraffin only) [52]. The other study found a significantly higher success rate in the synbiotic group compared with the placebo group [53].

Defecation Frequency

Defecation frequency was reported in both studies and was significantly higher in the group receiving both liquid paraffin and the synbiotic [52] and a significant improvement in the synbiotic but not placebo group after treatment. No between-group comparison was executed [53].

Adverse Events

Adverse events were reported in both studies, but none were observed in the synbiotic-only treatment groups. In contrast, 39 children receiving liquid paraffin as control or in addition to a synbiotic reported seepage [52].

DIETARY INTERVENTIONS

Three studies, including 295 children, investigated the effect of a dietary intervention [56,60,61]. Two studies investigated the effect of a cow's milk elimination diet versus a diet containing dairy (in a subpopulation with constipation as a possible manifestation of cow's milk allergy) [60,61], and 1 investigated the effect of an increase in water intake, or the consumption of hyperosmolar liquids, versus normal liquid intake [56]. A high risk of bias was found in all 3 studies.

Meta-analysis

The meta-analysis of the 2 studies evaluating a cow's milk-free diet to a diet containing dairy, with considerable heterogeneity, showed a significant effect of the cow's milk-free diet on treatment success (**Figure 4**).

Treatment Success

Treatment success was reported as a combination of outcomes in 1 study, which reported a significantly higher treatment success rate in the cow's milk elimination diet group [60]. The authors of the other study concluded that constipation can be a manifestation of intolerance of, or an allergic reaction to, cow's milk [61]. The authors of the study investigating higher water intake and hyperosmolar liquids found no significant effect of fluid intake on constipation symptoms [56].

Defecation Frequency

Defecation frequency was reported in all studies. Children receiving a cow's milk-free diet had a significantly higher defecation frequency compared with those receiving a diet containing cow's milk [60,61]. An increase in water intake or hyperosmolar liquid had no significant effect on defecation frequency [56].

Adverse Events

The 2 studies including a cow's milk diet reported that none of the children receiving a cow's milk diet had an acute allergic reaction [60,61].

ORAL SUPPLEMENTS

Cassia Fistula Emulsion

Two studies, including a total of 190 children, investigated the effect of *Cassia Fistula* emulsion compared with laxative treatment (mineral oil [64] and PEG [65]), with a high risk of bias in both studies. Meta-analyses showed evidence for a higher treatment success rate and increased defecation frequency in the *Cassia Fistula* emulsion group compared with control treatment (**Figures 4 and 5**). Treatment success was defined in both studies, and *Cassia Fistula* emulsion was found to be more effective than treatment with mineral oil [64] and as effective as treatment with PEG [65]. Defecation frequency was reported in both studies and was significantly higher in the *Cassia Fistula* emulsion groups. Both studies reported adverse events. In children using *Cassia Fistula* emulsion, diarrhea was the most common side effect reported in 25%-32% of children, all in whom the diarrhea resolved after a 25% dose decrease. Medication refusal because of taste was similar in both treatment groups in both studies.

Descurainia Sophia Seeds (Flixweed)

One study, including 120 children, investigated the effect of flixweed compared with PEG, with a high risk of bias [67]. Treatment success rates and defecation frequency were not significantly different between the groups. Adverse events were not clearly reported, except that in the flixweed group fewer children required rescue medication and more children (30%) disliked the taste.

Xiao'er Biantong Granules

One study, including 480 children, investigated the effect of Chinese patent medicine Xiao'er Biantong granules compared with placebo [68]. A high risk of bias was found. Treatment success rates and defecation frequency were significantly higher in the Xiao'er Biantong granules group. There were no differences in observed adverse events between groups, all of which were mild with favorable prognosis.

Green Banana Biomass

One study, including 80 children, investigated the effect of green banana biomass and included 5 different treatment groups, with a high risk of bias (**Supplementary Table 1,** https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/) [69]. Treatment success was not defined by the authors. No between-group comparisons were made. Adverse events were reported, but none were observed.

Black Strap Molasses (Sugar Cane Extract)

One study, including 92 children, investigated the effect of black strap molasses compared with PEG, with some concerns for bias [58]. Treatment success and the proportion of children with at least 3 bowel movements per week did not significantly differ between groups. Adverse events were reported and included transient abdominal pain which disappeared over time in both treatment groups (I, n=4; PEG, n=7).

BIOFEEDBACK

Four studies, including 320 children, investigated the effect of biofeedback, of which 3 studied the effect of the addition of biofeedback to laxative treatment [74-76] and 1 studied the effect of the addition of home biofeedback to biofeedback in the laboratory [102]. A high risks of bias was found in all studies.

Meta-analysis

A meta-analysis on treatment success, including the 3 studies, which investigated the additional effect of biofeedback to laxative treatment [74-76], showed considerable levels of heterogeneity and no evidence for benefit of the addition of biofeedback (**Figure 4**).

Treatment Success

Treatment success was defined by the authors and reported in all studies. Treatment success rates were higher in the biofeedback group in 2 studies [74,76], were not different between groups in 1 study [75] and were higher in the group receiving additional home biofeedback in 1 study [102].

Defecation Frequency

Defecation frequency was reported in 1 study, which found no benefit of the addition of biofeedback training at home compared with biofeedback in the laboratory [102].

Adverse Events

Adverse events were not reported in any of the studies.

ELECTRICAL STIMULATION AND CRYOTHERAPY

Four studies, including 237 children, investigated the use of electrical stimulation and/or cryotherapy [78-81]. Two studies investigated the effect of abdominal interferential electrical stimulation (versus sham [78] or no stimulation [79]) as an addition to treatment with pelvic floor muscle exercises and laxatives when necessary. One study investigated the effect of abdominal interferential electrical stimulation versus sham stimulation [80]. One study investigated not only the effect of percutaneous abdominal electrical stimulation but also looked at the effect of local cryotherapy and the combination of the 2 (cryoelectroneurostimulation) [81]. A high risk of bias was found for all studies.

Meta-analysis

The meta-analysis on treatment success including 3 of the studies which defined treatment success [78,79,81] showed a significant effect of the addition of abdominal electrical stimulation to conventional treatment (**Figure 4**).

Treatment Success

Treatment success was reported in 3 of the 4 studies, and all studies showed benefit of the addition of electrical stimulation to conventional treatment [78,79,81]. The addition of cryotherapy also significantly increased treatment success rates compared with conventional treatment alone [81]. Cryoelectroneurostimulation significantly increased treatment success rates compared with the other 3 treatment groups [81]. The authors of 1 study did not define treatment success, nor did they compare outcomes between groups [80].

Defecation Frequency

Defecation frequency was reported in 3 of the 4 studies, of which 2 found a significantly higher defecation frequency in the group receiving additional electrical stimulation compared with those receiving conventional treatment [78,79]. The addition of cryotherapy alone significantly increased defecation frequency compared with conventional treatment, and cryoelectroneurostimulation significantly increased defecation frequency compared with the other 3 treatment groups [81].

Adverse Events

Adverse events were reported in 3 of the 4 studies; none were observed.

MASSAGE THERAPY

Three studies, including 256 children, investigated the effect of massage therapy [84,85,87]. Two studies investigated the effect of the addition of daily sessions of Chinese abdominal massage (Tui Na) to treatment with Chinese herbal medicine [84,85]. The other study investigated the effect of a 10-minute foot reflexology massage for 5 days a week as addition to regular advice including dietary advice and toilet training [87]. A high risk of bias was found in all studies (**Figure 3D**).

Meta-analysis

A meta-analysis on treatment success using the proportions of children who were completely cured in the 2 studies investigating the effect of the addition of Chinese abdominal massage [84,85] showed low levels of heterogeneity and no significant effect of the addition of Chinese abdominal massage (**Figure 4**).

Treatment Success

Treatment success was reported in the 2 studies investigating the effect of Chinese abdominal massage. Authors reported that a higher number of the children receiving Chinese abdominal massage were cured, although not completely cured, see meta-analysis. The authors of the study investigating the effect of foot reflexology found no differences between groups after 4 weeks of treatment [87].

Defecation Frequency

Defecation frequency was only reported in the study investigating the effect of foot reflexology, which did not show any significant difference between the groups [87].

Adverse Events

Adverse events were not reported in any of the studies.

OTHER AND COMBINED TREATMENTS

Pelvic Physiotherapy

Although multiple studies describe the use of pelvic muscles exercises in the treatment of children with functional constipation [78,79,86,88], only 1 study, including 53 children, specifically evaluated the effect of the addition of pelvic muscle exercises to laxative treatment,

with a low risk of bias [88]. Treatment success rates were significantly higher in the group that received additional pelvic physiotherapy. Improvement rates of children defecating at least 3 times per week did not differ between groups. Adverse events were not reported.

Behavioral Therapy

One study, including 134 children, evaluated the additional benefit of 12 sessions of behavioral therapy to laxative treatment with toilet training, with a high risk of bias [89]. Both treatment success rates and defecation frequency were not significantly different between groups indicating no evidence for the addition of behavioral therapy. Adverse events were not reported.

Dry Cupping

One study, including 120 children, compared the effect of dry cupping therapy to conventional treatment with PEG, with a high risk of bias [91]. Treatment success rates were higher in the group receiving conventional treatment. Defecation frequency was not different between groups. Adverse events were not reported.

Combination Therapy

One study, including 72 children, investigated the combined effect of the addition of abdominal muscle training, breathing exercises, and abdominal massage to treatment with magnesium hydroxide, with a high risk of bias [86]. Treatment success was not defined by the authors. Defecation frequency was higher in the group receiving the combination therapy. Adverse events were reported; none were observed.

DISCUSSION

A total of 52 RCTs were analyzed, including 4592 children, with a wide variety of interventions. Meta-analyses for treatment success and defecation frequency showed that a cow's milk exclusion diet (n=2 in a subpopulation with constipation as a possible manifestation of cow's milk allergy), abdominal electrical stimulation (n=3), and *Cassia Fistula* emulsion (n=2) may be effective. Evidence from studies not included in the meta-analyses, indicated that some prebiotic and fiber mixtures, Xiao'er Biantong granules, and abdominal massage are promising therapies. In contrast, studies showed no benefit for the use of probiotics, synbiotics, an increase in water intake, dry cupping, or additional biofeedback or behavioral therapy. Studies were heterogeneous with respect to study design, diagnostic criteria for functional constipation, study population, study intervention, duration of treatment and follow-up, and outcome measures. Adverse events were reported by the majority of the studies (33 of 52). Overall, adverse events of studied interventions were uncommon. If adverse events were observed, they were mild and

mostly consisted of transient abdominal pain, diarrhea, or other gastrointestinal symptoms. No serious adverse events were reported. Additionally, an overall high risk of bias was found across the majority of studies. Therefore, the evidence found in this systematic review should be interpreted with caution.

We found that some prebiotic and fiber mixtures may be effective treatments, whereas no evidence was found for the use of probiotics or synbiotics. This difference may be explained by the fact that fibers and prebiotics stimulate fecal bulking via their own mass and the ability of insoluble fibers to bind water directly [50]. In accordance with this finding, numerous trials in healthy infants with infant formulas supplemented with prebiotics and/or fibers report stool softening effects [103]. Moreover, associations have been found between childhood constipation and low consumption of fiber [103,105], fruits and vegetables [104,106-108], and frequent consumption of fast foods [108]. As with laxatives, a dose-response effect is likely to be present for the effects of fibers and prebiotics. Some of the included studies used a low dose of fibers and prebiotics, which may explain the observed ineffectiveness, besides that some substrates might have no effect on functional constipation symptoms [46,97,98]. Adequate dosing regimens have not yet been established, and studies investigating which fiber and prebiotic mixtures to use, including dose-response effects, are needed. Of the studies included in this systematic review, only 3 evaluated the effects of treatment on microbiota composition. Future studies should take into account the actual differences in intestinal microbiota composition, working mechanisms, and metabolite profiles before and after intervention to clarify host-microbe interactions and identify possible differences between responders and non-responders to move towards personalized intestinal microbiome-directed medicine or nonpharmacologic treatments.

Several studies investigated the effects of oral supplements or dietary changes, other than prebiotics, probiotics, or synbiotics, on constipation symptoms. In addition, 19 studies (37%) gave general dietary advice to all included children, often consisting of frequent consumption of fruits and vegetables and a normal fiber and fluid intake. This systematic review shows a lack of evidence for the benefit of a particular dietary intervention or supplement. Future studies may focus on investigating the effects of *Cassia Fistula* emulsion, Xiao'er Biantong granules, or black strap molasses as alternative laxative treatment. Current adult guidelines on functional constipation consider Chinese herbal medicine, like Xiao'er Biantong granules, effective, but clearly state that it is unknown which formulation and dosage is best to use [109]. Flixweed and green banana biomass seem less attractive options, because approximately one-third of children disliked the taste of flixweed, and green banana biomass alone did not seem to be an effective treatment [67,69]. Evidence from 2 studies with a high risk of bias suggested that a cow's milk-free diet may be useful in children with constipation as a manifestation of an underlying cow's milk allergy [60,61]. However, the generalizability of these findings is limited, because the authors of both studies described that their study populations represent a select patient population of children not responsive to conventional treatment, and one of the participating centers had considerable experience in the treatment of food allergies [61].

Another subset of the identified interventions—namely, biofeedback and pelvic physiotherapy—

target the act of defecation, because stool withholding is a major contributing factor in the onset and persistence of childhood constipation. By teaching children how to control their pelvic floor, in addition to laxative therapy to soften stools, they may relearn how to defecate. Indeed, after biofeedback training, the majority of constipated children were able to relax their pelvic floor, but this was not related to successful outcomes [75]. The addition of pelvic physiotherapy with a more extensive approach may contribute to better outcomes [88]. However, this might only be the case in children with symptoms refractory to conventional treatment, because a large study in a primary care setting did not find an additional benefit of pelvic physiotherapy [110].

Massage therapy, [86] abdominal electrical stimulation [78-81], and cryotherapy might directly enhance colonic motility [81]. Although evidence is limited and the mode of action remains incompletely understood, these interventions may have a positive effect on functional constipation in children. More rigorous and uniform studies using a standardized approach should be performed before these interventions can be recommended.

The main limitations of this review arise from the nature of the included studies. The actual therapeutic effect size is uncertain owing to possible publication bias, the majority of studies (71%) were conducted in a tertiary care setting, therefore limiting the generalizability of these findings. Additionally, the risk of bias within studies was overall high, especially in the overall risk of bias and bias in the selection of the reported results. Moreover, a meta-analysis was only possible for a proportion of studies owing to the lack of reported outcomes or differences in investigated treatment. Therefore, heterogeneity was only assessed for studies included in the meta-analysis and was found to be high for many studies. Also, large differences in effectiveness may exist between individual interventions, like probiotic strains or prebiotic substrates, which may differ greatly in their potential therapeutic effect.

Future research should focus on conducting high quality multicenter trials and follow current trial recommendations [16] using outcomes described in the COS [11]. Trials may focus on the most promising interventions found in this review: specific prebiotic and fiber mixtures, abdominal electrical stimulation, *Cassia Fistula* emulsion, and Xiao'er Biantong granules. Future studies may also investigate interventions of interest of which no trials were found like personalized gut-microbiota interventions, chicory inulin [111], exercise [112], (electro) acupuncture [113-115], other noninvasive neuromodulating therapies like posterior tibial nerve stimulation [116], and virtual and digital interventions [117]. Because education and ongoing toilet training are considered key elements in the treatment of childhood constipation, interventions motivating children to defecate and improving the self-efficacy of children in their constipation treatment are likely to be of great value [118]. Last, more attention should be given to the costs and cost-effectiveness of treatments, because none of the currently included studies reported on the costs of the studied interventions [119.120].

To conclude, more rigorous evidence is needed to confirm the effectivity of nonpharmacologic interventions for children with functional constipation, before strong recommendations can be given to change current guidelines.

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The effect of fiber and prebiotics on children's gastrointestinal disorders and microbiome

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ABSTRACT

Introduction: The bacteria received upon birth are the start of colonization of the approximately 10^{14} bacteria that are present in the mature human gastrointestinal tract, better known as the microbiota. The intestinal microbiota is implicated in gastrointestinal health, nutrient metabolism and benefits such as prevention of infection. Dietary fiber, including prebiotics, escape digestion in the small intestine and reach the colon intact, where they are partially or completely fermented by the intestinal microbiota.

Areas covered: The possible interactions between dietary fiber, prebiotics and microbiota are discussed as well as how this relates to functional gastrointestinal disorders. During the first years of life the microbiota have not yet reached a stable state and is sensitive to disturbance by environmental factors. An imbalance in the microbiota early in life is found to be associated with several functional gastro- intestinal disorders such as colic, functional abdominal pain, irritable bowel syndrome and constipation.

Expert commentary: A better understanding of how gut microbial changes in early-life can impact gastrointestinal health might lead to new treatments or disease prevention. Nutritional strategies with fiber or prebiotics may support health due to modification of colonic microbiota composition and metabolic activity, for example by growth stimulation of *Bifidobacterium* and *Lactobacillus*.

Keywords: Intestinal Microbiota; Children; Dietary Fiber; Functional Gastrointestinal Disorders; Prebiotics; Oligosaccharides.

THE INTESTINAL TRACT AND DEVELOPING INTESTINAL MICROBIOTA

The thin epithelial layer lining the gastrointestinal (GI) tract is covered with the largest mucosal surface of the body. It has a dual function of absorbing nutrients as well as defending the body against a wide range of compounds that may be damaging, toxic, infectious, or carcinogenic [1, 2]. Within the intestinal tract a complex ecosystem of intestinal microbiota engages in a symbiotic association with the host [3]. The microbiota can interact with the human body to influence the host's response to the diet, while the host simultaneously can influence the intestinal microbiota by changes in the diet [4]. There are indications that colonization of the infant gut may already start *in utero* [5]. The microbes received upon birth will stimulate the colonization which will evolve until the age of 3 to 6 years when the ecosystem becomes relatively stable [6, 7]. Ultimately the microbiota reaches 10^{14} microbes in the mature adult gut, which equals the amount of human eukaryotic cells [8]. The intestinal microbiota has important roles in GI health among others for protection against pathogens, involvement in nutrient metabolism, vitamin synthesis and bioavailability of minerals [4]. Furthermore, there is an increasing evidence of its involvement in protection against some disorders, for example, inflammatory bowel disease, diabetes, obesity, and necrotizing enterocolitis [9]. Moreover, it is hypothesized that there is a critical window in the first 1000 days of life during which the influences on the microbiota and the immune system of infants can impact development of disease later in life [6]. Specifically, the composition of the intestinal microbiota plays an important role in the development of the immune system, although there are also direct microbiota independent effects described [10]. One prominent mechanism by which microbiota shape the immune response is via short-chain fatty acids (SCFA), the end products of microbial fermentation. In addition, SCFA are important host modulators and butyrate for example serves as an energy source for the host epithelial cells, and low levels of butyrate modify cytokine production profile of T_H-cells and promote intestinal epithelial barrier integrity. The SCFA acetate protects against intestinal inflammation via G-protein-couples receptor GPR43 [11].

A healthy intestinal microbiota consists of many different microbes, but before the age of 3 years the microbiota has a lower diversity compared to adults [12]. Remarkably, the inter-individual variability of the microbiome of children is higher compared to that of adults [6]. Upon birth, gut colonization commences with the facultative anaerobes, like *Enterobacteriaceae,* that are believed to lower the oxygen levels still present in the infant's gut. In a matter of days these bacteria will create more anaerobic conditions that give rise to strict anaerobes such as *Bifidobacteriacea* and *Clostridiaceae* [6, 13]. The most abundant bacterial families on average during the first 3 years of life are depicted in **Figure 1**. However, this average microbiota composition can be strongly influenced and modified dramatically by several factors.

Firstly, the mode of delivery is believed to be a major determinant of the intestinal microbiota colonization of newborns [14]. The intestinal microbiota of the newborn reflects the type of microbiota the infant encountered during birth [6]. Vaginally delivered infants have a intestinal

microbiota that resembles the microbiota of the maternal vagina, whereas infants born via a Caesarean section (C-section) have a intestinal microbiota that resembles skin microbiota [15-17].

Secondly, human-milk-feeding versus formula feeding has an impact on microbiota composition. Human milk introduces new microbial communities, and contains human milk oligosaccharides (HMOs), which selectively stimulate growth of, amongst others, Bifidobacteria and *Lactobacillus* spp. which are thought to play a role in health [6, 16]. For this reason, prebiotics such as galacto-oligosaccharides, long chain fructo-oligosaccharides (lcFOS) and/or inulin are currently being added to infant and follow-on formula [18, 19]. The microbiota of formula fed babies contains more diverse species resembling an adult-like microbiota, and this is influenced by the presence or not of prebiotics in the formula milk [17].

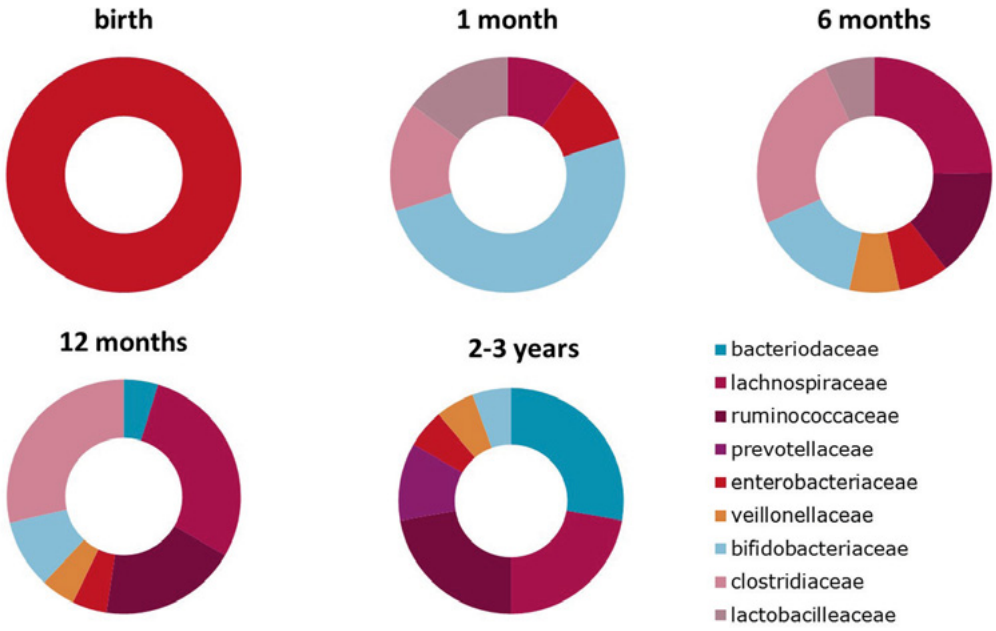

Figure 1 | Intestinal microbiota colonization from birth up until the age of 3. Adapted and retrieved from [3–5].

Thirdly, the weaning period, where the child receives a variety of solid foods modifies the intestinal microbiota. The change of composition in intestinal microbiota strongly depends on the newly available substrates and the withdrawal of human- or formula milk [6]. Prior to the introduction of solid foods, the infant intestinal microbiome harbors genes encoding enzymes that can degrade non-digestible polysaccharides of plant origin. Thereby, the infant microbiome is capable of metabolizing simple plant-derived foods containing polysaccharides and fibers [20, 21]. Failure to transfer fiber and prebiotic fermenting microbes from mother to offspring is considered a potential issue for long term health over the generations [22].

Finally, other factors that can influence the composition of the microbiota such as the use of pre- and postnatal antibiotics, premature birth, geographic influences, host genetics, diet, stress, and hygiene are reviewed elsewhere [6, 23-25].

Adjusting the diet by adding for example dietary fibers or prebiotics, gives the opportunity to modulate the intestinal microbiota and exert effects on health.

DIETARY FIBER AND PREBIOTICS

The role of dietary fiber was already discussed by Hippocrates in the medical literature in the 4th century BC. His findings focused on the health benefits and laxative effect of wholegrain bread [26]. Over the course of history many references can be found regarding dietary fiber in relation to GI disorders such as functional constipation and other health effects. More recently the concept of prebiotics was introduced. Both definitions of dietary fiber and prebiotics partly overlap. The main difference is that prebiotics selectively stimulate certain microbiota species [27, 28], while not all fibers show prebiotic properties [29].

Many definitions exist for dietary fiber as it can relate to chemical compounds defined by structure or functional properties [28]. Recent definitions are not only based on their chemical features by the total dietary fiber (TDF) method, but also on their physiological effects [29]. Therefore, dietary fibers are often defined as non-digestible carbohydrates and lignin that are intrinsic and intact in plants [29]. Or as non-starch polysaccharides, resistant starches, and oligosaccharides [30, 31]. Moreover, from a regulatory perspective the definition of dietary fiber differs between countries or regions, this difference is mainly based on the degree of polymerization (DP) of the polymer [32, 33]. Even though there is no universal definition for dietary fiber, all definitions hold that: 'Dietary fiber is a group of carbohydrate polymers, oligomers, and lignin that escape digestion in the small intestine and reach the colon intact, where they are partially or completely fermented by the intestinal microbiota' [28]. Additionally, dietary fiber also contributes to fecal bulking directly via their own mass and/or by the mass of the water that they attract and indirectly by stimulating growth of colonic microbiota leading to an increase in microbial biomass [34].

Prebiotics are referred to as: 'Selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health' [35]. This in contrast to probiotics: 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [36]. Synbiotics are defined as: 'A product containing both pro- and prebiotics' [27].

An overview of different types of dietary fiber is presented in **Figure 2**. The Low Molecular Weight Dietary Fiber (LMWDF) subgroup is best known for their prebiotic properties [28]. Most prebiotics are nondigestible oligosaccharides such as manno-, pectic-, soybean-, isomalto-, (trans)galacto- and xylo-oligosaccharides [37, 38]. The vast majority of prebiotic studies have focused on the prebiotics inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS), and therefore these will be discussed in more detail, descriptions shown in **Table 1**.

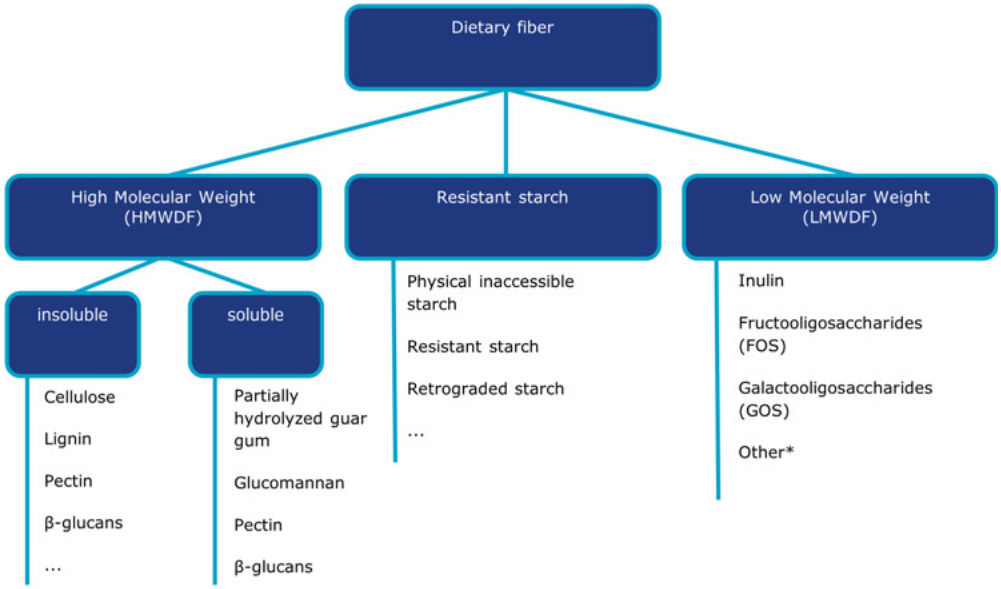

Figure 2 | Dietary fiber. Adapted and retrieved from [1, 2]. *This includes manno-, pectic-, soybean-, isomalto-, transgalacto- and xylo-oligosaccharide, and polydextrose [5, 8].

Our diet comprises various plants materials that contain FOS and inulin, such as onion, garlic, wheat, banana, chicory and some cereals [39, 44]. Despite the large variety of plants that contain inulin, the most commonly used source is chicory (*Cichorium intybus*) where inulin is extracted from the fresh roots [34]. In contrast to FOS and inulin, GOS have a dairy origin and are produced by the enzymatic conversion of lactose using beta-galactosidase [40]. Methods of manufacturing inulin, other fructans, and GOS are summarized in **Table 1**. One of the main differences is the DP which may have a significant impact on the fermentation location; for example low and high DP fructans tend to be fermented more in the proximal and distal colon, respectively [41, 45].

One of the main effects of inulin, FOS and/or GOS is that, even when consumed in small amounts (0.24-0.8 g/100mL formula in infants or 1.5-5 g/day in young children) the growth of *Bifidobacterium* and *Lactobacillus* species are stimulated [42, 46]. These species are commonly found in human-milk-fed babies mainly due to fermentation of HMOs, but tend to be at lower levels in formula fed infants depending on the addition of prebiotics [42]. Studies in infants have shown that supplementing infant formula with prebiotics results in an increase in *Bifidobacterium* and *Lactobacillus* [18, 19, 47]. Addition of prebiotics to infant formula can also result in physiological benefits such as effects on allergy and incidence of infection [48].

Table 1 | Description of fructans and GOS [40-43].

Type	composition	Method of manufacturing	Degree of polymerization
Native Inulin	β(2-1) fructans	Extraction from inulin-rich plant material, often chicory root	2-60 Average: 9-12
lc-inulin (lcFOS)	β(2-1) fructans	Produced from native inulin from chicory root	10-60 Average: >21
sc-inulin	β(2-1) fructans	Produced from native inulin from chicory root	2 - 10
Oligofructose (FOS)	β(2-1) fructans	Enzymatic degradation of inulin from chicory root or other plant material	2-10 Average: 4
(sc)FOS	β(2-1) fructans	Enzymatic synthesis from sucrose	sc: 3-5
GOS	Chains of galactose with a terminal glucose	Produced enzymatically from lactose	sc: 2-8 Average: 3

FOS: fructooligosaccharides, GOS: galactooligosaccharides, lc: long-chain, sc: short-chain

Interestingly, it was found that microbial genes facilitating the breakdown of plant-derived fibers are already present after 100 days of life, despite an exclusive human-milk diet [21]. This indicates that during this period the infant microbiome becomes metabolically ready to receive simple plant-derived fibers. The intestinal microbiota use their carbohydrate hydrolyzing enzymes to multiply and produce SCFAs, gasses (hydrogen, carbon dioxide, hydrogen sulphide, and methane), lactate, and other products [49]. SCFAs are absorbed by the human gut and subsequently metabolized [43]. The difference in intestinal microbiota of human-milk- and formula-fed infants results in differential production of SCFAs. In human-milk-fed infants lactate is the predominant product and butyrate is usually absent, whereas in formula fed infants acetate is the predominant SCFA and small amounts of butyrate are detected [50]. However, a study in infants showed that the addition of 90% GOS and 10% FOS to infant formula can shift the SCFA profile and pH closer to that observed in human-milk-fed infants, and compared to infants fed control formula [51]. After weaning the production of butyrate increases, an important SCFA as it is the preferred energy source for colonic epithelial cells. Moreover, butyrate is considered a key nutrient for determining metabolic activity and growth of epithelial cells of the colon [29]. Hence, lactate, butyrate, acetate, and other SCFAs are important not only as a source of energy for epithelial cells, but also to reduce fecal pH and thereby inhibit growth of pathogens [29].

Table 2 | Key terms, first tree rows apply to all searches in combination with one of the bottom three rows

OR Child[a]
OR Infant[a]
OR Adolescen[a]
OR Pediatric[a]
OR Dietary fiber
OR Inulin
OR Prebiotic[a]
OR Oligosaccharid[a]
OR Fiber
OR Non-digestible oligosaccharide[a]
OR Placebo
OR Control
OR intervention
OR Clinical study
OR Clinical trial
OR RCT
OR Trial[a]
OR Cross-over
AND Constipation OR constipat[a] OR Obstruction OR Obstipation
AND FAP OR IBS OR functional abdominal pain OR IBS OR irritable bowel syndrome
AND Colic OR infant colic OR colic pain

* includes any extension to the listed word
FAP: functional abdominal pain, IBS: irritable bowel syndrome, RCT: randomized controlled trial

INTESTINAL MICROBIOTA, DIETARY FIBER AND PREBIOTICS IN EARLY LIFE FUNCTIONAL GASTROINTESTINAL DISORDERS (FGIDS)

An imbalance and/or reduced microbial diversity has been associated with a wide variety of functional gastrointestinal disorders (FGIDs) in children such as colic, irritable bowel syndrome (IBS), constipation and diarrhea, but also with other diseases such as allergy [9, 46]. In addition, many diseases later in life seem to be associated with the intestinal microbiota early in life, for example inflammatory bowel disease, celiac disease, obesity and allergic reactions [9, 46]. The impact of dietary fiber and/or prebiotics on different FGIDs in interventions with infants and children will be discussed below. The Cochrane Library and PubMed were searched for relevant studies using the key search terms both as MeSH and key words are listed in **Table 2**. Studies published in English were included. Trials in children from birth until the age of 18 years were eligible for inclusion. Additional strategies for identifying studies included searching the references lists of the relevant studies found as well as review articles.

COLIC

Definition, prevalence and etiology

Infant colic is a common disturbance and has a worldwide average prevalence of 21% in children younger than 12 months of age [52]. One definition is based on Wessel's description; 'an infant who, otherwise healthy and well-fed, has paroxysms of irritability, fussing or crying lasting for a total of more than three hours a day and occurring on more than three days in any one week' [53]. Other symptoms include drawing up of knees, excessive flatulence and no relief upon feeding, mainly in the late afternoon and early evening. However, the Wessel criteria have recently undergone revisions in the Rome IV criteria [54] as follows 'For clinical purposes, the diagnostic criteria must include all of the following: 1) An infant who is <5 months of age when the symptoms start and stop; 2) Recurrent and prolonged period of infant crying, fussing or irritability reported by caregivers that occur without obvious cause and cannot be prevented or resolved by caregivers; 3) No evidence of infant failure to thrive, fever, or illness'.

The etiology of excessive crying remains unclear, but in the majority of cases colic probably represents the upper end of the normal developmental "crying curve" of healthy infants [54]. Only 5.1% of infants presenting with excessive crying at an emergency department had underlying organic cause, of which urinary tract infection was the most prevalent condition [55]. Others have suggested an important role for environmental factors, such as psychosocial issues, domestic violence, inadequate parent-infant interaction, or parental anxiety [58]. In contrast to this, a relationship between gastrointestinal causes, such as lactose intolerance, cow's milk allergy and gastroesophageal reflux disease, and the excessive crying, has been suggested [56]. In recent years, it has been further suggested that aberrancies in the infant intestinal microbiota affect gut motor function and gas production, thereby leading to excessive crying.

Available treatments for infant colic range from drug therapies and nutritional interventions to behavioral interventions, however there is no standard care [57].

Intestinal microbiota and colic

Several studies were performed to investigate the differences in intestinal microbiota between healthy infants and infants with colic. In the first study in 2004, Savino et al. [58] detected a significantly lower prevalence of *Lactobacilli* spp. (P=0.044) and a higher abundance of gram-negative bacteria in infants with colic [58]. Subsequent studies reported that *Proteobacteria* were positively correlated with an increase in crying and fussiness, and especially *Klebsiella* and *Escherichia* were predominant in the fecal samples of infants with colic [59, 60]. Proteobacteria are phyla that contain many opportunistic pathogens, and thus may increase the abundance of inflammatory bacteria. On the other hand, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* were reduced in infants with colic compared to controls. Specifically, bifidobacteria were significantly reduced at 1 week after birth in infants with colic (P=0.049) and lactobacilli were significantly reduced 2 weeks after birth in infants with colic (P=0.023) [59]. These groups contain many beneficial bacteria typical for a healthy infant microbiota, thus their reduction may also be an indication of decreased gut health. The latter is supported by a study

in preterm infants [61]. This study found a higher percentage of a *Firmicute, Clostridium histolyticum* in contrast to a significantly higher proportion of the *Lactobacillus-Lactococcus–Enterococcus* group in excessive criers compared to content infants *(P*=0.005). Moreover, infants with colic generally show a less diverse microbiota compared to controls [59, 60]. This points towards the possibility that children with colic might have less beneficial bacteria, because a lower richness in infants (many bifidobacteria and lactobacilli, but a lower overall diversity) is in general found to be beneficial [23, 59, 60]. It is noteworthy that comparisons were sometimes hampered when only the microbial phylum level was reported in comparison to studies with microbial genus level specifications. Furthermore, several studies were excluded from this review as they used outdated culture- or broth-based techniques to identify microbes, or used a probiotic prior to fecal sampling which might have influenced the microbiota of infants with colic.

Influence of dietary fiber and prebiotics

Only one study was found that investigated the treatment effect of fiber on infant colic (**Table 3**). This study by Treem et al. [62] evaluated the effect of soy polysaccharide versus a placebo in 27 children, aged 2 to 8 weeks with colic (defined as crying plus fussing for more than 3 hours a day for at least 3 days of a 6-day baseline period) in a double-blind, randomized, crossover study. No significant differences were found in the average daily time spent by the infants for fussing and crying with ingestion of soy fiber [62]. An observational prospective study by Savino et al. [63] in 214 infants with colic, aged up to 3 months (mean: 1.35 ± 0.77 months) evaluated a formula containing 90% GOS, 10% lc-FOS, sn-2 palmitic acid and partially hydrolyzed proteins. This study showed a reduction in frequency of colic in 79% of children (from 4.1±2.0 per day to 2.0±1.8) at the end of the study [63]. In order to confirm the effect of the formula, a prospective randomized controlled study was conducted with the same formula with added simethicone (6mg/day) (**Table 3**). In this study [64] 199 infants, aged up to 4 months (mean: 1.39±0.84 months), completed the study. Infants who received the new formula had a statistically significant (*P*<0.0001) but also clinically relevant decrease in colic episodes compared to the control formula. Colic episodes decreased from 5.99±1.84 per day to 2.47±1.94 per day after 1 week in comparison to 5.14±1.88 per day to 3.72±1.98 after 1 week in the control group. Moreover, at day 14 the crying episodes were significantly different (*P*<0.0001) between the two groups (1.76±1.60 for new formula compared to 3.32±2.06 in the control formula) [64].

Pärtty et al., [61] investigated the effect of GOS:polydextrose 1:1 versus a probiotic and a placebo in 94 preterm infants (gestational age 32-36 weeks), aged 1-60 days in a randomized, double-blind, placebo-controlled study. A total of 27 out of 94 were classified as excessive criers, while this was significantly less in the prebiotic and probiotic group than in the placebo group (19% vs 19% vs 47%, P=0.02).

Further research is needed not only to understand the delicate balance of the intestinal microbiota in colicky infants, but also large randomized controlled studies to investigate if the effect found by Savino et al. [64] is caused by the prebiotics, the other compounds found in formula milk, such as sn-2 palmitic acid (as this can reduce the amount of calcium soaps in the gut and thereby improve the consistency of stools) or the combination of compounds [65].

Table 3 | Summary of intervention studies for infant colic

Study	Intervention	Power	Age group	duration	Dosage	Disease	Outcome
Dietary fiber							
Treem et al., 1991 [62]	Soy fiber vs placebo	27	2-8 weeks	9 days	1.1g/100 mL	Infant colic	No significant difference in average daily time crying and fussing between fiber and placebo. Significant increase in stool frequency in fiber group.
Prebiotics							
Savino et al., 2003 [63]	90% GOS, 10% lc-FOS, sn-2 palmitic acid, partially hydrolyzed proteins	214	>3 months	14 days	0.8g/100 mL	Infant colic	reduction in frequency of colic in 79% of children (from 4.1±2.0 per day to 2.0±1.8) at the end of the study
Savino et al., 2006 [64]	90% GOS, 10% lc-FOS, sn-2 palmitic acid, partially hydrolyzed proteins vs standard formula + 6mg/kg simethicone	199	0-4 months	14 days	0.8g/100 mL	Infant colic	Significant and clinically relevant decrease in colic episodes between prebiotic and control group after 7 and after 14 days.
Partty et al., 2013 [61]	Polydextrose, GOS 1:1, probiotic or placebo	94	1-60 days	60 days	1-30 days: 600mg/day, 31-60 days: 1200mg/day	Colic in preterm infants	Significantly less excessive criers in the prebiotic group vs placebo.

Abbreviations; FOS: fructooligosaccharides, GOS: galactooligosaccharides, lc-: Long chain

FUNCTIONAL CONSTIPATION (FC)

Definition, prevalence and etiology

Functional constipation (FC) in children is a common GI disorder with a worldwide prevalence ranging from 0.7% to 29.6% (defined here as defecation frequency of <3/wk) [66]. Complaints include infrequent bowel movement, painful defecation due to hard and/or large stools, fecal incontinence, and abdominal pain [67]. The etiology of FC is still incompletely understood but is likely to be multifactorial. Some important factors in children include withholding behavior of stools, psychosocial factors, behavioral disorders, parental child-rearing attitudes, low fiber intake, and the intestinal microbiota composition [67, 68].

Intestinal microbiota and FC

In the last decades only a few studies reported on microbiota in children with constipation. These studies gave different results, which might be caused by the differences in study populations. Two studies were conducted in otherwise healthy children with FC [69], while another study was conducted in obese children with FC [70]. Zoppi et al. [69] found a significant increase in clostridia ($P<0.001$) and bifidobacteria ($P<0.02$) in children with FC compared to healthy children [69]. An increased abundance of bifidobacteria was confirmed in the study of de Meij et al. in constipated children [71]. In obese children with FC this effect was not seen [70]. In this study population a significant decrease of *Prevotella* ($P=0.010$) and increase in several genera of firmicutes was seen ($P<0.05$). These differences may be explained by obesity, which has been associated with a particular intestinal microbiota composition [72]. De Meij et al. [71] also used a supervised statistical learning method in which they were able to discriminate the microbiota of constipated children from healthy controls with 82% accuracy [71].

In practice the population of children with constipation is very heterogeneous, therefore it may be useful to link altered intestinal microbiota signatures to specific subgroups of children. Moreover, in order to draw conclusions, well conducted large studies are needed in otherwise healthy children with FC, but also in specific subgroups such as children with constipation and obesity. The conventional approach for FC treatment includes dietary advise, a toilet program and laxatives [73].

Influence of dietary fiber and prebiotics

An overview of four RCTs that studied the effect of dietary fiber on constipation in children is presented in **Table 4**. Loening-Baucke et al. [74] evaluated the effect of glucomannan (a fiber gel polysaccharide from the tubers of the Japanese Konjac plant) and placebo in 31 children, 4.5 to 11.7 years of age (mean: 7±2 years) with chronic FC in a double-blind, randomized, crossover study. Significantly more children were successfully treated while on fiber (45% with ≥ 3 bowel movements/wk and / or ≤ 1 soiling episodes/3 weeks with no abdominal pain in the last 3 weeks of each 4-week treatment period) as compared with placebo treatment 13%; $P<0.02$). It is noteworthy to mention that 71% of the children had a low initial dietary fiber intake [74].

Secondly, Castillejo et al. [75] compared the effect of cocoa husk supplement (4g cocoa husk and 1g of betafructosans) to a placebo in 48 children, aged between 3 and 10 years (mean: 6.3±2.2 years) with chronic FC in a parallel, randomized, double-blind, controlled trial. This study used a combination of dietary intervention or placebo with toilet training and showed a significant reduction in the percentage of patients with hard stools in the cocoa husk group compared to the placebo group (41,7% versus 75%; P=0.017). However, no significant differences were found in defecation frequency or pain during defecation, as reported by parents. Both the treatment and placebo group had a mean basal dietary fiber intake near the recommended daily allowance (age +10g/day, 12.3±4.1 g/day and 13.4±5.6 g/day respectively) [75].

The third study by Üstündağ et al. [76] evaluated the effect of fiber (partially hydrolyzed guar gum) and lactulose in 61 children, 4 to 16 years of age with chronic FC in a prospective, randomized, controlled study. Both groups showed a significant (P<0.05) improvement in defecation frequency (increase from 4±0.7 to 5±1.7 in PHGG group and from 4±0.7 to 6±1.1 in the lactulose group), stool consistency and abdominal pain. However, children in the lactulose group had significantly more bowel movements after treatment compared to the PHGG group (P<0.05) [76].

The last study by Chmielewska et al. [77] investigated the effect of glucomannan compared to a placebo (maltodextrin in the same dosage) in 72 children, aged 3 to 16 years (mean: 6.1±3.3 years for the glucomannan group and 5.9±2.5 in the placebo group) with chronic FC in a double-blind, placebo-controlled, randomized study. No significant differences were found between the glucomannan and placebo group in terms of treatment success (defined as 3 or more bowel movements with no episodes of soiling during the last week of product consumption). Stool consistency score was significantly (P<0.0001) higher at week 1 in the glucomannan group compared to placebo (2.9±1.2 versus 1.7±1.5 respectively), but lower at week 3 (P=0.008) and similar at weeks 2 and 4. Stool frequency was higher in the glucomannan group only in week 3 (p=0.007). Abdominal pain episodes were more frequent in the glucomannan group compared to placebo in week 1 (P=0.04) and week 4 (p>0.0001) but were similar in week 2 and 3 [77].

In conclusion, studies that investigated the effect of dietary fiber in children with chronic functional constipation are highly heterogeneous, not only in study design, population, duration, follow-up, dosages of treatment and types of fibers used, but also in primary outcomes. Prebiotics may be beneficial for children with FC due to multiple factors, e.g. by their fermentation in the colon they increase microbial numbers and biomass, and they influence the intestinal microbiome [82]. Five RCTs investigated the effect of prebiotics on FC in children, as summarized in **Table 4**.

The first study by Bongers et al. [65] investigated the effect of a formula containing 90% GOS, 10% lc-FOS, sn-2 palmitic acid and partially hydrolyzed whey proteins compared to placebo in 35 children, aged 3 to 20 weeks with chronic FC in a double-blind, randomized cross-over trial. Improvement in stool consistency was found more often in the prebiotic group, however it did not reach statistical significance (90% in the prebiotic group, 50% in the placebo group, P=0.14). Only 25 infants completed the full cross-over study, in this analysis stool consistency

was significantly different between both formulae (17% had soft stools in the prebiotic group, and hard stools in the placebo group, whereas no infants had soft stools in the placebo group and hard stools in the prebiotic group, P=0.046).

The second study by Kokke et al. [78] evaluated the effect of a prebiotic and fiber mixture (GOS, inulin, soy fiber, resistant starch) versus lactulose in 97 children, aged 1 to 13 years, median (range)5.5 (1-12) and 5.0 (1-12) years in the prebiotic and lactulose group, respectively, with chronic FC in a prospective, double-blind, controlled study. No difference was found between groups after the treatment period in defecation frequency, fecal incontinence frequency, abdominal pain, flatulence and the need for step-up medication. However, stool consistency was softer in the lactulose group (P=0.01) [78].

The third study by Weber et al. [79] investigated the effect of a prebiotic and fiber mixture (FOS, inulin, gum arabic, resistant starch, soy fiber, cellulose) versus a placebo in 54 children, aged 4 to 12 years (means 8.5±1.8 and 7.7±2.4 years for the prebiotic and placebo group, respectively), with chronic FC in a randomized, placebo-controlled, double-blind clinical trial. No significant difference was found in therapeutic failure between the prebiotic group (34.6%) and the placebo group (35.7%, P=0.933). A significant difference was found in the mean increase of stool frequency (0.53 in the prebiotic group compared to 0.23 in the placebo group, P=0.014). Moreover, the passage of non-hardened stool was higher in the fiber group; 60.0% in the prebiotic group, 16,7% in the placebo group (P=0.003) [79].

The fourth study by Beleli et al. [80] investigated the effect of GOS versus a placebo in 20 children, aged 4-16 years (mean 8.8±4.1), with chronic FC in a double-blind, placebo-controlled crossover study. There were significant changes for several parameters, namely an increase in bowel movement frequency (P<0.0001), decrease in stool consistency (P=0.0014) and relief of defecation straining (P<0.0001) [80].

Lastly, Closa-Monesterolo et al. [81] investigated the effect of inulin-type fructans (70% oligofructose, 30% lcFOS) versus a placebo in 17 children, 2 to 5 years of age (means 3.72±1.07 and 4.03±0.79 years in the prebiotic and placebo groups respectively), with chronic FC in a double-blind, randomized, placebo-controlled parallel group trial. Stools were significantly softer in the prebiotic group compared to placebo (1.63±0.64 versus 2.57±0.58 respectively, P=0.003). Moreover, stool consistency became softer in the prebiotics group (from 2.2 to 2.6 on the modified Bristol Stool Scale, P=0.040) over time while there was no change in the placebo group [81].

In conclusion, prebiotics in children with chronic functional constipation are highly heterogeneous, not only in study design, population, duration, follow-up, dosages of treatment and types of prebiotics used, but also in primary outcomes. However, there seems to be a trend towards softer stools in studies that used prebiotics in children with FC. As for infant colic, studies on the role of the intestinal microbiota in children with functional constipation in comparison to healthy children is clearly needed as well as large, high-quality RCTs with fibers and/or prebiotics.

Table 4 | Summary of intervention studies in children with functional constipation

Study	Intervention	Power	Age group	Duration	Dosage	Disease	Outcome
Dietary fiber							
Loening-Baucke et al., 2004 [74]	Glucomannan vs placebo	31	4-12 years	8 weeks (2 x 4 weeks cross-over)	100mg/kg body weight per day (max 5 g/day)	Chronic functional constipation	No significant change in defecation or fecal incontinence frequency. Significant difference in the percentage of children with <3 bowel movements per week; 19% glucomannan vs 52% placebo and abdominal pain; 10% glucomannan vs 42% placebo
Castillejo et al., 2006 [75]	Cocoa husk and betafructosans vs placebo	48	3-10 years	4 weeks	3-6 years: 2 x 5.2g per day 7-10 years: 2 x 10,4g per day	Chronic functional constipation	No significant differences in defecation frequency or pain during defecation, reported by parents.
Üstündağ et al., 2010 [76]	PHGG vs lactulose	61	4-16 years	4 weeks	4-6 years: 3g per day 6-12 years: 4g per day 12-16 years: 5g per day	Chronic functional constipation	Significant improvement in defecation frequency, stool consistency and abdominal pain was found in both groups. Significant more bowel movements in the lactulose group.
Chmielewska et al., 2011 [77]	Glucomannan vs placebo	72	3-16 years	4 weeks	2 x 1,26 g per day	Chronic functional constipation	No significant differences in treatment success between both groups. Higher stool frequency in the glucomannan group, but only at week 3 significant.
Prebiotics							
Bongers et al., 2007 [65]	Sn-2 palmitic acid and oligosaccharide mix formula (90% GOS, 10% lc-FOS) vs control formula	35 (24 full cross-over)	3-20 weeks	6 weeks	0.8g/100 ml	Chronic functional constipation	Period 1; Non-significant improvement of stool consistency in OS-formula group. Period 2; significant improvement in stool consistency in OS-formula group compared to control formula.

Table 4 | continued

Study	Intervention	Power	Age group	Duration	Dosage	Disease	Outcome
Kokke et al., 2008 [78]	Fiber mixture (GOS, inulin, soy fiber, resistant starch) vs lactulose	97	1-12 years	13 weeks	10g per day	Chronic functional constipation	No significant difference in defecation or fecal incontinence frequency, abdominal pain, flatulence and the need for step-up medication. Consistency of stools was significant softer in lactulose group.
Weber et al., 2014 [79]	Fiber mixture (FOS, inulin, gum arabic, resistant starch, soy fiber, cellulose) vs placebo	54	4-12 years	4 weeks	<18kg bw: 3.8 g per day, >18 kg bw: 7.6 g per day	Chronic functional constipation	No significant difference in colonic transit time and therapeutic failure. Significant change in daily bowel movements and passage of non-hardened stools was significant in the fiber group compared to placebo.
Beleli et al., 2015 [80]	GOS vs placebo	20	4-16 years	75 days	1.7g/day	Chronic functional constipation	Significant increase of bowel movement, relief of defecation straining and decrease in stool consistency in GOS vs placebo
Closa-Monasterolo et al., 2016 [81]	Inulin-type fructans (70:30 sc-FOS:inulin) vs placebo	17	2-5 years	8 weeks	2 x 2g per day	Chronic functional constipation	Significant improved stool consistency in inulin group

Abbreviations; bw: body weight, FOS: fructooligosaccharides, GOS: galacto-oligosaccharides, lc-FOS: long-chain fructo-oligosaccharides , OS: oligosaccharide, PHGG: partially hydrolyzed guar gum, sc-FOS: short-chain fructo-oligosaccharides

FUNCTIONAL ABDOMINAL PAIN (FAP) AND IRRITABLE BOWEL SYNDROME (IBS)

Definition, prevalence and etiology

Functional abdominal pain and irritable bowel syndrome are common FIGDs in children with an estimated worldwide prevalence of 13.5% for 4-18 year-old children [83]. These disorders are associated with a reduced quality of life [84], excess use of health care services [85-87], school absenteeism, and co-morbid anxiety and depression [88-90]. About 30% of health care visits from children aged 4 to 16 years are due to abdominal pain [91]. FAP and IBS are two FGIDs that, after appropriate medical evaluation, cannot be attributed to another medical condition [92]. FAP and IBS are diagnosed according to the Rome IV criteria [54]. Complaints of IBS include abdominal discomfort or pain, and altered bowel habits. Four types of IBS can be distinguished based on bowel dysfunction: diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), alternating stool forms (IBS-A), and unsubtyped (IBS-U) [93]. Despite its high prevalence the cause of IBS is not fully understood and it is unlikely that one single factor will be the cause for all subtypes of IBS. Gut hypersensitivity and altered gut motility are implicated in both FAP and IBS [94]. Multiple risk factors have been linked, including hypersensitivity to food products, psychological factors such as child abuse, stress, depression and anxiety, genetic factors, and alterations of the intestinal microbiota [91, 95].

Pharmacological options for the treatment of FAP and IBS include antispasmodics, antidepressants, anti-reflux agents, antihistaminic agents, and laxatives. Nonpharmacological options include cognitive behavioral therapy, hypnotherapy, dietary interventions, and pre-, pro- or synbiotics [95].

Intestinal microbiota, FAP and IBS

In terms of microbiological differences in children with IBS compared to healthy children, one study found a significantly greater percentage of *γ-Proteobacteria* ($P>0.05$) (a group containing many opportunistic pathogens). Furthermore *Haemophilus*, *Dorea* and *Veillonella* were more abundant with reduced potential butyrate-producing *Eubacterium* and *Anaerovarax* [91]. However, in the second study in children with IBS-D, the proportions of *Veillonella*, *Prevotella*, *Lactobacillus*, and *Parasporobacterium* were increased together with reducing members, also described as beneficial, *Bifidobacterium* and *Verrucomicrobium* [96]. Moreover, the species *Haemophilus parainfluenzae* was identified as a prominent component in children with IBS. In addition, specific IBS subtypes could be successfully classified according to their intestinal microbiota with accuracies exceeding 95% [91]. This indicates that there are not only microbial differences between healthy children and children with IBS, but also differences according to the IBS subtypes.

Influence of dietary fiber and prebiotics

Five RCTs were identified that studied the effect of dietary fiber in children with functional abdominal pain or irritable bowel syndrome (**Table 5**).

The first study by Feldman et al. [97] dates back to 1985. This study investigated the effect of corn fiber versus a placebo in 52 children, aged 5 to 15 years (mean; 9.37 years), with simple, idiopathic, recurrent abdominal pain in a randomized, double-blind, placebo-controlled study. A statistically significant and clinically relevant decrease in pain attacks (at least 50% less) was found in 13 children in the fiber group, compared to 7 in the placebo group (P=0.04) [97].

A year later Christensen [98] investigated the effect of ispaghula husk (seed coats of the plant *Plantago ovata Forssk*) versus a placebo in 31 children, aged 3 to 14 years, with recurrent abdominal pain in a double-blind, randomized, controlled trial. No significant differences were found in the number of abdominal pain episodes between both groups [98].

More recently Romano et al. [99] studied the effect of partially hydrolyzed guar gum versus a placebo in 60 children, aged 8 to 16 years (means of 12.3±2.0 and 13.1±1.5 years in the fiber and placebo group, respectively), with chronic abdominal pain and irritable bowel syndrome in a randomized, double-blind, placebo-controlled study. A significant higher level of efficacy was found for the fiber group compared to the control group (43% versus 5%, P=0.025), reduced clinical symptoms of the Birmingham IBS score (media 0±1 versus 4±1, P=0.025) and also normalized bowel habit (40% versus 13.3%, P=0.025).

The most recent study by Horvath et al [100] investigated the effect of glucomannan versus a placebo in 84 children, aged 7 to 17 years (means of 11.6±3.0 and 11.3±2.5 in the fiber and placebo group, respectively), with abdominal pain-related FGIDs in a double-blind, placebo-controlled, randomized trial. No differences were found for the parameters 'no pain' and 'treatment success' (defined as no pain or a decrease ≥ 2/6 points on the FACES Pain Scale Revised) between groups. Moreover, no significant differences were found in the secondary outcomes either (i.e. abdominal cramps, abdominal bloating, nausea or vomiting and stool consistency) [100].

Shulman et al. [101] performed a randomized, double-blind study in 103 children (mean: 13±3 years) with IBS seen at primary or tertiary care settings. Children were assigned to groups given psyllium (n=37) or placebo (maltodextrin, n=47). Children in the psyllium group had a greater reduction in the mean number of pain episodes than children in the placebo group (mean reduction of 8.2±1.2 and 4.1±1.3 after receiving psyllium or placebo, respectively; P=0.03); the level of pain intensity did not differ between the groups. At the end of the study period, the percentage of stools that were normal (Bristol scale scores, 3-5), breath hydrogen or methane production, intestinal permeability, and microbiota composition, were similar between groups. However, a limitation of the study mentioned above is that of the 3 primary outcomes (i.e., change in the severity of abdominal pain, frequency of abdominal pain, and the proportion of stools that were normal), only change in abdominal pain frequency showed a significant benefit (P=0.03) for children treated with psyllium [105]. In contrast there was no significant difference in the total abdominal pain frequency after treatment between groups, whereas the third primary outcome even showed a trend toward a negative effect of psyllium compared with placebo.

In conclusion, three studies with fibers showed a clinically and statistically significant improvement in symptoms [97] and pain attacks [99] in children with FAP or IBS. On the

contrary, another two studies did not find any significant differences or changes in the number of abdominal pain episodes [98, 100]. These contradictory findings might be due to the overall low methodological quality of the two studies [97, 99]; in addition, all the studies used different types of fibers, different dosages and different primary outcomes. Moreover, the name and definitions of 'FAP' and 'IBS' disorders have changed over time which makes it hard to compare studies. No RCTs were identified that had investigated the effect of prebiotics on FAP and IBS in children so far and thereby recommendations cannot be provided. This emphasizes the need for well conducted RCTs investigating the effect of prebiotics in children with FAP or IBS. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) are short-chain carbohydrates which are implicated in IBS. Mechanisms may involve poor absorption of some FODMAPS (notably fructose) in the small intestine thereby distending the small intestine with water due to osmotic load, and/or they reach the colon (e.g. inulin) where they are fermented by the microbiota producing gas and flatulence. Such effects are implicated in symptoms experienced by IBS patients [106]. It seems contradictory that fermentable oligosaccharides, some of which are prebiotics, might improve IBS symptoms, and yet exclusion on a low FODMAP diet might also relief symptoms for some patients with IBS. This can be due to the multifactorial etiology of IBS and heterogeneity of symptoms. The efficacy of a low FODMAP diet has been reported in children in two studies (**Table 5**).

Chumpitazi et al. [102] investigated if a diet low in FODMAPs decreased IBS symptoms in a pilot study with 8 children, aged 7 to 16 years. Significant decreases were found compared to baseline in pain frequency (from 11.5±6.3 to 6.3±6.8, $P<0.05$), pain severity (from 1.8±1.1 to 0.8±0.7, $P<0.05$) and pain-related interference with activities (from 9.9±7.9 to 6.3±7.2, $P<0.05$). Moreover, 4 children were identified as responders (50%, responders, defined as; >50% decrease in abdominal pain and pain frequency while on the FODMAP diet) [102]. Subsequently, Chumpitazi et al. [103] conducted a second study to evaluate the efficacy of a diet low in FODMAPs versus a typical American childhood diet in 33 children, aged 7-17 years, with IBS in a double-blind, crossover trial. Compared to the baseline, children had fewer episodes of abdominal pain during the low FODMAP diet ($P<0.01$), but more episodes during the typical American childhood diet ($P<0.01$) [103].

These two pediatric IBS studies on a low FODMAP diet show a decrease in abdominal pain, however, both studies have low power and only investigated short-term effects. Therefore, more interventions that are sufficiently powered are needed to investigate long-term safety, efficacy and effects in children with FAP or IBS [104].

Table 5 | Summary of studies in children with FAP or IBS

Study	Intervention	Power	Age group	Duration	Dosage	Disease	Outcome
Dietary fiber							
Feldman et al., 1985 [97]	Corn fiber vs placebo	52	5-15 years	6 weeks	2 x 5g corn starch per day	RAP	Clinically and statistically significant decrease in pain attacks in corn fiber group; 50% in corn fiber group vs 27% in placebo group
Christensen, 1986 [98]	Ispaghula husk vs placebo	31	3-14 years	6 weeks	2 x 5mL solution per day, amount of fiber not given	RAP	no difference in the number of episodes of abdominal pain
Romano et al., 2013 [99]	PHGG vs placebo	60	8-16 years	4 weeks	5g PHGG per day	IBS-C, IBS-D or CAP	Clinical symptom improvement and improvement in bowel habits in PHGG group
Horvath et al., 2013 [100]	Glucomannan vs placebo	84	7-17 years	4 weeks	2 x 1.26 g glucomannan per day	FIGDs	No significant differences
Shulman et al., 2016 [101]	Psyllium fiber vs placebo	103	7-18 years	6 weeks	7-11 years of age: 6 g/day 12-18 years of age: 12 g/day	IBS	Significant reduction in the mean number of pain episodes in the psyllium group. No differences between groups in pain intensity, percentage of normal stools, breath hydrogen and methane production, intestinal permeability and microbiome composition
FODMAP							
Chumpitazi et al., 2014 [102]	Low FODMAP compared to baseline period	8	7-16 years	2 weeks	n/a	IBS	Significant decrease in the number of pain episodes, mean and max pain severity and pain limiting activities
Chumpitazi et al., 2015 [103]	Low FODMAP vs typical American childhood diet	33	7-17 years	2 weeks	n/a	IBS	Significant decrease in abdominal pain frequency in low FODMAP group

Abbreviations; CAP: chronic abdominal pain, FIGDs: functional gastrointestinal disorders, FODMAP: fermentable oligosaccharides disaccharides monosaccharides and polyols, IBS: irritable bowel syndrome, IBS-C: irritable bowel syndrome constipation-predominant, IBS-D: irritable bowel syndrome diarrhea-predominant, PHGG: partially hydrolyzed guar gum, RAP: recurrent abdominal pain

CONCLUSION

In this review differences in the composition of the microbiota between healthy children and children with FGIDs are described. There are indications for the presence of a specific microbial signature in the intestinal microbiota of infants with colic. The limited data for children with IBS also suggest that the intestinal microbiota composition is different compared to healthy controls. In contrast, the data for the microbiota composition of constipated children in comparison with healthy controls is contradictory. Currently, the differences in analysis methods, reporting of the level of taxonomy rank and high inter-individual variability, prevent strong conclusions from being drawn, and thus clearly more data are required. Furthermore, the function of the microbial groups and impact on health is often not completely clear, which makes it hard to formulate hypotheses on potential mechanisms. Studies on the crosstalk between the microbiome and the host are ongoing and technical advances in analyzing genomes, transcriptomes and proteomes will help to clarify the roles of the intestinal microbiome in health and disease.

In addition, this review stresses the need for well-designed large randomized controlled trials evaluating the effect of different dietary fibers and prebiotics in infant colic, constipation, FAP and IBS. The studies as described in this review are heterogeneous in design, population, duration, follow-up, dosages of treatment and types of fibers or prebiotics used as well as primary outcomes, which makes it difficult to draw general conclusions on the influence of fibers and prebiotics in FGIDs in children.

EXPERT COMMENTARY

FGIDs are a prevalent and serious issue in the pediatric population which have a significant impact on quality of life of patients and patient families, besides health costs [84, 87, 107, 108]. Modification of the intestinal microbiota via diet and foodstuffs provides a powerful route to influence health. Increasing evidence suggests associations between the microbiome and health outcomes. Differences have been identified between healthy children and children with diseases of the intestinal tract. Moreover, there is evidence that the intestinal microbiota can affect health in the long run. However, it remains a challenge to determine whether there is a causal link between the intestinal microbiota and the disease state for many diseases. Causality has been shown in animal models for some diseases e.g. obesity [109], but it is essential to underpin causality in humans as well. This will require large prospective cohort studies in order to investigate the development of the intestinal microbiome in healthy compared to a disease state. A better understanding of the intestinal microbiota in healthy and children with a disease is essential in order to improve our understanding of the role of the intestinal microbiota in disease development. Furthermore, more studies in children are needed to study the effect of dietary fiber and prebiotics for the full range of GI diseases and other diseases.

FIVE-YEAR VIEW

Although the number of studies that address the complexity and dynamics of the intestinal microbiome is increasing, the knowledge so far does not bridge the gap between pathogenesis in the host, individual microbes and alterations in the gut microbial metabolism and function. Understanding of the host-microbe interactions is vital to be able to assign specific bacterial entities or microbial communities that can use specific fibers or prebiotics, of which the resulting microbiota composition and fermentation products of microbiota metabolism may promote health. In the future, the use of supervised machine learning and data-processing algorithms, that can predict the response of an individual to a given food based on their microbiome, might be used to predict the response of individuals to dietary interventions, and thereby positively influence health outcomes. Ideally, microbiota analysis might be used to specifically design individual recommendations in terms of personalized food, specific prebiotics and/or fibers in order to promote health outcomes.

In addition to the research on the intestinal microbiota, studies are needed to investigate the development of FGIDs in children. Importantly, large studies that assess the microbiota in both healthy children with and without FGIDs are needed. Furthermore, interventions are required that determine if certain prebiotics and/or fibers show clinically relevant effects. The more we understand the complexity of the intestinal microbiome, the more we will be able to recommend specific prebiotics and/or other food ingredients in order to promote health outcomes.

KEY ISSUES

- FGIDs, including colic, functional abdominal pain, irritable bowel syndrome and functional constipation are common problems in children worldwide. The involvement of the intestinal microbiome is clear, but proof for causality and how to adapt the intestinal microbiota for the better is still scarce in children.
- Microbiological differences exist between healthy children and children with several FGIDs, however giving a proper conclusion is hard due to differences in analysis methods, reporting of the level of taxonomy rank and high inter-individual variability.
- There is a lack of large randomized placebo controlled trials evaluating the effect of different fibers and prebiotics in children with FGIDs

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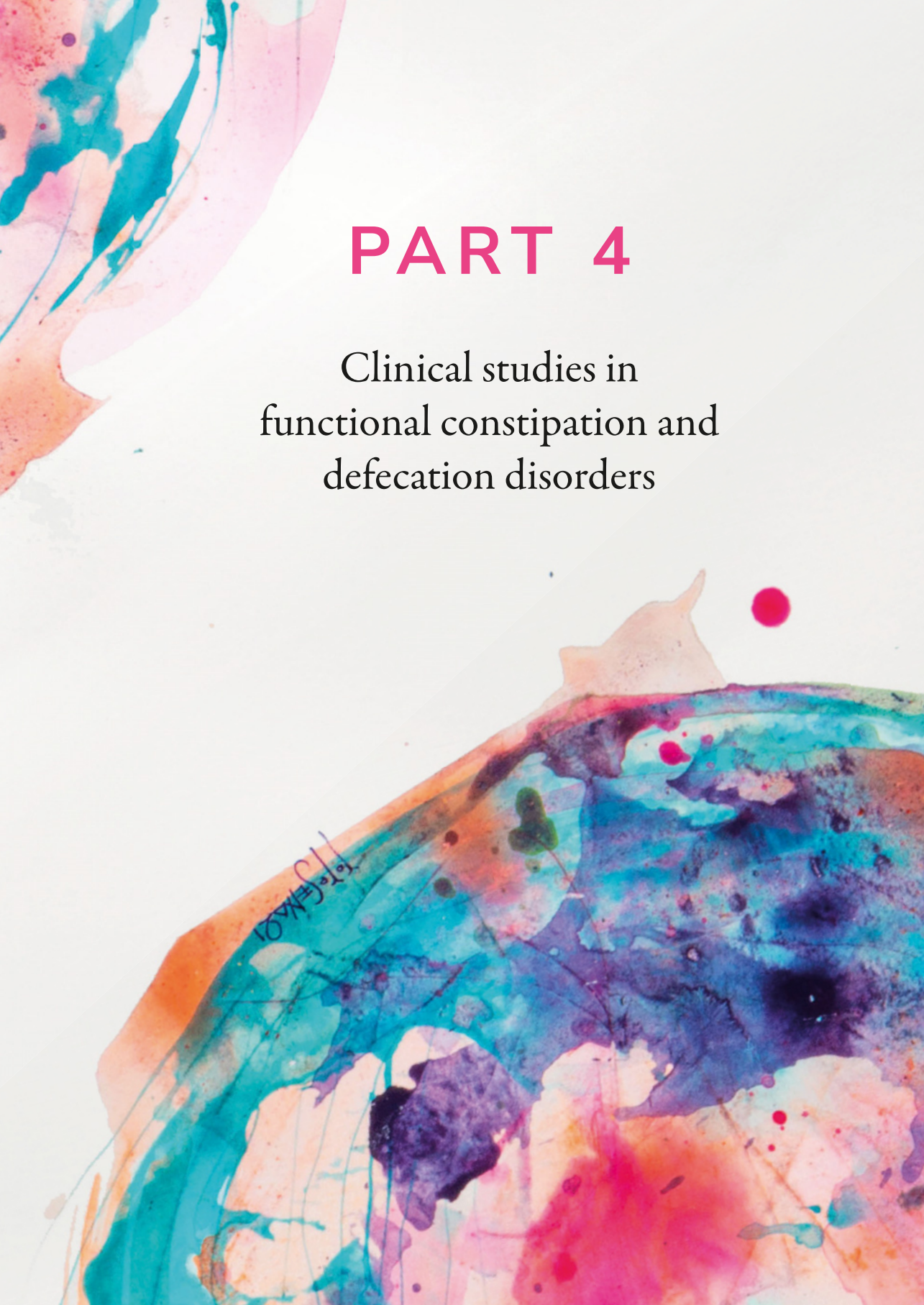
'We moeten niet grijpen naar valse zekerheden en dogmatisme, maar leren omgaan met dubbelzinnigheid, ambiguïteit. Dat is geen wegkijken, dat is goed kijken.'

WAAROM IK VAN SIMONE DE BEAUVOIR HOUD

PART 4

Clinical studies in functional constipation and defecation disorders



A randomized, double-blind, placebo-controlled study to evaluate the effects of inulin on gut intestinal microbiota and bowel habit in adults with functional constipation

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ABSTRACT

Background: Functional constipation (FC) is a widespread condition represented by infrequent and mostly hard bowel movements that substantially affect the patients' quality of life. Supplementing FC patients with a prebiotic fiber could potentially alleviate symptoms by intestinal microbiome modulation.

Objectives: To investigate the effect of daily 12 g inulin intake in adults with FC on stool frequency (SF) and consistency (SC), constipation symptoms (PAC-SYM), quality of life (PAC-QOL) and intestinal microbiota composition by 16s rRNA gene sequencing.

Methods: A randomized, double-blind, placebo-controlled, cross-over trial with a two-week run-in followed by two four-week intervention periods with 12 g inulin or placebo maltodextrin separated by a four-week washout in 40 adults with FC according to Rome III Criteria.

Results: Subjects were 37.32 ± 11.26 years old and 92.5% were female. No within-individual differences between inulin and placebo were detected, but a remarkable carry-over effect of inulin was observed in nearly all secondary outcomes together with 40% fecal samples missing after cross-over. Therefore, we analyzed the run-in and first intervention period as a parallel trial reporting between group-differences. Median weekly SF increased after inulin intake compared to placebo (4.0 [2.75, 4.50] vs 2.50 [2.38, 3.50], p=0.046). Similarly, mean SC increased after inulin intake compared to placebo (2.72±0.22 vs 2.24±0.14; p=0.04). PAC-SYM and PAC-QoL scores also improved above the adopted concept of 'minimally important differences' after inulin, but not after placebo intake, reflected in less rectal tearing and burning (inulin -0.66 vs placebo -0.47, p=0.036) and improved treatment satisfaction (inulin -1.23 vs placebo -0.53, p=0.05). The relative abundances of several bacterial genera were modulated by inulin, but there were no changes for placebo (p>0.10). An 1.3-fold increase in relative abundance levels of bifidobacteria was observed for inulin (p=0.02; q=0.36). Furthermore, only following inulin intake relative abundance of *Anaerostipes* and *Subdoligranulum* spp. increased with a simultaneous decrease in several genera of the *Ruminococcaeae* family.

Conclusion: Daily consumption of 12 g inulin has potential to alleviate FC by improving SF and SC as well as PAC-SYM and PAC-QoL. Observed effects are concomitant with changes in intestinal microbiota composition reflected in an increase in relative abundance of potential butyrate producers and a reduction in constipation-associated genera of the *Ruminococcaeae* family.

INTRODUCTION

Functional constipation (FC) is a widespread healthcare problem in the general population. The exact prevalence of FC depends on the definition used, but in children pooled worldwide prevalence was reported to be 9.5% (ranging from 0.5% to 32.2%) and in adults 14% (ranging from 2.5% to 79%) [1-4]. FC is characterized by irregular, difficult and/or painful to pass hard stools and may be accompanied by abdominal pain. Moreover, fecal incontinence, despite not being considered as one of the diagnostic criteria, may co-exist [5]. FC is a clinical diagnosis based on history and physical examination and is defined according to the Rome criteria, of which the Rome IV criteria are the most recent (**Box 1**) [6-8]. The etiology of FC is poorly understood, but is likely multifactorial and may include psychological factors, lifestyle factors, stress and stressful life events, genetic factors, colonic dysmotility or impaired anorectal function and the intestinal microbiome [2].

Box 1 | Rome III and IV criteria for functional constipation for adults [6,19]. No diagnostic differences exist between Rome III and IV other than that functional bowel disorders are considered to be on a continuum rather than as independent entities [6].

Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

1. Must include two or more of the following:**
   a. Straining during more than ¼ (25%) of defecations
   b. Lumpy or hard stools (Bristol Stool Form Scale 1-2) more than ¼ (25%) of defecations
   c. Sensation of incomplete evacuation more than ¼ (25%) of defecations
   d. Sensation of anorectal obstruction/blockage more than ¼ (25%) of defecations
   e. Manual maneuvers to facilitate more than ¼ (25%) of defecations (e.g., digital evacuation, support of the pelvic floor)
   f. Fewer than three spontaneous bowel movements per week
2. Loose stools are rarely present without the use of laxatives
3. Insufficient criteria for irritable bowel syndrome

**For research studies, patients meeting criteria for opioid-induced constipation (OIC) should not be given a diagnosis of FC because it is difficult to distinguish between opioid side effects and other causes of constipation. However, clinicians recognize that these two conditions may overlap.

International guidelines such as from the National Institute for Health and Care Excellence and the European Society of Neurogastroenterology and Motility advise normal fiber and fluid intake, regular physical activity and pharmacological treatment with e.g. osmotic laxatives [9, 10]. Despite these available pharmacological treatments, many people seek for help by means of alternative or complementary (nonpharmacological) medicine, and in a large survey study, 28% of participants reported to be dissatisfied with their treatment [11]. The above-mentioned nonpharmacological treatments may also include dietary fiber supplementation, which might be of interest in the treatment of FC, since low fiber consumption has been associated with an increased incidence of constipation [12]. Additionally, fiber intake may also contribute to fecal bulking by stimulating growth of certain members of the intestinal microbiota, such as bifidobacteria, leading to an increase

in microbial biomass. Moreover, microbial fiber breakdown may lead to an increase in short chain fatty acids (SCFAs), which may exert osmotic effects (**Figure 1**) [2, 13, 14]. Lastly, other bacterial metabolites such as ferulic acid and quercetin have been associated with an increase in intestinal motility [14]. Besides clinical effects of fibers on FC, one study found that an increase in dietary fiber was associated with considerable direct medical cost-savings in patients with constipation, potentially exceeding $12 billion annually among adults in the US [15].

Some dietary fibers may also be classified as prebiotics, which are defined by the International Scientific Association of Pro- and Prebiotics as: 'a substrate that is selectively utilized by the host microorganisms conferring a health benefit' [16]. Inulin, extracted from chicory roots is a well-established prebiotic [16]. Inulin passes though the upper gastrointestinal tract undigested, reaching the colon intact where it is fermented by the intestinal microbiota [17]. Native chicory inulin currently holds a European Food Safety Authority (EFSA) approved health claim on the maintenance of normal defecation by increasing stool frequency (SF). Besides specific changes induced by inulin in intestinal microbiota composition, several studies indicated that inulin has the potential to alleviate constipation symptoms [18].

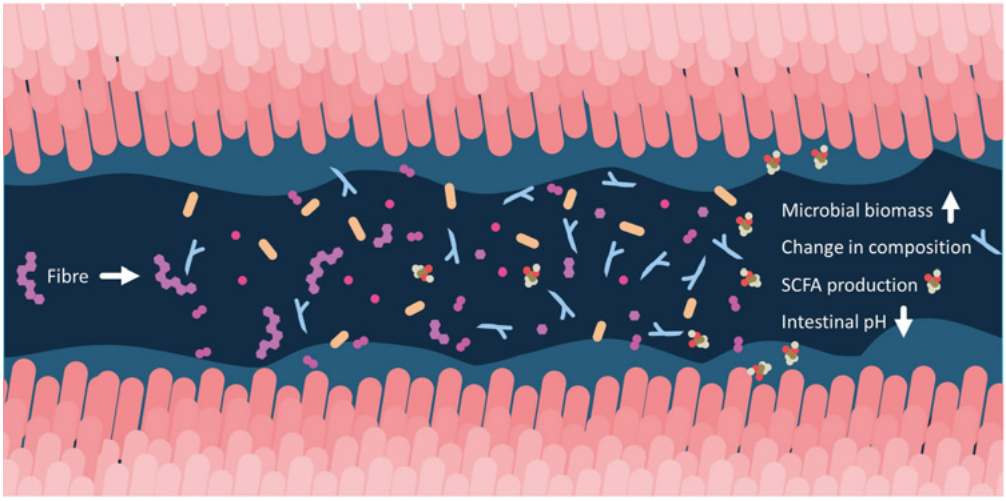

Figure 1 | Potential working mechanisms of prebiotic fibers such as chicory fructo-oligosaccharides. Prebiotic fibers are not digested in the small intestine and reach the colon intact. Fermentation of these fibers in the large intestine may increase peristalsis through intestinal distention by increasing stool volume, a decrease in intestinal pH and the production of short chain fatty acids, which may induce more colonic peristalsis.

The aim of this study was to investigate the effect of the consumption of 12 g inulin versus placebo on SF and stool consistency (SC), constipation symptoms, quality of life (QOL), physical activity, resort to laxatives and intestinal microbiota composition in FC subjects.

MATERIALS AND METHODS

STUDY DESIGN

This study was a randomized, double-blind, placebo controlled, cross-over trial. Subjects were recruited through a clinical research organization's database, general practitioners offices, hospital clinics, and adverts in local newspapers in the surroundings of Cork, Ireland. Subjects were screened and after enrollment participants entered a two week run-in period during which they were randomized to receive either 12 g inulin (Frutafit® HD native inulin, Sensus B.V., Roosendaal, the Netherlands) or placebo maltodextrin (MD20, Avebe, Foxhol, the Netherlands) for four weeks of intervention. The first intervention period was followed by a wash-out period of four weeks and a second cross-over intervention period of four weeks. During the study a variety of measurements were performed at specific time points (**Figure 2**). In the **Supplementary material**, data can be found for the full cross-over trial. Periods will be referred to as: run-in: day -14 to -1, intervention period 1: day 0 to day 28, wash-out: day 29 to day 56, intervention period 2: day 57 to day 84.

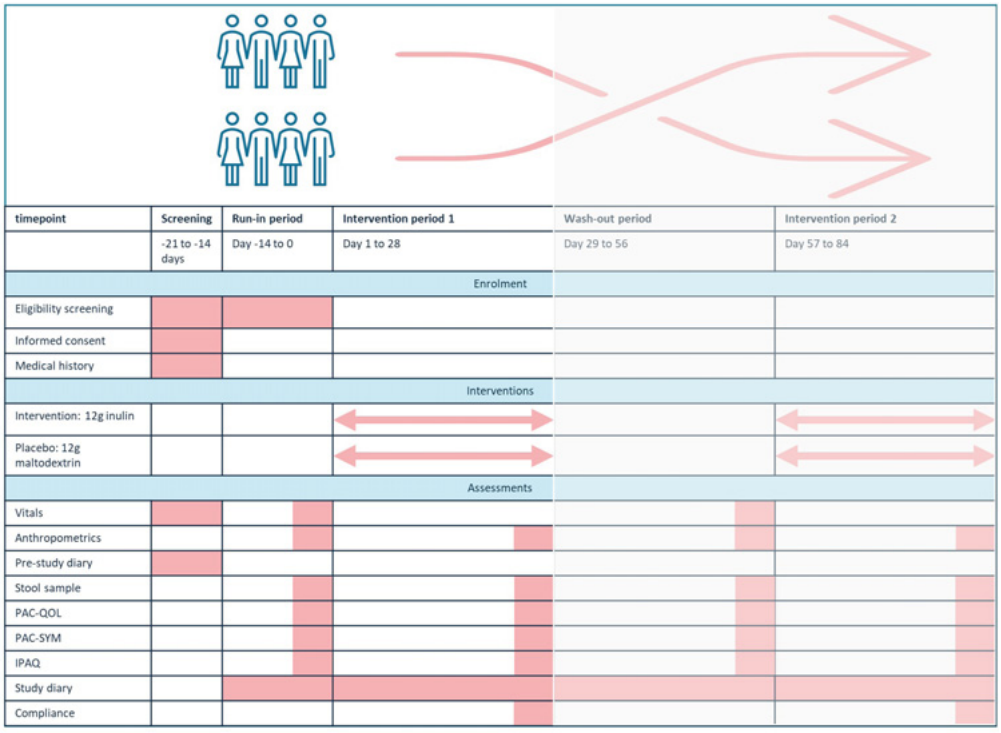

Figure 2 | schematic overview of the study in terms of enrolment, interventions and assessments. The original study is depicted, where only the brightly colored part of the trial is reported in this study. Pink indicates the moment at which a certain measurement was conducted or requested from study subjects. Adapted from the SPIRIT checklist [83].

This study was conducted at Atlantia Clinical Trials, Cork, Ireland, in accordance with the principles of the Declaration of Helsinki and in compliance with International Council for Harmonization Good Clinical Practice, approved by the Cork Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork. Reference ECM 4 (v) 01/09/15, and registered at ClinicalTrials.gov (NCT05447481). Written informed consent was obtained from all study participants.

PARALLEL STUDY ANALYSES

This study was designed and initially analyzed as cross-over design to assess within-individual differences between inulin and placebo intake. However, when testing for a possible carry-over effect we detected that an improvement in SF as well as constipation-related symptoms and quality of life persisted through the wash-out into the second intervention period. Moreover, 40% of the subjects (n=15) of both arms did not provide a fecal sample during wash-out and the second intervention period, which substantially impaired the detection of within-individual changes in microbiota composition. Therefore, here we report first the main outcomes of the cross-over trial as within-subject differences between inulin and placebo intake. Then we proceed to assess the first phase of the study only (run-in and intervention period 1) as parallel trial and reporting between-group differences in inulin and placebo intake.

SUBJECTS

Inclusion criteria for subjects were; 18 to 75 years of age, diagnosed with FC according to the Rome III criteria (**Box 1**) [19]. Subjects were evaluated to be in good general health, as determined by the investigator, and asked to continue their normal diet, but not take any pro- or prebiotic products/supplements or dietary fiber supplements for the duration of the study. Subjects were excluded when they were hypersensitive to any components of the test product or had an acute or chronic, unstable and untreated disease or any condition which contraindicated entry to the study. Also, subjects with a history of laxative abuse, or drug and/or alcohol abuse were excluded. Subjects taking any probiotic or prebiotic product or supplement within two weeks of the screening visit were excluded. Lastly, women who wished to become pregnant during the study, were pregnant or were lactating were excluded as well.

POWER CALCULATION

A total sample size of 39 subjects was required, taking a drop-out rate of 10% into account, to detect a minimal difference of 1 bowel movement per week between inulin treatment and placebo with a standard deviation (SD) of 2, using a power of 80% and a significance level of 5% based on a two-sided Wilcoxon non-parametric test [20].

RANDOMIZATION, ALLOCATION CONCEALMENT, BLINDING AND INTERVENTION

Subjects were equally and randomly assigned to both groups. The randomization was performed by an independent statistician, and used the uniform random number function in SPSS (IBM Corp. IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp). Study products were similar in flavor, appearance and packaging and both subjects and research personnel involved in this study were blinded, ensuring true allocation concealment. After initial blinded data analysis for internal use data was unblinded, and data-analysis was performed unblinded.

Subjects consumed two doses of 6 g per day for four weeks of either inulin (Frutafit® Inulin HD) or placebo maltodextrin (MD20), except for the first three days of the study period in which they only took one dose of 6 g per day. The study product was provided in dark plastic 120 ml bottles, the subjects were instructed to add 60 ml of water to the bottle and shake until the study product had dissolved. As marker of compliance subjects were instructed to return all used and unused study product, and compliance was checked by counting the number of empty bottles. Each participant would receive 60 bottles, where 53 was the total expected number to be used to be 100% compliant; compliance was calculated as: (the total number of bottles consumed/53)*100. Maltodextrin was chosen as placebo as it is a digestible carbohydrate, making it also a suitable placebo for interventions investigating the intestinal microbiota composition [21]. Subjects received all bottles of the respective intervention products on day 0 for the first four weeks of intervention.

MEASUREMENTS

The effect of inulin supplementation on FC in adults was assessed as follows. The primary outcome, as measured via bowel diaries, was the change in the number of bowel movements per week from run-in to the last two weeks of intervention period 1 of inulin versus the placebo. Secondary outcomes were the change in the following parameters for inulin compared to placebo: (1) SC as measured by the Bristol Stool Form Scale (BSFS), (2) meeting the Rome III criteria as dichotomous outcome, (3) Patient Assessment of Constipation – Symptoms (PAC-SYM) scores, (4) Patient Assessment of Constipation – Quality Of Life (PAC-QOL), (5) International Physical Activity Questionnaire (IPAQ), (6) resort to laxatives, and (7) intestinal microbiota composition [22-24]. The PAC-SYM is a retrospective questionnaire (recall period of two weeks) with 12 items for assessing the severity of patient-reported symptoms. This tool creates an overall score and three subscores of abdominal symptoms (four items), rectal symptoms (three items) and stool symptoms (five items) [23]. The PAC-QOL is a retrospective questionnaire (recall period of two weeks) with 28 items for assessing a patient-reported quality of life of the impact of constipation symptoms. The PAC-QOL creates an overall score and four subscores of worries/concerns (eleven items), physical discomfort (four items), psychosocial discomfort (eight items) and satisfaction (five items). For the PAC-SYM a difference of -0.6 is regarded as the minimal important difference and a slightly larger difference

of -0.75 was suggested to be used in placebo-controlled clinical trials [25]. For the PAC-QOL similar minimally important differences are defined: a change of -0.5 or more is considered the minimum important difference for the overall score. For both the PAC-SYM and PAC-QOL a lower score on the overall score or subscale scores indicates less severe symptoms and a higher quality of life, respectively. Besides these outcomes also blood pressure (BP), heart rate (HR), and temperature as well as anthropometric measures including weight, height and body mass index (BMI) and medication use during the trial were recorded.

MICROBIOTA ANALYSIS

Subjects were asked to provide a fecal sample in the run-in period and at the end of each intervention period and the wash-out (**Figure 2**). Samples were collected at home, stored in home freezers until they were transferred to the study center in cooler bags with a frozen ice pack and stored at -20°C. DNA was extracted using a repeated bead-beating step and the Maxwell® 16 instrument (Promega, Leiden, The Netherlands). 0.25 g of fecal material was added to a bead-beating tube with 700 µl Stool Transport and Recovery (STAR) buffer, 0.5 g of sterilized zirconia beads (0.1 mm), and five glass beads (2.5 mm). These tubes containing the fecal sample were bead-beaten three times (60 s × 5.5 ms) and incubated for 15 min at 95 °C at 300 rpm. Samples were then centrifuged for 5 min at 4 °C and 14,000 g and supernatants transferred to sterile tubes. Pellets were re-processed using 300 µl STAR buffer and both supernatants were pooled. DNA purification was performed with a customized kit (AS1220; Promega) using 250 µl of the final supernatant pool. DNA was eluted in 50 µl of DNAse- and RNAse-free water and its concentration measured using a DS-11 FX+ Spectrophotometer/Fluorometer (DeNovix Inc., Wilmington, USA). The V4 region of the 16S ribosomal RNA (rRNA) gene was amplified in duplicate PCR reactions for each sample in a total reaction volume of 50 µl. Primers used for this were 515F (5'-GTGTGYCAGCMGCCGCGGTAA-3') and 806R (5'-CCGGACTACNVGGGTWTCTAAT-3'). The master mix contained 1 µl of a unique barcoded primer, 515F-n and 806R-n (10 µM stock concentration), 1 µl dNTPs mixture (200 µM), 0.5 µl Phusion Green Hot Start II High-Fidelity DNA Polymerase (2 U/µl; Thermo Scientific, Landsmeer, The Netherlands), 10 µl 5× Phusion Green HF Buffer, and 36.5 µl DNAse- and RNAse-free water. The amplification program included 30 s of an initial denaturation step at 98°C, followed by 25 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 10 s, elongation at 72 °C for 10 s, and a final extension step at 72 °C for 7 min. The PCR product was visualized in 1% agarose gel (~290 bp) and purified with CleanPCR kit (CleanNA, Alphen aan den Rijn, The Netherlands). The concentration of the purified PCR product was measured with Qubit dsDNA BR Assay Kit (Invitrogen, California, USA), and 200 ng of microbial DNA from each sample was pooled for the creation of the final amplicon library which was sequenced (150 bp, paired-end) on the Illumina HiSeq. 2000 platform (GATC Biotech, Constance, Germany).

STATISTICAL ANALYSIS

All statistical analyses were performed only on an intention-to-treat basis as there were no drop-outs, except for one in the second period of the trial. Data were analyzed using R (version 4.0 or higher) [26], and the packages 'tidyverse' [27], 'ggstatsplot' [28], 'ggplot2' [29], 'phyloseq' [30], 'phyloseqCompanion' [31], 'microbiome' [32], 'mare' [33] and 'rstatix'[34]. Data were checked for normality by visual inspection of Q-Q plots and accordingly analyzed by parametric or non-parametric methods. If not indicated otherwise, parametric tests were performed. Results for outcomes from the bowel diaries (stool frequency, stool consistency, Rome criteria, resort to laxatives) were summarized over the last two weeks of each period and averaged to reflect measurements per week (e.g. SF as bowel movements per week). Since the run-in period was two weeks, these full two weeks were taken into account. Similarly, as the PAC-SYM and PAC-QOL scores are based on a two-week recall periods, also these outcomes are expressed for the same time periods. Lastly, the IPAQ score is a recall over the past seven days and was filled-out at the end of run-in, both intervention periods and the wash-out. Hence, differences in all these outcomes were calculated for the full-crossover as within-individual differences (comparing per person outcomes after inulin intake vs placebo intake) and are reported in the supplementary material. For the analysis of the first trial phase (run-in and intervention period 1) as a parallel trial, we report differences in all outcomes as between-groups differences at the end of intervention period 1, and changes within groups between intervention period 1 and run-in were reported.

MICROBIOTA DATA PROCESSING AND ANALYSIS

Data filtering and taxonomy assignment was performed using the NG-Tax pipeline using default settings [35]. A table, based on Amplicon Sequence Variants (ASV), was created for each sample with the most abundant sequences. Low abundance ASVs were discarded, using a minimum relative abundance threshold of 0.1% [36, 37]. For quality control purposes, two inhouse assembled mock communities were included in the library and compared to their theoretical composition. Moreover, a negative control of the DNA extraction and purification procedure and a water blank were included. Alpha and beta diversity were calculated and visualized using the Microbiome R package [38], which relies among others on the phyloseq [30] and vegan package [39]. Alpha diversity analyses provided within sample information on richness (number of species), and/or evenness (the relative abundance of those species), diversity (combination of richness and evenness) as well as dominance of abundant species and beta diversity was calculated as a measure of variation between samples. Changes in bacterial taxa were calculated and tested using the mare package [33], which relies among others on the 'vegan' [39], 'MASS' [40] and 'glmmADMB' [41] packages. Details of such analyses have been previously described elsewhere [42]. In short, the mare package includes subject as random-factor for dependent data and offers the possibility to analyze data using zero-inflated negative binominal models as well as models

excluding samples where respective taxa are not observed (non-zero models). These outcomes were further substantiated by Linear discriminant analysis Effect Size (LEfSe) analysis [43].

RESULTS

PARTICIPANT CHARACTERISTICS

Forty participants, either consuming first inulin (n=20) or consuming first placebo (n=20), were included and completed the study. The majority of subjects were female (92.5%) with an age (mean ± SD) of 37.32 ± 11.26. **Table 1** depicts the baseline characteristics. No differences were found at baseline between groups (p>0.05). A CONSORT flowchart for enrolment and analysis is presented in **Figure 3** [44]. All participants were above the cut-off limit of 80% consumption (range 92.5% - 113.2%). One participant dropped-out in the second intervention period of the trial. None of the subjects from either of the groups used laxatives during the run-in or the intervention period.

Table 1 | baseline characteristics. All data given are mean ± SD unless otherwise stated.

	Inulin (n=20)	Placebo (n=20)	p-value
Age years	37.05 ± 9.46	37.59 ± 13.06	p=0.88
Gender (female/male)	18/2	19/1	P>0.99
Blood pressure systolic	112.1 ± 12.7	114.1 ± 14.2	P=0.66
Blood pressure diastolic	74.63 ± 9.08	73.28 ± 7.68	P=0.63
Pulse	73.00 ± 11.77	77.22 ± 8.98	p=0.23
Body temperature	36.28 ± 0.31	36.26 ± 0.30	p=0.78
BMI (median [IQR])	24.34 [2.42]	25.53 [5.55]	p=0.60‡
Stool frequency (median [IQR])	2 [1.5-2.0]	2 [2.0-2.12]	p>0.99‡
Stool consistency	1.84 ± 0.57	1.75 ± 0.54	p=0.60
Meeting Rome III criteria	20	20	p>0.99
PAC-SYM	1.98 ± 0.53	1.93 ± 0.60	p=0.76
PAC-QOL	2.04 ± 0.62	1.94 ± 0.62	p=0.60
Physical activity % high, moderate, low	15%, 60%, 25%	20%, 60%, 20%	n.a.
Concomitant medication n (%)	11 (55%)	7 (35%)	n.a.
Resort to laxatives	0	0	p>0.99
Adverse events	0	0	p>0.99

‡ indicates non-parametric testing

CROSS-OVER ASSESSMENT AND SUFFICIENCY OF WASH-OUT

We tested for a possible carry-over effect by comparing primary and secondary outcome variables assessed during wash-out to their run-in levels. While we did not find a carry-over effect on SF, we unexpectedly observed substantial differences between wash-out and run-in for both arms in SC (run-in 1.81 (0.13) vs wash-out 2.05 (0.22), p=0.040) and total PAC-SYM (run-in 1.95 (0.09) vs wash-out 1.77 (0.12), p=0.045), for the latter a lower score indicating an improvement (**Supplementary Table 2**). Moreover, the arm receiving inulin first showed a persisting improved scores for nearly all subscores of the PAC-SYM as well as total and subscores of PAC-QOL (**Supplementary Table 3**).

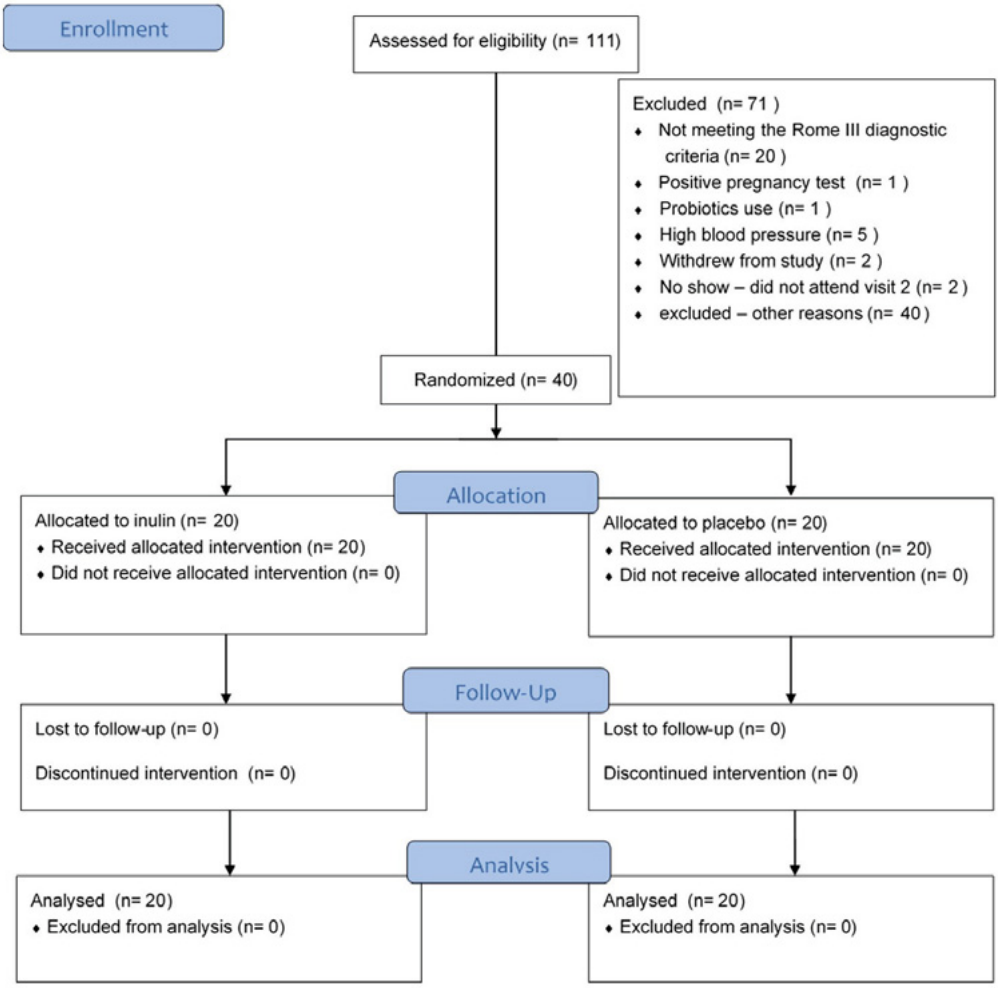

Figure 3 | CONSORT flowchart of participants

Intestinal microbiota composition also remained impacted, which was reflected in alpha-diversity metrices measuring lower richness (Chao1 index:, p=0.049), lower evenness (Simpson index p=0.033), lower diversity (inverse Simpson index: p=0.017, Shannon index: p=0.016) and higher dominance of the most abundant species (Simpson index: 0.038) after washout compared to run-in. Altogether, these analyses indicated that neither the group receiving inulin first, nor the group receiving the placebo first started the second period of the trial as they had started in the first period. No within-individual differences were found for any of the outcomes of SF, SC, PAC-SYM total or subscores and PAC-QOL total and subscores for the full cross-over trial (**Supplementary Table 4**), as a possible consequence of this carry-over issue (**Supplementary Tables 1-7**). Due to the remarkable carry-over effect and the fact that during the second intervention period of the trial 15 participants did not provide a fecal sample, we report between-group differences based on the first phase (run-in and period 1) of the study and assessed as a parallel design.

STOOL FREQUENCY, AS AVERAGES AND CATEGORICAL DATA, BETWEEN GROUPS AND OVER TIME

Median weekly SF [IQR] increased in both groups from run-in to the end of intervention period 1 from 2.00 [1.50-2.00] to 4.00 [2.75-4.50] ($p<0.001$, non-parametric testing) after inulin intake and from 2.00 [2.00-2.12] to 2.50 [2.38-3.50] ($p<0.001$, non-parametric testing) after placebo intake. However, this increase in weekly SF was substantially higher for inulin intake with a median difference from placebo intake of +1.50 (95%CI[0.03, 0.64], p=0.046 (**Figure 4A**).

During run-in only 2.5% and 0% defecated more than 3 times per week in the groups that received inulin or placebo, respectively, while at the end of the treatment period 1, that rose to 55% and 25% for inulin compared to placebo intake (p=0.012), , respectively (**Supplementary Table 5** and **Figure 5A**).

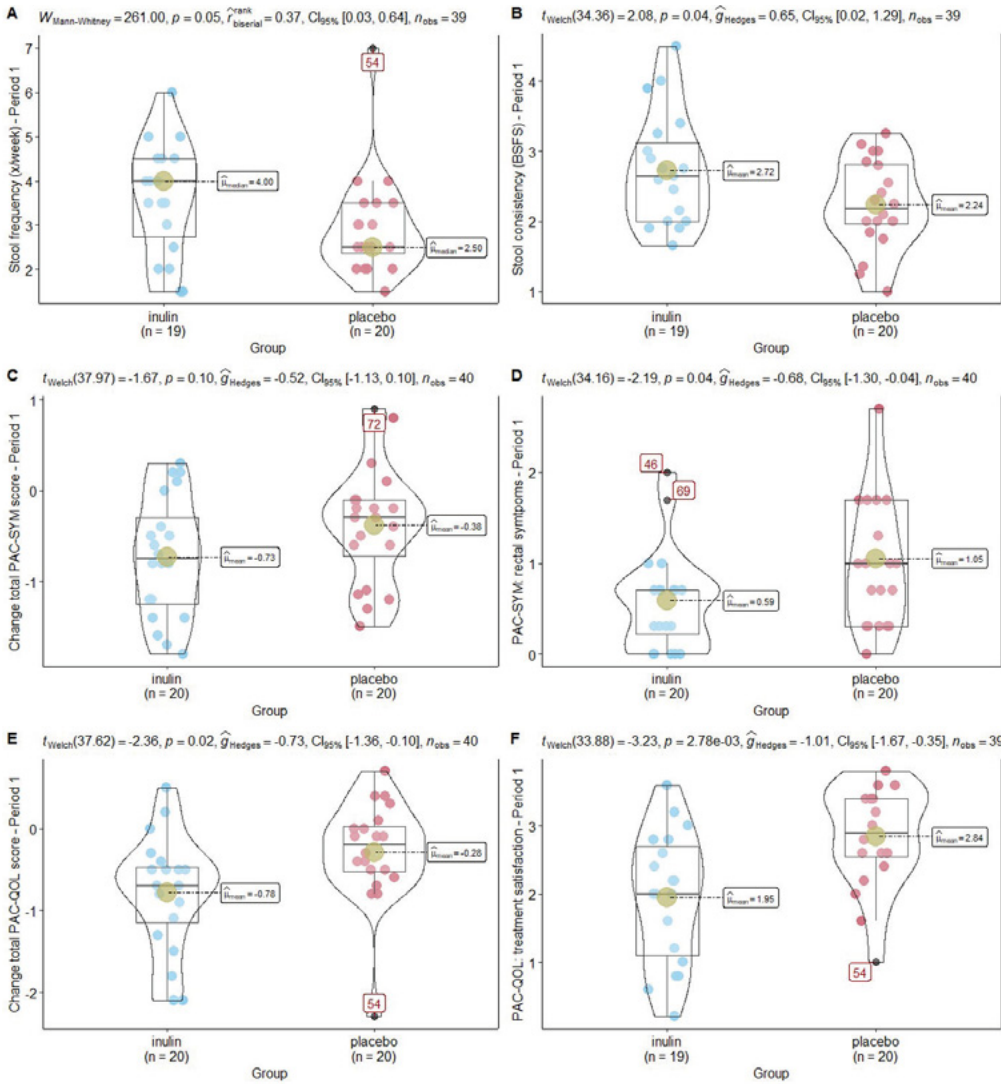

Figure 4 | violin plots for clinical outcomes. **A |** stool frequency between groups at the end of intervention period 1; **B |** stool consistency between groups at the end of intervention period 1; **C |** PAC-SYM total score change between groups from baseline to the end of intervention period 1; **D |** PAC-SYM subscore for rectal symptoms between groups from baseline to the end of intervention period 1; **E |** PAC-QOL total score change between groups from baseline to the end of intervention period 1. **F |** PAC-QOL subscore for treatment satisfaction between groups from baseline to the end of intervention period 1. Numbers indicated in a red square represent statistical outliers, but were taken into account for the analyses. Respective tests are indicated in each graph.

STOOL CONSISTENCY, AS AVERAGES AND CATEGORICAL, DATA BETWEEN GROUPS AND OVER TIME

Mean SC (SEM) increased in both groups from run-in to the end of intervention period 1 with 1.84 (0.13) to 2.72 (0.18) after inulin intake and 1.77 (0.11) to 2.24 (0.14) after placebo intake. However, SC improved more than placebo after inulin intake with a mean difference from placebo of +0.48 (95%CI[0.02, 1.29]), p=0.045 (**Figure 4B** and **Supplementary Table 2**). Stools improved towards softer stool types in both groups (**Figure 5B**). Grouping the BSFS scores into clinically relevant groups of hard (type 1, 2), normal (type 3, 4, 5) and soft (type 6, 7) stools indicated that the percentage of participants with hard stools decreased after inulin intake from 85% to 53% (p=0.004) compared to 87% to 51% after placebo intake (p<0.001), but was not different between groups (inulin: 53% vs placebo: 51%, p>0.99) (**Supplementary Table 6**).

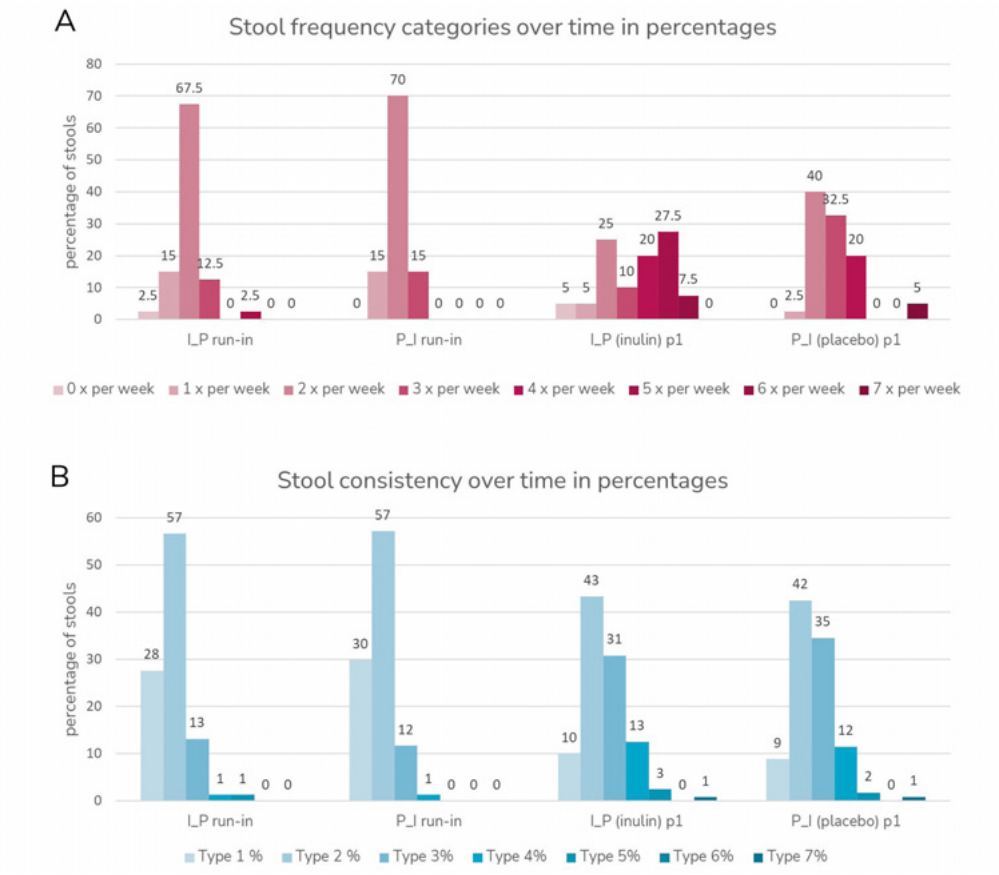

Figure 5 | the distribution of **A |** stool frequency and **B |** stool consistency categories over time. I_P indicates the group that started with inulin intake while P_I indicates the group that started with placebo intake.

ROME CRITERIA AS DICHOTOMOUS OUTCOME BETWEEN GROUPS AND OVER TIME

During the run-in period, all participants met the Rome criteria III for FC (box 1) [45]. This changed over time, albeit not significantly, with eight participants meeting the Rome criteria after inulin intake at the end of the intervention period compared to 14 after placebo intake (p=0.11).

PAC-SYM SCORES AND SUBSCORES BETWEEN GROUPS AND OVER TIME AS MEASURE OF SYMPTOMS

The mean PAC-SYM total scores (SEM) improved in both groups from run-in to the end of intervention period 1 with 1.98 (0.12) to 1.25 (0.13) for inulin intake and 1.93 (0.13) to 1.54 (0.16) for placebo intake, but with a larger change after inulin intake (a change of -0.73 (0.15) compared to -0.38 (0.14) after placebo intake, p=0.10) (**Figure 4C**). The magnitude of change in scores for PAC-SYM has been reported to be of relevance due to the definitions of the minimally important and clinically relevant limits [23, 25]. Hence, the change after inulin intake reached the defined minimal important difference (defined as >-0.6) and close to the more conservative threshold for clinical trials (defined as >-0.75), while this was not the case for placebo intake. Moreover, using these reference values, all subscales showed a similar pattern of improvement after inulin intake, but not after placebo intake: (1) 'abdominal symptoms' improved after inulin intake with -0.79 (0.18) compared to after placebo intake -0.26 (0.20) (p=0.055); (2) 'rectal symptoms', including rectal burning and rectal tearing/bleeding, improved -0.66 (0.16) after inulin intake compared to -0.47 (0.20) after placebo intake (p=0.48); (3) 'stool related aspects' improved after inulin intake with -0.71 (0.20) compared to -0.45 (0.14) after placebo intake (p=0.295) (**Supplemental Table 3**). Moreover, only 'rectal symptoms' was significantly different between groups after intervention period 1 (p=0.036) (**Figure 4D**).

PAC-QOL SCORES AND SUBSCORES BETWEEN GROUPS AND OVER TIME AS MEASURE OF QUALITY OF LIFE

The change after inulin intake in overall PAC-QOL was higher compared to placebo and exceeded the defined minimum important difference [24]. After inulin intake PAC-QOL improved from 2.04 (0.14) to 1.26 (0.14), change: -0.78 (0.16) compared to 1.94 (0.14) to 1.66 (0.16), change: -0.28 (0.14) after placebo intake. As for PAC-SYM the change in scores for PAC-QOL has been reported to be of relevance due to the defined minimum important difference. Again, we observed a larger change after inulin intake with -0.78 (0.16) compared to -0.28 (0.14) after placebo intake (p=0.023) (**Figure 4E**). Hence, again after inulin intake a minimum important difference was found (defined as >-0.5) in overall quality of life [24], while this was not the case after placebo intake. For the subscores of PAC-QOL no minimum important differences are defined, but the subscore for 'treatment satisfaction' differed between

groups with a lower score after inulin than placebo intake (inulin: 1.95 (0.22), placebo: 2.84 (0.17); p=0.003) (**Figure 4F**) and the improvement after inulin intake with -1.23 (0.27) was pronouncedly larger compared to -0.53 (0.21) after placebo intake, p=0.05. No differences were found for the other subscores (**Supplemental Table 3**).

IPAQ SCORE BETWEEN GROUPS AND OVER TIME AS MEASURE OF PHYSICAL ACTIVITY

Significant differences between groups were found in physical activity in the intervention period, but not in the run-in period; run-in $X^2(1, N=40)=1.27$, p=0.53 versus period 1 $X^2(1, N=38)=18.8$, p<0.001 (**Table 2**), indicating higher physical activities in the group with placebo intake. Data for the full cross-over can be found in **Supplementary Table 7**.

Table 2 | IPAQ score

	inulin	placebo	p-value
Run-in			
High	**15.0%**	**20.0%**	
Moderate	**60.0%**	**60.0%**	
Low	**25.0%**	**20.0%**	Chi-square statistic: 1.27, p-value: 0.53
Period 1			
High	**26.3%**	**36.8%**	
Moderate	**36.8%**	**52.6%**	
Low	**36.8%**	**10.5%**	Chi-square statistic: 18.8, p-value: 0.00008

CHANGES IN INTESTINAL MICROBIOTA COMPOSITION

Intestinal microbiota composition assessed by within sample α-diversity changed in subjects consuming inulin, reflected in lower richness (Chao-index p<0.01), lower evenness (Simpson p=0.01) and related lower diversity (inverse Simpson-index p<0.01, Shannon-index p<0.01) as well as higher abundance of dominant taxa (Simpson p=0.026). None of these metrices changed in the placebo group or differed after the intervention period between groups. We did not observe changes in overall intestinal microbiota composition assessed by β-diversity based on pairwise Bray-Curtis dissimilarities (taking into account the relative abundance of observed microbial taxa) between samples neither over time (PERMANOVA inulin p=0.350, placebo p=0.750) nor after intervention period 1 (group explaining 2.2% of variation, PERMANOVA p=0.670). Similarly, no differences were found for weighted UniFrac distances taking into account the relative abundance and phylogenetic relatedness of observed taxa, neither over time (PERMANOVA inulin p=0.296, placebo p=0.138) nor between groups after intervention

period 1 (group explaining 4.4% of the variation, PERMANOVA p=0.117) (**Supplementary Figure 1**). Whereas the overall intestinal microbiota composition did not differ, we observed several changes in the 1% most abundant taxa after inulin intake, which were not observed in the placebo group (p>0.05, q>0.1 **Supplementary Table 8** and **Figure 6**). For individual relative abundances per timepoint, one outlier in the placebo group was found that had a high relative abundance in *Akkermansia* spp. (**Supplementary figure 2,** only placebo, **box 2**). The largest change in relative abundance was observed in *Anaerostipes* spp., which increased with 2.00-fold from 1.13% to 2.26% (non-zero model p<0.01, q=0.02), followed by *Subdoligranulum* spp, which increased with 1.47-fold from 4.10% to 6.15% (p=0.01, q=0.13). *Bifidobacterium* spp. relative abundance increased with a 1.30 fold-change from 14.75% to 19.24% (p=0.24, q=0.25), which was different from placebo. Furthermore, we observed a decrease in relative abundance in three *Ruminococcaceae* genera, which were *Eubacterium coprostanoligenes group*, *Ruminococcus* 2 spp and *Ruminococcus* UC002 (**Supplementary Table 8**). Several other less abundant *Ruminococcaceae* genera decreased in relative abundance after inulin differently from placebo (data not shown), among which the *Ruminococcaceae* NK4A214 group and *Ruminiclostridium* 5, which were also identified by LEfSe analysis to discriminate the intestinal microbiota of subjects after inulin intake from that of subjects consuming the placebo (**Supplementary Figure 3**).

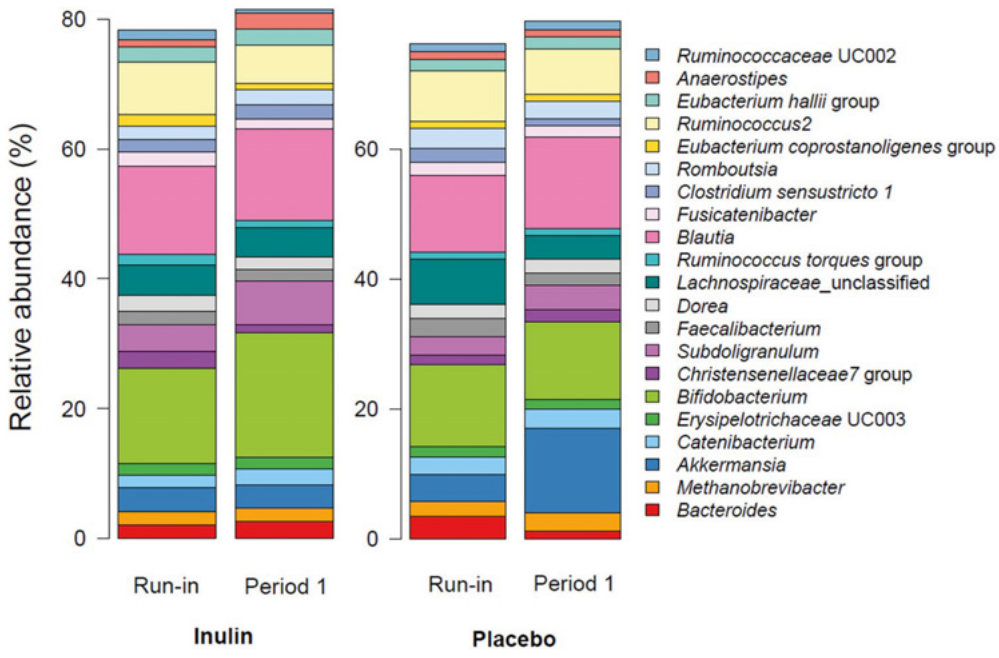

Figure 6 | Relative abundance of the most abundant genera (>1% relative abundance) after inulin and after placebo intake.

ADVERSE EVENTS

During the intervention, seven participants reported possible inulin-related adverse events. The most frequently reported adverse event was flatulence (n=6, 50% moderate and 50% severe intensity), followed by bloating (n=3, 66% moderate and 33% severe intensity), nausea (n=1, moderate intensity) and stomach cramps (n=1, moderate intensity). A complete overview of possibly related and unrelated adverse events during the whole cross-over is given in **Supplementary Table 1**.

DISCUSSION AND CONCLUSION

This study assessed the effect of the consumption of 12 g inulin versus placebo which resulted in an increase in SF and SC compared to placebo. PAC-SYM and PAC-QOL scores improved above minimally important differences after inulin, but not after placebo intake, reflected in less rectal tearing and burning and improved treatment satisfaction. Several bacterial genera were modulated by inulin intake, but there were no changes for the placebo. An 1.3-fold increase in relative abundance levels of bifidobacteria was observed for inulin and relative abundance of *Anaerostipes* and *Subdoligranulum* spp. increased with a simultaneous decrease in several genera of the *Ruminococcaeae* family, which distinguished it from placebo.

The study was designed as cross-over trial, with the aim to assess the effect of inulin as within-individual difference by comparing a subject's response to inulin versus the placebo. Assessing these personal responses bears great informative value for outcomes characterized by high individuality such as intestinal microbiota composition. However, a primary bias in these cross-over designs is a potential carry-over effect of the treatment [46]. Currently, no guidelines exist for an appropriate wash-out period for microbiota studies in humans [47]. Here, a four-week wash-out was estimated to be sufficient to eliminate any carry-over effect even in the intestinal microbiota composition. Evidence of several studies, as summarized by Roberfroid, showed that the effect of inulin-type fructans on the intestinal microbiota should progressively disappear within one to two weeks when intake stops [48]. In line with this, two other cross-over studies appeared successful in this respect and did not report such carry-over effects, but both used lower doses of 3g-8g per day [49, 50]. The most recent cross-over trial was a study in healthy subjects with inulin-type fructans investigating intestinal microbiota composition in low dietary fiber consumers, although the doses used were lower at approximately 3 or 7 g [49]. Another cross-over study in subjects with constipation also gave a 12 g dose [17, 50]. Nevertheless, we observed a substantial carry-over effect in both arms of the current trial, which interestingly was not observed in the primary, but rather in the secondary outcomes, namely SC, improvement in total PAC-SYM score and changes in fecal microbiota α-diversity. This indicates that a wash-out of four weeks appears insufficient for symptom and quality of life assessment in an intestinal-microbiota directed intervention study for functional gastrointestinal disorders such as FC [48]. While different approaches exist to deal with a possible carry-over effect, they have received some critique [51]. Some guidelines indicate that a parallel study design is preferred over a cross-over design in specifically functional gastrointestinal disorders in adult and child populations, for example,

treatment success, order-prone subjective outcomes assessment and time-dependent changes in symptoms are recognized biases in trial designs for such disorders [52-54]. Altogether, this study shows that, in contrast to what was considered during the setup of this study, the advantages of a cross-over design of less variability in outcomes within subjects and the possibility to use a smaller sample size with similar statistical power may be outweighed by carry-over effects. This limitation might be diminished with a longer wash-out period, but will come with its respective challenges of e.g. potentially higher drop-out rates because of the longer study duration. Here we decided to drop the second period of the intervention, due to following reasons. Firstly, the carry-over effect persisted especially in the outcomes assessing the subject's constipation-related symptoms and QoL. Remarkably, all scores of PAC-SYM and PAC-QOL were improved during wash-out compared to run-in for the arm receiving first inulin, despite their bowel movements returning to run-in levels. These PAC scores reflect the patients' experience of their condition, indicating that their symptoms and the impact thereof on their daily life were still improved when starting into the second intervention period of the trial. Hence, these subjects very likely experienced the second trial intervention period (consuming placebo) differently from how they would have experienced the placebo intake during the first intervention period. Besides the bias introduced by the carry-over effect, secondly also missing samples pose a substantial bias in cross-over designs. Unfortunately, it was not possible to accurately assess within-individual microbiota changes for the full cross-over as about 40% of the subjects did not collect a fecal sample during the wash-out and second intervention period. Taking into account the bias introduced by the carry-over effect, along with the bias introduced by the missing samples, we focused on the first, more trustworthy part of the trial to assess changes based on between-group differences in line with a parallel study design.

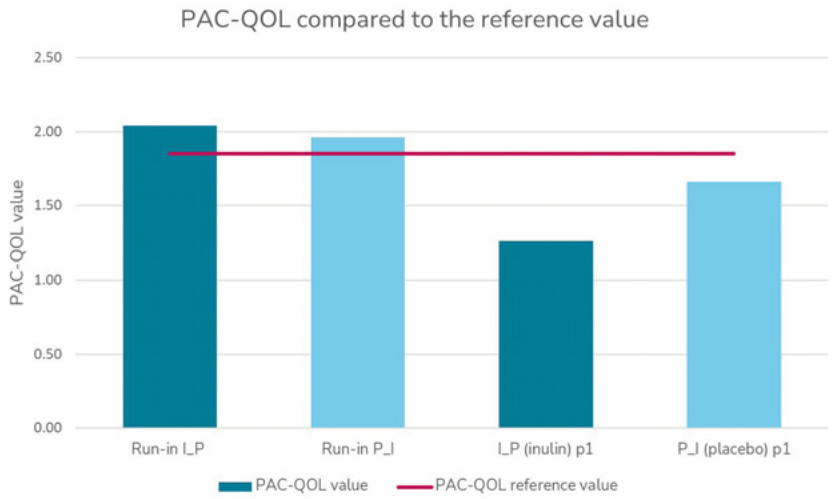

Figure 7 | Quality of life in our population at run-in and after intervention period 1 (p1) compared to the reference value. A higher QOL score represents a lower QOL. I_P indicates the group that started with inulin intake while P_I indicates the group that started with placebo intake.

Native chicory inulin currently holds an EFSA health claim on the maintenance of normal defecation by increasing SF [55]. In this study we support this effect of inulin consumption on median [IQR] SF (inulin 4.00 [2.75-4.50] times per week vs placebo 2.50 [2.38-3.50] times per week, p=0.05). In addition, we observed a higher increase after inulin intake in the percentage of participants defecating more than 3 times per week (inulin 55% vs placebo 25%, p=0.012). Besides SF, SC has been reported earlier to be associated with intestinal microbiota composition [56, 57]. In addition to this finding, SC, besides SF, is of major interest as it was reported by a study in patients as one of the top two severe symptoms in FC, together with straining [58]. The mean (SEM) SC (after inulin intake of 2.72 (0.18) compared to 2.24 (0.14) after placebo intake p=0.045) could therefore be considered more important from a patient's perspective. SF and SC are both part of the diagnostic criteria, but the Rome criteria for FC include more symptoms. We therefore considered it to be of value to also evaluate whether participants met the Rome criteria during the study. We found no big differences between groups; both substantially improved. When further looking into the patient perspective, we compared the PAC-QOL scores to the original validation of the PAC-QOL. Both groups of our population started in the run-in with a slightly higher score (SEM), indicating a lower QOL: 2.04 (0.14) in the group starting with inulin intake and 1.94 (0.14) in the group starting with placebo intake compared to the reference score of 1.85. However, after intervention both groups decreased below this reference score: 1.26 (0.14) after inulin intake and 1.66 (0.16) after placebo intake (**Figure 7**). To further investigate how clinical outcomes related to each other, a Venn diagram was created (**Figure 8**). Despite that there was no clear correlation between clinical outcomes, the Venn diagram showed n=14 participants after inulin intake that improved in any of the five clinical outcomes (SF, SF, Rome criteria, PAC-SYM or PAC-QOL). The diagram shows that there is improvement for n=7 participants on all five aspects. For physical activity, we found a significant difference in the intervention period. This effect was mainly driven by a shift from individuals after inulin intake from 'moderate' to 'low' activity, while this did not happen after placebo intake. On the contrary, there was a slight increase after placebo intake of participants shifting to 'high' activity levels. Since physical activity is known to be a risk factor in the etiology of FC, it could have influenced FC symptoms, possibly contributing to the placebo effect that was observed in several outcomes [2]. For instance, the average SF after placebo intake increased with 47.5%, SC increased with 28% and QOL improved with 30%. It is known that rather large placebo effects can occur in studies, especially when subjective and/or patient-reported outcome measures are used [59]. Our study is no exception in this. However, this placebo effect is a confounding factor in the assessment of the efficacy of an intervention [60].

In terms of medication use, we found that none of the participants were taking laxatives either in the run-in period or in the intervention period. It was remarkable, however, that none of the patients were on laxatives, while meeting the Rome criteria. This may be due to the chronic nature of FC; lifestyle advice, and the use of fiber or laxatives on demand may suffice for a part of this patient population [9]. Moreover, it was reported in a large survey study that 28% of participants reported to be dissatisfied with their treatment and 83% was interested in other treatments [11]. Moreover, the level of dissatisfaction for (dietary) fiber treatments was lowest

(16%) compared to other laxatives such as macrogol (32%) or rectal laxatives (32%). Therefore, further investigating the effects of other (dietary) fibers such as inulin may be of interest from the patient perspective [11]. In relation to this, we found mostly mild adverse events of moderate intensity for inulin consumption for flatulence, bloating, nausea and stomach cramps.

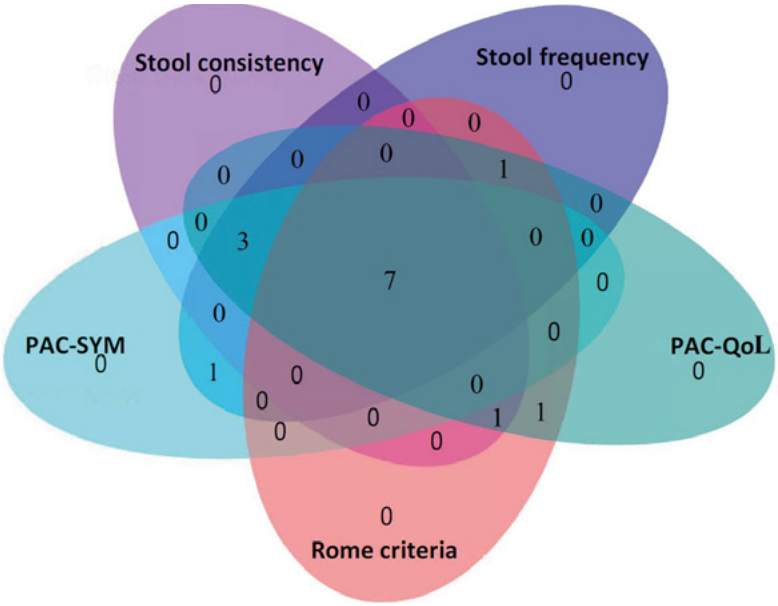

Figure 8 | Venn diagram representing the co-occurrence of improvements after inulin intake in stool consistency, stool frequency, total PAC-SYM and PAC-QOL score as well as Rome criteria for the n=14 responders

Inulin is known for its bifidogenic effect, which has been suggested to possibly relate to bifidobacteria's ability to intracellularly degrade inulin, outcompeting other taxa [61, 62]. Indeed we observed a small increase in the relative abundance of *Bifidobacterium* spp by 1.3-fold after inulin intake that was different from the placebo group. An increase of this magnitude has been found before after five-week 10 g inulin intake in subjects with low SF [17, 63]. Interestingly, subjects in this study already had a high relative abundance of bifidobacteria at baseline (~15%), which could possibly relate to the high proportion of women in this trial (92%) as sex-specific higher levels of *Bifidobacterium* spp. have been reported for women [64]. Moreover, it has been reported that the bifidogenic effect of inulin is smaller in subjects with high initial bifidobacteria levels [65]. The high abundance and increase in bifidobacteria following inulin intake, likely also explains the observed changes in α-diversity. The observed decrease in richness and evenness was concomitant with a higher dominance index for abundant taxa and has been previously reported [66]. Similar to the bifidogenic effect also *Anaerostipes* spp. have been frequently reported to be increased following inulin consumption, and also here we observed a substantial two-fold

increase. *Anaerostipes* spp. can possibly cross-feed on inulin-breakdown products released by *Bifidobacterium* spp. and thereby produce butyrate [42, 67, 68], an SCFA used by colonocytes as energy source contributing to gut homeostasis [68]. Besides, *Anaerostipes* also *Subdoligranulum* spp. increased with 1.47-fold, a genus whose members possibly also produce butyrate and whose abundance has recently been related to positive metabolic health outcomes such as lower insulin resistance, fat mass and inflammation markers [69]. Several genera within the family *Ruminococcaeae* decreased in relative abundance in the intervention group and compared to placebo. This was also confirmed by LEfSE analysis that was used to discriminate subjects that consumed placebo from those that had consumed inulin. Intriguingly, cross-sectional studies from Italy, USA and Russia have reported *Ruminococcaeae* to be an abundant taxon in FC compared to healthy controls [70-72]. Hence, a decrease in genera belong to this family likely points towards a beneficial modulation of the intestinal microbial environment induced by inulin intake on the intestinal microbiota. The exact mechanism by which inulin via modulating the intestinal microbiota impacts bowel function is not fully understood. SCFAs produced through microbial fermentation of inulin can exert osmotic effects and together with increased microbial biomass increase fecal bulk, beneficially impacting stool softness and defecation regularity. Other potential mechanisms have been proposed, such as the role of 5-HT in colonic motility, which is a neurotransmitter also under the influence of the intestinal microbiota [73].

Strengths of this study include that we report the relevant outcomes as proposed by a core outcome set (COS), despite that this COS was designed for studies in children. COS are standardized sets of outcomes developed to reduce heterogeneity between studies, make pooling of results easier, reduce the risk of reporting bias and they are more likely to report clinically relevant outcomes that are relevant from the perspective of a wide range of stakeholders, such as patients and healthcare professionals [76]. Currently, there is no COS available to evaluate the outcomes of therapeutic trials in FC in adults. This COS for trials in children might give an indication of what could be considered important outcomes in therapeutic trials for FC, taking into account the limitations that this COS was developed for trials in children with FC aged 0-18 years [77]. The COS includes: (1) SF, (2) SC, (3) painful defecation, (4) QOL, (5) side effects of treatment, (6) fecal incontinence, (7) abdominal pain, (8) school attendance. Compared to the outcomes reported in our trial, we included several of the above outcomes, except for the outcomes that are, in adults, used for the diagnosis of IBS– constipation predominant: outcome 3, and 7. Moreover, fecal incontinence in adults is regarded a separate diagnosis, therefore not taken into account in our study [78]. Lastly, school attendance is not relevant in adults, however, the IPAQ may give an indication on the physical activity an individual is capable of as a proxy of a comparable outcome. Beside these clinical outcomes, this study also took into account the intestinal microbiota composition to further investigate its potential role in FC in adults.

This study has several limitations of which the greatest limitation, as elaborately discussed above, is that we found clear carry-over effects which introduces substantial bias for the analysis of the study as cross-over trial. We chose to report outcomes as between-group differences based on the run-in and first period of the study only, which introduced bias due to, among others, smaller sample size. Moreover, in the second intervention period missing data and missing fecal

samples made it difficult to calculate within-subject differences and to interpret data. Hence, we decided to report mainly the data of the study which we can confidently report, being aware of the limitations of doing so. Another limitation was that in the current study we did not measure SCFA composition, but in order to further elucidate the role of SCFAs in the pathogenesis of FC, case-control or cohort studies could consider taking SCFA measurements into account [80]. Lastly, an important factor in FC that was not taken into account in this study was nutritional intake. Beside the fact that nutritional intake is known to be a major driver of intestinal microbiota composition, important factors in FC are fiber and fluid intake [9, 10, 81, 82]. Such data on dietary intake would have added information for the interpretation of the data of the full cross-over trial to exclude the possibility of changes in dietary habits.

Summarizing, we found an increase in median SF after inulin intake compared to the placebo. In addition, the percentage of participants with more than three defecations per week increased and SC improved after inulin compared to placebo intake. Moreover, the change of the PAC-SYM and PAC-QOL scores was above the minimally important difference after inulin intake but not after placebo intake. Lastly, microbiota composition analysis showed an increase in relative abundance of bifidobacteria that differed from placebo, where no changes in bacterial taxa were observed. Besides the bifidogenic effect of inulin, also an increase in relative abundance of potential butyrate-producers, namely *Anaerostipes* and *Subdoligranulum* spp., was observed as well as a decrease in constipation-associated genera belonging to the *Ruminocacceae* family. Therefore, we can conclude that the daily consumption of 12 g inulin has the potential to alleviate FC by improving SF and SC as well as constipation related symptoms and QOL. These improvements are concomitant with changes in intestinal microbiota composition, related to inulin's bifidogenic effect and taxa likely associated with improvements in gut-health and constipation.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 | Adverse events for the full cross-over trial

	Inulin intake	Placebo intake
Number of subjects with AEs	14	5
Total number of AEs	20	5
Unrelated[a]	1	2
Unlikely related[b]	1	0
Possibly related[c]	18	3
Probably related	0	0
Definitely related	0	0

[a]Included: headache, sciatica and gastroenteritis , [b]Included: headache and toothache, [c]Included: flatulence, stomach cramps, bloating, abdominal cramps, thirst, nausea and cramps.

Supplementary Table 2 | Clinical outcomes for each arm and phase of the cross-over trial including their time-dependent within-group changes (estimated differences) over run-in and over wash-out.

Stool frequency		Run-in	Period 1	Wash-out	Period 2
I_P					
	Median [IQR]	2.00 [1.5-2.0]	4.00 [2.8-4.5]	2.00 [2.0-3.0]	3.00 [2.3-4.5]
	estimated difference$_{run\text{-}in}$ ((95% CI)		+1.75 (1.00, 2.5)	+0.25 (-0.50, 1.25)	+1.00 (0.50, 1.75)
	estimated difference$_{wash\text{-}out}$ (95% CI)				+1.75 (0.75, 2.50)
P_I					
	Median [IQR]	2.00 [2.0-2.1]	2.50 [2.4-3.5]	1.75 [1.4-2.5]	3.25 [2.9-3.6]
	estimated difference$_{run\text{-}in}$ (95% CI)		+1.00 (0.75, 1.50)	-0.50 (-0.75, 0.00)	+1.50 (1.00, 2.00)
	estimated difference$_{wash\text{-}out}$ (95% CI)				+1.25 (1.00, 1.50)
Stool consistency		Run-in	Period 1	Wash-out	Period 2
I_P					
	Average (SEM)	1.84 (0.13)	2.72 (0.18)	2.18 (0.22)	2.86 (0.27)
	estimated difference$_{run\text{-}in}$ (95% CI)		+0.82 (0.43, 1.21)	+0.33 (-0.08, 0.75)	+0.93 (0.37, 1.50)
	estimated difference$_{wash\text{-}out}$ (95% CI)				+0.67 (0.27, 1.08)
P_I					
	Average (SEM)	1.77 (0.11)	2.24 (0.14)	1.91 (0.16)	2.28 (0.14)
	estimated difference$_{run\text{-}in}$ (95% CI)		+0.47 (0.21, 0.73)	+0.16 (-0.10, 0.42)	+0.54 (0.23, 0.85)
	estimated difference$_{wash\text{-}out}$ (95% CI)				+0.39 (0.08, 0.70)
PAC-SYM					
I_P					
	Average (SEM)	1.98 (0.12)	1.25 (0.13)	1.54 (0.17)	1.35 (0.19)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.73 (-1.04, -0.42)	-0.44 (-0.71, -0.15)	-0.63 (-0.97, -0.30)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.20 (-0.50, 0.11)

Supplementary Table 2 | continued

P_I					
	Average (SEM)	1.93 (0.13)	1.54 (0.16)	1.99 (0.16)	1.52 (0.16)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.38 (-0.69, 0.08)	+0.06 (-0.15, 0.27)	-0.44 (-0.81, -0.07)
	estimated difference$_{wash\text{-}out}$ (95%CI)				-0.51 (-0.84, -0.18)
PAC-QOL					
I_P					
	Average (SEM)	2.04 (0.14)	1.26 (0.14)	1.74 (0.17)	1.50 (0.21)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.78 (-1.11, -0.46)	-0.31 (-0.57, -0.04)	-0.54 (-0.95, -0.14)
	estimated difference$_{wash\text{-}out}$ (95%CI)				-0.24 (-0.54, 0.06)
P_I					
	Average (SEM)	1.94 (0.14)	1.66 (0.16)	2.02 (0.15)	1.62 (0.15)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.28 (-0.58, 0.01)	+0.08 (-0.06, 0.23)	-0.35 (-0.71, 0.01)
	estimated difference$_{wash\text{-}out}$ (95%CI)				-0.45 (-0.80, -0.09)

I_P: this is the group that started with the inulin and ended with the placebo period. P_I: this is the group that started with the placebo and ended with the inulin period.
IQR: inter quartile range, PAC-SYM: patient assessment of constipation-symptoms, PAC-QOL: patient assessment of constipation quality of life, SEM: standard error of the mean

Supplementary Table 3 | PAC-SYM and PAC-QOL subscores for each arm and phase of the cross-over trial including their time-dependent within-group changes (estimated differences) over run-in and over wash-out.

		Run-in	Period 1	Wash-out	Period 2
PAC-SYM subscores					
Subscore abdominal symptoms (SEM)					
I_P					
	Average (SEM)	2.12 (0.12)	1.33 (0.15)	1.52 (0.20)	1.51 (0.21)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.79 (-1.16, -0.42)	-0.60 (-0.95, -0.25)	-0.61 (-1.00, -0.24)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.01 (-0.38, 0.35)
P_I					
	Average (SEM)	1.97 (0.18)	1.70 (0.20)	2.08 (0.22)	1.69 (0.22)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.27 (-0.68, 0.15)	0.11 (-0.15, 0.39)	-0.31 (-0.80, 0.18)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.46 (-0.89, -0.03)
Subscore rectal symptoms (SEM)					
I_P					
	Average (SEM)	1.25 (0.16)	0.59 (0.12)	0.93 (0.20)	0.82 (0.19)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.66 (-1.00, -0.32)	-0.32 (-0.62, -0.01)	-0.43 (-0.65, -0.19)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.11 (-0.43, 0.22)
P_I					
	Average (SEM)	1.52 (0.14)	1.05 (0.17)	1.29 (0.17)	0.89 (0.15)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.47 (-0.89, -0.05)	-0.23 (-0.44, -0.02)	-0.63 (-1.02, -0.28)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.40 (-0.77, -0.07)

Supplementary Table 3 | continued

Subscore stool-related aspects (SEM)					
I_P					
	Average (SEM)	2.29 (0.18)	1.58 (0.17)	1.96 (0.20)	1.07 (0.13)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.71 (-1.12, -0.30)	-0.33 (-0.69, 0.03)	-1.22 (-1.61, -0.83)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.89 (-1.36, -0.42)
P_I					
	Average (SEM)	2.15 (0.17)	1.70 (0.20)	2.30 (0.19)	1.07 (0.13)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.45 (-0.75, -0.15)	0.15 (-0.12, 0.42)	-1.13 (-1.56, -0.70)
	estimated difference$_{wash\text{-}out}$ (995% CI)				-1.26 (1.70, -0.82)
PAC-QOL subscores					
Subscore physical discomfort (SEM)					
I_P					
	Average (SEM)	2.37 (0.12)	1.36 (0.16)	1.81 (0.19)	1.55 (0.22)
	estimated difference$_{run\text{-}in}$ (95% CI)		-1.01 (-1.35, -0.64)	-0.56 (-0.88, -0.22)	-0.82 (-1.20, -0.42)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.26 (-0.58, 0.06)
P_I					
	Average (SEM)	2.28 (0.18)	1.73 (0.21)	2.28 (0.23)	1.76 (0.21)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.55 (-0.95, -0.16)	0.00 (-0.29, 0.28)	-0.57 (-1.08, -0.07)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.61 (-1.10, -0.12)
Subscore psychosocial discomfort (SEM)					
I_P					
	Average (SEM)	1.58 (0.19)	0.93 (0.13)	1.25 (0.21)	1.28 (0.25)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.65 (-1.03, -0.27)	-0.33 (-0.62, -0.03)	-0.30 (-0.72, 0.12)
	estimated difference$_{wash\text{-}out}$ (95% CI)				0.03 (-0.26, 0.31)

Supplementary Table 3 | continued

P_I					
	Average (SEM)	1.28 (0.18)	1.10 (0.18)	1.32 (0.20)	1.07 (0.17)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.18 (-0.51, 0.15)	0.04 (-0.22, 0.30)	-0.27 (-0.68, 0.14)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.30 (-0.77, 0.18)
Subscore worries discomfort (SEM)					
I_P					
	Average (SEM)	1.83 (0.17)	1.16 (0.17)	1.62 (0.20)	1.46 (0.24)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.67 (-1.02, -0.34)	-0.21 (-0.46, 0.04)	-0.37 (-0.77, 0.02)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.16 (-0.47, 0.14)
P_I					
	Average (SEM)	1.75 (0.15)	1.50 (0.17)	1.93 (0.18)	1.48 (0.15)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.25 (-0.57, 0.05)	0.18 (-0.03, 0.37)	-0.31 (-0.67, -0.04)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.50 (-0.87, -0.14)
Subscore treatment satisfaction (SEM)					
I_P					
	Average (SEM)	3.18 (0.13)	1.95 (0.22)	2.75 (0.17)	1.94 (0.24)
	estimated difference$_{run\text{-}in}$ (95% CI)		-1.23 (-1.80, -0.66)	-0.47 (-0.85, -0.09)	-1.29 (-1.88, -0.70)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.81 (-1.34, -0.28)
P_I					
	Average (SEM)	3.38 (0.11)	2.84 (0.17)	3.19 (0.13)	2.66 (0.21)
	estimated difference$_{run\text{-}in}$ (95% CI)		-0.54 (-0.98, -0.08)	-0.21 (-0.48, 0.06)	-0.64 (-1.12, -0.15)
	estimated difference$_{wash\text{-}out}$ (95% CI)				-0.52 (-0.86, -0.17)

I_P: this is the group that started with the inulin and ended with the placebo period. P_I: this is the group that started with the placebo and ended with the inulin period.
CI: confidence interval, PAC-SYM: patient assessment of constipation-symptoms, PAC-QOL: patient assessment of constipation quality of life, SEM: standard error of the mean

Supplementary Table 4 | Within-individual differences calculated based on the full cross-over for all outcomes (neglecting any carry-over effect). Reported as means (SEM) unless otherwise specified.

	Inulin	Placebo	Estimated ted within-individual difference (95CI)	p-value
Stool frequency*	3.5 [1.25, 2.75]	3.0 [1.75, 2.25]	0.25 (-0.50, 1.25)	0.285
Stool consistency	2.51 (0.12)	2.53 (0.15)	-0.00 (-0.45, 0.44)	0.99
PAC-SYM total	1.38 (0.11)	1.45 (0.13)	-0.09 (-0.47, 0.28)	0.614
Abdominal symptoms	1.51 (0.13)	1.61 (0.15)	-0.11 (-0.53, 0.32)	0.622
Rectal symptoms	0.74 (0.09)	0.94 (0.13)	-0.23 (-0.59, 0.13)	0.207
Stool-related aspects	1.33 (0.13)	1.39 (0.14)	-0.09 (-0.41, 0.24)	0.593
PAC-QOL total	1.44 (0.10)	1.58 (0.13)	-0.18 (-0.56, 0.21)	0.358
Physical discomfort	1.56 (0.13)	1.64 (0.15)	-0.13 (-0.56, 0.31)	0.564
Psychosocial discomfort	1.00 (0.11)	1.19 (0.15)	-0.23 (-0.65, 0.20)	0.285
Worries discomfort	1.31 (0.12)	1.48 (0.15)	-0.19 (-0.63, 0.25)	0.383
Treatment satisfaction	2.31 (0.16)	2.39 (0.16)	-0.13 (-0.67, 0.41)	0.638

*Reported as median [IQR]. I_P: this is the group that started with the inulin and ended with the placebo period. P_I: this is the group that started with the placebo and ended with the inulin period. IQR: inter quartile range, PAC-SYM: patient assessment of constipation-symptoms, PAC-QOL: patient assessment of constipation quality of life, SEM: standard error of the mean

Supplementary Table 5 | Proportion of subjects within each stool frequency category per intervention arm for each phase of the trial

		0 x per week %	1 x per week %	2 x per week %	3 x per week %	4 x per week %	5 x per week %	6 x per week %	7 x per week %
***Before** cross-over*									
Run-in	I_P	2.5	15	67.5	12.5	0	2.5	0	0
	P_I	0	15	70	15	0	0	0	0
Period 1	I_P: Inulin intake	5	5	25	10	20	27.5	7.5	0
	P_I: Placebo intake	0	2.5	40	32.5	20	0	0	5
***After cross-over** (excluded due to carry-over)*									
Washout	I_P	15	5	45	17.5	15	0	2.5	0
	P_I	17.5	22.5	35	22.5	2.5	0	0	0
Period 2	I_P: Placebo intake	12.5	2.5	22.5	15	22.5	15	5	5
	P_I: Inulin intake	7.5	0	15	37.5	35	5	0	0

I_P: this is the group that started with the inulin and ended with the placebo period. P_I: this is the group that started with the placebo and ended with the inulin period.

Supplementary Table 6 | Proportion of subjects within a stool consistency category per intervention arm and phase.

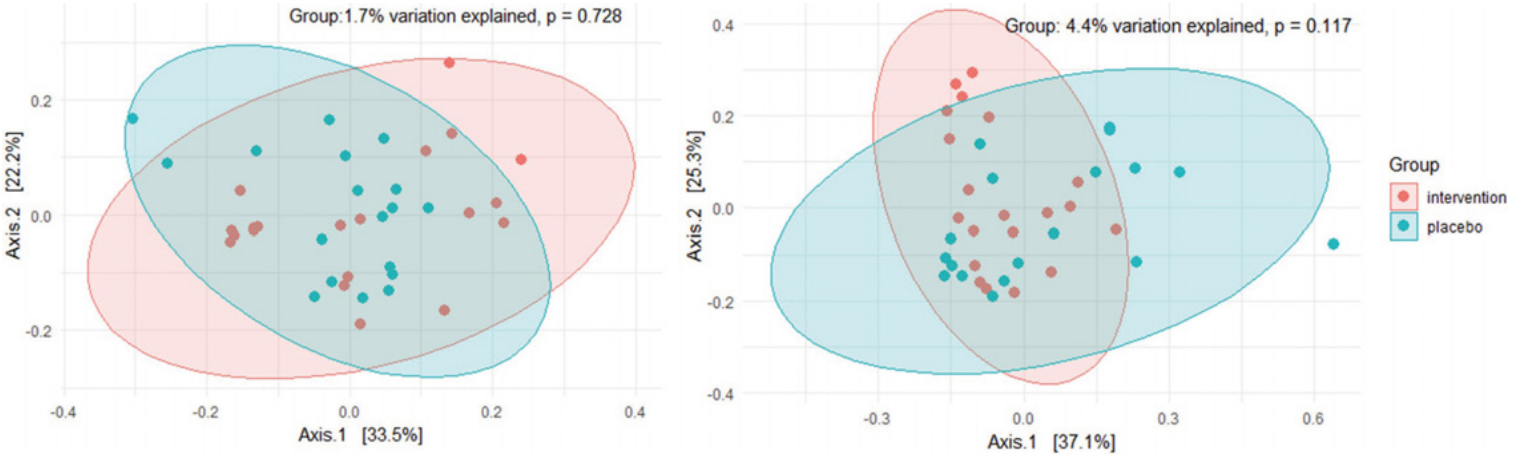
		Type 1 %	Type 2 %	Type 3%	Type 4%	Type 5%	Type 6%	Type 7%
Before cross-over								
Run-in	I_P	27.6	56.6	13.2	1.3	1.3	0.0	0.0
	P_I	29.9	57.1	11.7	1.3	0.0	0.0	0.0
Period 1	I_P: Inulin intake	10.0	43.3	30.8	12.5	2.5	0.0	0.8
	P_I: Placebo intake	8.8	42.5	34.5	11.5	1.8	0.0	0.9
After cross-over *(excluded due to carry-over)*								
Washout	I_P	31.6	38.0	13.9	13.9	0.0	2.5	0.0
	P_I	28.8	45.8	25.4	0.0	0.0	0.0	0.0
Period 2	I_P: Placebo intake	10.1	34.9	25.7	22.0	1.8	1.8	3.7
	P_I: Inulin intake	15.2	46.7	29.5	8.6	0.0	0.0	0.0

I_P: this is the group that started with the inulin and ended with the placebo period. P_I: this is the group that started with the placebo and ended with the inulin period.

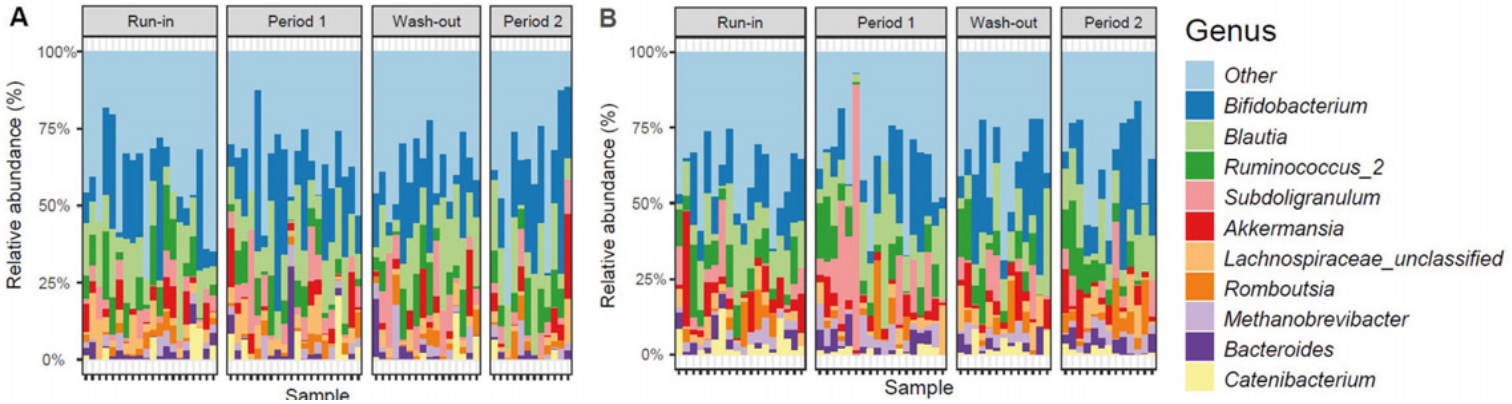
Supplementary Table 7 | IPAQ score

	I_P	P_I	p-value
Run-in			
High	**15.0%**	**20.0%**	
Moderate	**60.0%**	**60.0%**	
Low	**25.0%**	**20.0%**	Chi-square statistic: 1.27, p-value: 0.53
P1			
High	**26.3%**	**36.8%**	
Moderate	**36.8%**	**52.6%**	
Low	**36.8%**	**10.5%**	Chi-square statistic: 18.8, p-value: 0.00008
Wash-out			
High	**30.0%**	**26.3%**	
Moderate	**50.0%**	**52.6%**	
Low	**20.0%**	**21.1%**	Chi-square statistic: 0.39, p-value: 0.82
P2			
High	**35.0%**	**20.0%**	
Moderate	**35.0%**	**60.0%**	
Low	**30.0%**	**20.0%**	Chi-square statistic: 12.7, p-value: 0.0017

I_P: this is the group that started with the inulin and ended with the placebo period. P_I: this is the group that started with the placebo and ended with the inulin period.



Supplementary Figure 1 | Principle Coordinate Analysis based on weighted UniFrac distances to assess differences in overall gut micorbiota composition (β-diversity) between groups at A | run-in and B |at the end of intervention period 1.



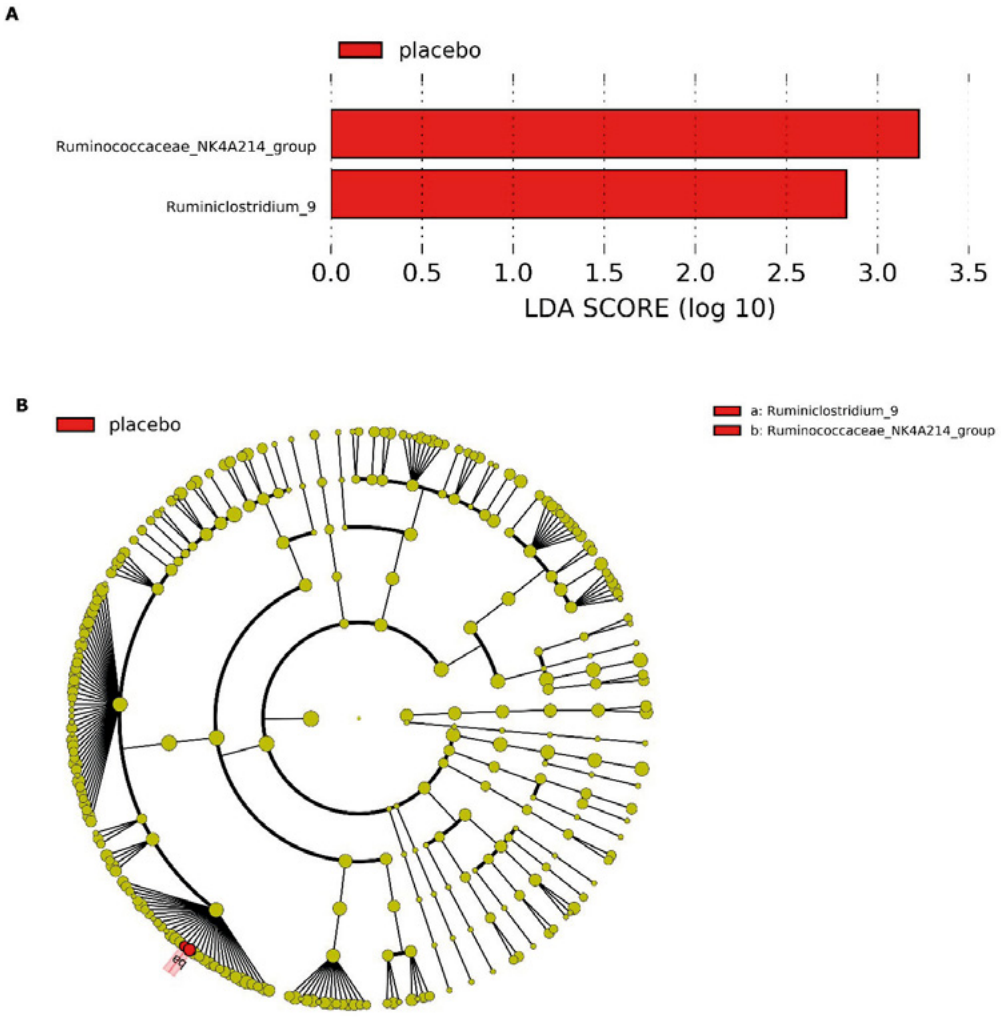
Supplementary Figure 2 | Relative abundances for top 10 taxa at genus level for each sample collected during each part of the full cross-over for **A |** the group receiving first inulin and **B |** the group receiving first the placebo. The category "other" comprises all taxa that are not within the top 10 abundant taxa. The number of samples is decreasing over from run-in to wash-out and period 2 due to missing samples.

Supplementary Table 8 | changes in mean relative abundances of abundant taxa (1% abundance) in both groups before and after 12 g/day inulin or placebo intake for 4 weeks. ‡P and q-values reported between brackets are derived from the non-zero model as implemented in the Mare package (excluding subjects without the respective taxa).

	Inulin intake					Placebo intake					Differences between groups			
											Relative abundance at T1 inulin vs placebo		Fold change ΔT1 inulin vs placebo	
Taxon	**T0**	**T1**	**Fold ΔT1**	**p-value**	**q-value**	**T0**	**T1**	**Fold ΔT1**	**p-value**	**q-value**	**p-value**	**q-value**	**p-value**	**q-value**
Bifidobacterium	14.75%	19.24%	1.30	0.593	0.928	12.63%	12.01%	0.95	-	-	0.290	0.508	**0.024**	**0.254**
Eubacterium coprostanoligenes group	1.63%	0.98%	0.60	**0.002** *(0.01)*‡	0.059 *(0.06)*‡	1.09%	1.09%	1.00	0.563	0.934	**<0.001**	**<0.001**	**0.016**	**0.254**
Methanobrevibacter	2.06%	2.04%	0.99	0.833	0.928	2.28%	2.84%	1.25	-	-	**<0.001**	**<0.001**	0.103	0.362
Ruminococcus torques group	1.63%	1.17%	0.72	0.221	0.928	1.19%	1.02%	0.86	0.823	0.934	0.788	0.851	0.061	0.362
Fusicatenibacter	2.10%	1.46%	0.70	0.350	0.928	1.92%	1.63%	0.85	0.790	0.934	**<0.001**	**<0.001**	0.102	0.362
Ruminococcaceae UC002	1.45%	0.65%	0.45	0.240 *(0.01)*‡	0.928 *(0.06)*‡	1.30%	1.37%	1.05	0.934	0.934	**<0.001**	**<0.001**	0.097	0.362
Christensenellaceae 7 group	2.28%	1.23%	0.54	0.346 *(0.04)*‡	0.928 *(0.13)*‡	1.44%	1.80%	1.25	0.105	0.764	**<0.001**	**<0.001**	0.150	0.451
Erysipelotrichaceae UC003	1.60%	1.73%	1.09	0.916	0.928	1.50%	1.48%	0.99	0.304	0.811	**<0.001**	**<0.001**	0.215	0.538
Subdoligranulum	4.10%	6.15%	1.50	**0.013** *(0.01)*‡	0.127 *(0.06)*‡	2.83%	3.82%	1.35	0.441	0.882	0.227	0.434	0.231	0.538
Blautia	13.57%	14.12%	1.04	0.612	0.928	11.83%	13.44%	1.14	0.590	0.934	0.803	0.851	0.317	0.665
Bacteroides	2.02%	1.95%	0.97	-	-	3.31%	1.12%	0.34	0.115 *(0.04)*‡	0.764 *(0.22)*‡	0.400	0.595	0.566	0.824

Supplementary Table 8 | continued

	Inulin intake					Placebo intake					Differences between groups			
											Relative abundance at T1 inulin vs placebo		Fold change ΔT1 inulin vs placebo	
Taxon	**T0**	**T1**	**Fold ΔT1**	**p-value**	**q-value**	**T0**	**T1**	**Fold ΔT1**	**p-value**	**q-value**	**p-value**	**q-value**	**p-value**	**q-value**
Akkermansia	3.75%	3.50%	0.93	0.927	0.928	4.10%	11.34%	2.77	0.191 *(0.03)*‡	0.764 *(0.22)*‡	0.510 *(0.02)*‡	0.669 *(0.20)*‡	0.488	0.824
Catenibacterium	1.91%	2.23%	1.17	0.658	0.928	2.76%	2.58%	0.93	0.700	0.934	**<0.001**	**<0.001**	0.583	0.824
Unclassified genus Lachnospiraceae	4.66%	4.48%	0.96	0.896	0.928	6.18%	3.64%	0.59	-	-	0.425	0.595	0.433	0.824
Rombautsia	2.06%	2.27%	1.10	0.877	0.928	2.99%	2.49%	0.83	-	-	0.548	0.677	0.589	0.824
Ruminococcus 2	8.13%	5.90%	0.73	0.427 *(0.04)*‡	0.928 *(0.13)*‡	7.79%	7.00%	0.90	0.400	0.882	**<0.001**	**<0.001**	0.651	0.854
Faecalibacterium	2.13%	1.74%	0.81	0.600	0.928	2.80%	1.93%	0.69	0.270	0.811	0.915	0.915	0.813	0.865
Dorea	2.47%	1.96%	0.80	0.398	0.928	2.14%	2.05%	0.96	-	-	**<0.001**	**<0.001**	0.737	0.865
Eubacterium hallii group	2.31%	2.50%	1.08	0.740	0.928	1.81%	1.94%	1.07	0.881	0.934	**<0.001**	**<0.001**	0.824	0.865
Anaerostipes	1.13%	2.26%	2.00	0.150 *(0.001)*‡	0.928 *(0.02)*‡	1.10%	1.04%	0.95	0.909	0.934	0.811 *(0.002)*‡	0.851 *(0.04)*‡	0.822	0.865
Clostridium sensustricto 1	1.95%	2.22%	1.14	0.850	0.928	2.22%	1.04%	0.47	0.180 *(0.03)*‡	0.764 *(0.22)*‡	0.344 *(0.04)*‡	0.555 *(0.23)*‡	0.953	0.953



Supplementary Figure 3 | LEfSe outcomes for taxa discriminating after the intervention subjects that consumed placebo from those that consumed inulin. (A) LDA effect sizes and corresponding (B) cladogram of discriminant genera.

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Effect of prebiotic oligosaccharides on bowel habit and the intestinal microbiota in children with functional constipation (Inside study): study protocol for a randomized, placebo-controlled, multi-center trial.

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Slightly adapted version submitted to *Trials*

ABSTRACT

Background: Functional constipation (FC) in children is a common gastrointestinal disorder with a worldwide pooled prevalence of 9.5%. Complaints include infrequent bowel movements, painful defecation due to hard and/or large stools, faecal incontinence, and abdominal pain. Prebiotic oligosaccharides have been shown to relieve constipation symptoms in young adults and elderly. However, sufficient evidence is lacking linking additional prebiotic intake to improve symptoms in children with FC. We hypothesise that prebiotic oligosaccharides are able to relieve symptoms of constipation in young children as well.

Methods: In the present randomised, double-blind, placebo-controlled, multi-centre study, we will study the effects of two prebiotic oligosaccharides in comparison to a placebo on constipation symptoms in children of 1-5 years (12 to 72 months) of age diagnosed with FC according to the Rome IV criteria for functional gastrointestinal disorders. The primary outcome measure will be change in stool consistency. Secondary outcomes include stool frequency and stool consistency in number of cases (%). Tertiary outcomes include among others painful defecation, use of rescue medication, and quality of life; In addition the impact on intestinal microbiome outcomes such as faecal microbiota composition and metabolites will be investigated. Participants start with a run-in period, after which they will receive supplements delivered in tins with scoops for eight weeks, containing one of the two prebiotic oligosaccharides or placebo, followed by a 4-week wash-out period.

Discussion: This randomised double-blind, placebo-controlled multi-centre study will investigate the effectiveness of prebiotic oligosaccharides in children aged 1-5 years with FC.

Trial registration: ClinicalTrials.gov NCT04282551.

Keywords: Gastroenterology; Nutrition; Intestinal Microbiota; Paediatrics; Oligosaccharides; Prebiotic; Functional Constipation; Stool Consistency; Stool Frequency; Bowel Habit.

INTRODUCTION

BACKGROUND AND RATIONALE

Functional constipation (FC) in children is a common gastrointestinal (GI) disorder with a worldwide prevalence ranging from 0.7% to 29.6%, with a pooled prevalence of 9.5% [1, 2]. Only a minority of patients with FC, both children and adults, seeks healthcare [3]. However, it is estimated that up to 25% of visits to a pediatric gastroenterologist are due to FC [4]. Complaints include infrequent bowel movement, painful defecation due to hard and/or large stools, fecal incontinence, and abdominal pain [4]. FC is a clinical diagnosis; the evaluation primarily consists of a thorough medical history and is based on the pediatric diagnostic Rome IV criteria for functional GI disorders (**Box 1**) [5-7]. Although the condition is rarely life-threatening, it strongly impairs quality of life. The impairment in health-related quality of life is comparable with conditions such as diabetes, rheumatoid arthritis, and chronic allergies [8].

The etiology of FC is still incompletely understood but is likely to be multifactorial. Some factors in children which have been described are withholding behavior, psychosocial factors such as stressful life events or behavioral problems, behavioral disorders, parental child-rearing attitudes, low fiber intake, and intestinal microbiota composition [4, 7, 9]. Standard treatment of FC in children includes demystification, education, toilet training, and laxative treatment with, among others, polyethylene glycol (PEG) [10, 11]. Laxatives such as PEG are safe, but adherence to laxatives is low, and except for the use of PEG, little is known about long-term effects of chronic laxative use [12, 13]. This may explain why 36.4% of parents of children with FC seek help in the form of food supplements and complementary or alternative medicine [14]. One of such alternatives might be prebiotic oligosaccharides. Galacto-oligosaccharides (GOS) and chicory fructo-oligosaccharides (FOS; synonym oligofructose) are oligosaccharides that belong to the category of prebiotics. Prebiotics are defined by the International Scientific Association for Pro- and Prebiotics (ISAPP) as "a substrate that is selectively utilized by the host microorganisms conferring a health benefit" [15]. GOS and FOS have been shown to selectively stimulate certain gut microbial species, mostly bifidobacteria and lactobacilli, and have demonstrated health benefits, and consequently are endorsed as prebiotics by ISAPP [16-18].

Prebiotic oligosaccharides are of interest due to several factors; (1) low fiber intake has been associated with FC, and oligosaccharides are also considered dietary fibers; (2) fermentation of oligosaccharides is known to increase the abundance of intestinal microbiota thereby increasing fecal bulk; (3) oligosaccharide-derived microbial fermentation products such as short chain fatty acids (SCFAs) have been described to give energy to colonic epithelial cells and may generate an osmotic effect in the gut, which can increase the water content of feces, leading to softening of stools, and finally (4) these prebiotics are known to modify the composition of the intestinal microbiota which may indirectly affect bowel habit via gut-brain signaling. These hypotheses are supported by the fact that prebiotic oligosaccharides have shown stool softening effects in trials in healthy infants and children with infant, follow-on and young child formulas supplemented with prebiotics [17-26].

In addition, some trials showed improvement in stool consistency in children with FC after the consumption of prebiotic oligosaccharides. However, evidence linking oligosaccharide and/or fiber intake to improved symptoms in children with FC is rather weak [11, 27-31]. This is not

only due to the low number of studies, but also small sample size of studies, overall poor quality of methods used, and incomplete reporting of results. Therefore, a large scale, well executed study is needed to investigate if the consumption of GOS or FOS can result in improved bowel habit and modify the intestinal microbiota in young children with FC.

OBJECTIVES

We hypothesize that consumption of GOS or chicory FOS will result in softer stools, improvement of some other constipation related symptoms and modifications of the intestinal microbiota in comparison to a placebo. Therefore, the aim of the study is to investigate the effect of GOS or FOS versus a placebo on bowel habits and microbiota in children with FC aged 1-5 years.

TRIAL DESIGN

This is a randomized, double-blind, placebo-controlled, multi-center trial with three arms: GOS, chicory FOS and placebo, in which GOS will be compared to placebo and FOS will be compared to placebo.

METHODS

This study (named 'Inside study') is a double-blind, randomized, placebo-controlled, multi-center trial. We aim to enroll 198 children, aged between 1 and 5 years, with FC according to the Rome IV criteria (**Box 1**). SPIRIT reporting guidelines were used (**Supplementary material 1,**

https://gitfront.io/r/user-1250640/K1PePFuTAg9S/Thesis-CarrieWegh/) [32].

Box 1 | Rome IV criteria for functional constipation

<4 years of age [5]	**Developmental age of >4 years [6]**
Must include 1 month of at least 2 of the following in infants up to 4 years of age: 1. 2 or fewer defecations per week 2. History of excessive stool retention 3. History of painful or hard bowel movements 4. History of large-diameter stools 5. Presence of a large fecal mass in the rectum In toilet-trained children, the following additional criteria may be used: 1. At least 1 episode/week of incontinence after the acquisition of toileting skills 2. History of large-diameter stools that may obstruct the toilet	Must include 2 or more of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome 1. 2 or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years 2. At least 1 episode of fecal incontinence per week 3. History of retentive posturing or excessive volitional stool retention 4. History of painful or hard bowel movements 5. Presence of a large fecal mass in the rectum 6. History of large diameter stools that can obstruct the toilet After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

STUDY SETTING

This study is coordinated by Wageningen University & Research, Laboratory of Microbiology. The study is conducted in The Netherlands. Patients from the outpatient clinics in the Emma Children's Hospital, Amsterdam, Amsterdam University Medical Centers Amsterdam (AUMC), DeKinderKliniek Almere, Spaarne Gasthuis Haarlem, Haaglanden MC Den Haag, Rijnstate ziekenhuis Arnhem and Maasstad ziekenhuis Rotterdam, will be recruited by their treating pediatric gastroenterologist. More participating centers may follow.

ELIGIBILITY CRITERIA

Participant screening

Eligible patients will be contacted by researchers of Wageningen University & Research to answer possible questions and verify whether or not people are willing to participate, to avoid an undesirable dependency situation with the treating pediatric gastroenterologist.

Inclusion criteria:

In order to be eligible to participate in this study, a subject must meet all of the following criteria, as considered by a medical doctor:

- Written informed consent obtained from parents or guardians of children meeting the eligibility criteria and those willing to comply with the requirements of the study.
- Aged 1-5 years (12 to 72 months at the day of inclusion).
- Children that meet/fulfil the Rome IV criteria for FC.

Exclusion criteria:

Any of the following criteria will result in exclusion of a potential subject from this study:

- Children who suffer from any GI complaints other than FC, known structural GI abnormalities, or previous GI surgery.
- Any condition that would make it unsafe for the child to participate. This can include developmental delays associated with musculoskeletal or neurologic conditions affecting the GI tract. Children with underlying cause of defecation disorder (for example: Hirschsprung's disease, spina bifida occulta, cystic fibrosis, or GI malformations).
- Children with clinically significant cardiac, vascular, liver, pulmonary, psychiatric disorders, severe renal insufficiency, human immunodeficiency virus, acquired immunodeficiency syndrome, hepatitis B or C or known abnormalities of hematology, urinalysis, or blood biochemistry, as checked by the inclusion questionnaire.
- Children who are lactose intolerant, or who are self-perceived lactose intolerant or for whom it is expected that low doses of lactose could lead to GI symptoms.
- Children who are allergic to cow's milk or fish.
- Use of antibiotics or other medicines or food supplements, and human milk-feeding, which can influence defecation and intestinal microbiota four weeks prior to the study run-in period.
- The use of infant formula, follow on formula, young child formula in the previous week prior to the study run-in period.
- Children on other supplements / medication that could affect bowel function, including e.g. fiber supplements, and pre-, pro- and synbiotics (excluding rescue medication) for the past four weeks.
- Children that participate in another clinical trial.

Informed consent procedure

Informed consent will be obtained by the researchers or treating pediatric gastroenterologist either at one of the outpatient clinics or at a home visit before the start of the study.

INTERVENTIONS

After the run-in period, participants will receive either Vivinal® GOS powder (FrieslandCampina, Amersfoort, The Netherlands), Frutalose® OFP chicory oligofructose (Sensus, Roosendaal, The Netherlands) or placebo (maltodextrin) supplements in tins with scoops. The substances are approved food grade ingredients, they have been previously used in other clinical trials and are used in several food products. All supplements were similar powders light in color with a pleasant taste. All supplements are in identical tins with scoops and were produced according to good manufacturing practice standards. One scoop (8.5 mL size) should be consumed per day, with half a dose in the first three days to avoid any potential side effects such as flatulence caused

by intestinal fermentation of GOS or FOS. The product should preferably be dissolved in warm or cold drinks such as milk or semi-solid products. The intervention product will be consumed for eight weeks (**Figure 1**).

Rescue medication should be used if the participant does not have a bowel movement for three consecutive days, being either microlax, 5 mL, sodium picosulphate pearls (1 droplet per 5 kg body mass) or glycerine (glycerol) suppositories (1 g, 2 g or 4 g). These types of laxatives were chosen as they have a mode of action based on provoking peristalsis, and thereby are expected to have minimal effect on intestinal microbiota composition [33]. This is in contrast to (fermentable) osmotic laxatives such as lactulose or PEG, which were found to influence intestinal microbiota composition [34].

In case rescue medication is required, a child remains in the study. Each use of rescue medication needs to be reported in the diary to differentiate between spontaneous bowel movements and those related to rescue medication use. To further exclude an influence of escape medication on intestinal microbiota outcomes, a stool sample should only be collected after a spontaneous defecation and at least three days after the last use of escape medication.

Figure 1 | Study protocol flow; Visit 1 is at day=1, Call 1 at day=7, Call 2 at day=63 (end of 9 weeks), Visit 2 at day=91 (end of 14 weeks), which will be a home visit to pick up samples and leftover product. During visits 1 and 2, anthropometrics (weight, height and head circumference) will be measured. *diary includes filling out stool consistency and frequency and compliance of taking the study product.
FFQ: food frequency questionnaire, FOS: chicory fructo-oligosaccharides, GOS: galacto-oligosaccharides, QOL: quality of life questionnaire

OUTCOMES

Primary Objective: The main study parameter is change in stool consistency, measured by the validated Dutch modified Bristol Stool Form Scale (mBSFS) [35]. This will be the mean difference in stool consistency of GOS versus placebo and FOS versus placebo at all time points (week 1, 3, 6, 9 and 13) and from baseline to week 9.

Secondary Objectives: The secondary study parameters will be:

- Changes in stool frequency between groups and over time.
- Changes in stool consistency in number of cases in a certain score of the mBSFS, as percentages.

Tertiary Objectives:

- Painful defecation.
- Meeting the Rome IV criteria at baseline, week 9 and week 13.
- Quality of life of the child, measured by the TAPQOL [36].
- GI symptoms, such as flatulence and bloating.
- Intestinal microbiome:
    - Total fecal microbiota composition, as measured by 16S ribosomal RNA (rRNA) gene sequencing
    - Fecal abundance of specific genera/species as measured by quantitative PCR analysis.
    - Fecal pH and fecal concentration of fermentation products such as short-chain and branched-chain fatty acids.
    - Correlations between stool characteristics and intestinal microbiota composition, fecal pH or fermentation products.
- Use of rescue medication.
- Fecal incontinence (only for completely potty-trained children).
- The amount of GOS, FOS or placebo supplement consumed, as indication of compliance, measured in both diaries as well as weighing the tins after the trial.
- Anthropometrics: weight, height and head circumference measured at baseline and the close-out visit after week 13.
- Dietary intake, as measured by a food frequency questionnaire.

PARTICIPANT TIMELINE

After randomization, patients will enter a one-week run-in period, after which they will either receive GOS, FOS or a placebo for eight weeks. Lastly, a four-week run-out period is in place to investigate whether a possible effect lasts or not. The SPIRIT flow of the study protocol is presented in **Figure 1** [32].

SAMPLE SIZE

A sample size calculation was performed for stool consistency on a scale from 1-5. We used the sample size formula n=2 x (Zα+Zβ)2 x (SD/D)2 per group. Using a probability α=0.05 and a power (1-ß) of 80%, the formula simplifies to n=2 x 7.9 x (SD/D)2 per group.

The effect sizes of GOS and FOS versus placebo were estimated based on a study by Closa-Monasterolo et al. who investigated the effect of a mix of chicory inulin with FOS on stool consistency in functionally constipated children aged 2-5 years [27]. Based on these data, an effect size of 0.35 was chosen, with an SD of 0.65. This results in a group size of 54.5. The total number of children to be recruited is 198, that is, 66 per arm assuming a drop-out rate of 20%.

ASSIGNMENT OF INTERVENTIONS: ALLOCATION AND BLINDING

Randomization is done by a computerized random-number generator in the Electronic Data Capture System Castor EDC via a variable block randomization of block sizes of 6 and 12, not stratified per center, to one of the three intervention arms [37]. For the study product, two codes per treatment arm, each consisting of two letters and one number were made. The list linking these codes to GOS, FOS or the placebo are only known by two people who are not involved in this study: one at Wageningen University & Research and one at FrieslandCampina. Therefore, the study can be conducted fully blinded for all parties involved. In case of an emergency, the study treatment can be unblinded after consultation of the principal investigator at Wageningen University & Research.

DATA COLLECTION AND MANAGEMENT

Plans for assessment and collection of outcomes

Data are collected via several means: a diary, weekly report, questionnaires and measurements and clinical symptom reporting during visits. Moreover, parents are asked to collect fecal samples.

Diary: The diary is sent daily in the morning via Castor EDC and contains questions on stool frequency and consistency for each defecation, reported for in weeks 1, 3, 6, 9 and 13. Moreover, it contains a question on the use of escape medication and on the amount of study product that was consumed for that day. Lastly, in the diary there is also room for reporting of other, not urgent, problems such as mild GI symptoms.

Weekly report: The weekly report contains questions on the consumption of the study product (recall), and has room for other, non-urgent, issues such as mild GI symptoms.

Questionnaires: Three questionnaires are used in this study. The first one is a general questionnaire, which is only filled out once at the start of the study. This questionnaire includes questions on e.g. duration of human-milk-feeding and previous antibiotic treatment. Two other questionnaires are a quality of life questionnaire, filled out in week 1, 9 and 13. Lastly, to correct for changes in dietary habits, a food frequency questionnaire is filled out in week 1, 9 and 13.

Clinical symptoms: The Rome IV criteria are confirmed at the inclusion visit, and are re-assessed at the end of the intervention period and during the close-out visit.

Fecal samples: Parents are asked to collect one fecal sample from weeks 1, 5, 9 and 13 from their child, and store it in a freezer until the close-out visit. Parents are instructed how to collect the sample, and are provided with fecal sample tubes with an attached scoop and bags to safely store the sample in their freezer. These tubes are labelled with participant number, duration that the sample was outside of the freezer, date and stool consistency according to the mBSFS. During the close-out visit, fecal samples will be collected and transported on dry-ice until they are stored in a -80oC freezer at Wageningen University & Research.

Other measurements: To ensure normal growth, anthropometrics (weight, height and head circumference) are measured during the inclusion and close-out visits by the researchers. Beside the reported consumption of the study product in the diaries, tins are weighed before and after the trial for each participant as an additional measure for compliance.

Data management and confidentiality

Collected data will be treated confidentially by the study staff associated with the project and according to Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation, in Dutch the Netherlands 'algemene veroderning gevevensbescherming' (AVG)) guidelines. Consequently, codes are not based on personal data and are automatically provided by the online system used, i.e. Castor EDC. Data will be reported in electronic case reports (eCRF) and names of the research subjects will be coded, and this code will be used for study products, diaries and questionnaires. The codes list with both the codes and the names of the study participants and other source data will only be accessible for the coordinating investigator and principal investigators, Medical Ethical committee (METC) and Health Care Inspectorate by a password protected file in a secured online drive.

STATISTICAL METHODS

Statistical analysis for primary, secondary and tertiary outcomes

Data will be presented as mean ± standard deviation (SD) if normally distributed, or median (interquartile range) when skewed. To test associations between continuous parameters, multiple linear regression will be used. For categorical or dichotomous outcomes, generalized estimation equations or mixed models for repeated measures will be used. Data will be tested for confounding or effect mediators, and confounding factors will be added to the regression. All data will be assessed using the statistical program R (The R Foundation for Statistical Computing, Vienna, Austria). A p-value of <0.05 will be considered statistically significant. In order to prevent p-hacking, a false discovery rate correction will be applied for microbiota analyses, of which a q-value of <0.1 is regarded statistically significant. Either GOS or chicory FOS versus the placebo will be analyzed for all parameters.

A variety of in-house R-scripts and the Phyloseq package will be used for microbiota composition analyses. To assess variation in microbiota composition, 16S rRNA gene sequence data will be tested for differences between groups in α-diversity (phylogenetic diversity; number of observed species and inverted Simpson's for evenness and richness) and β-diversity (Bray-Curtis dissimilarity distances, weighted and unweighted unifrac distances; methods for constrained and unconstrained ordinations; significance of variations by e.g. adonis test). Principal response curves will be used to check the development of the intestinal microbiota over time. Moreover, area under the curve assessment for microbiota will be done. For 16S rRNA gene sequence data, qPCR-based abundance of specific taxa selected based on sequence data and SCFA's (multiple) linear regression will be used to test the predictive power of the model.

Changes in stool pH and results of the TAPQOL and changes in stool characteristics will be tested by a repeated measure analysis. Differences between timepoints will be assessed using mixed models.

Monitoring

Data collection, storage and analysis will be the responsibility of the coordinating investigator and principal investigator. The principal investigator will monitor collection, storage and analysis. Moreover, monitoring is planned before enrolment of the first subject, after three subject inclusions, after 60% of intended subjects per site and after the last subject's last visit. (Serious) Adverse events (SAE/AE) will be monitored throughout the study. In accordance with the legal requirements in the Netherlands (article 10, subsection 1, Medical Research Involving Human Subjects Act (WMO)), the coordinating investigator will inform the subjects and the reviewing accredited METC if harmful events occur. When there are indications that the disadvantage of participation may be significantly greater than was described in the research proposal, the study will be suspended pending a further positive decision by the accredited METC. The principal investigator will take care that all subjects are kept informed.

DISCUSSION AND CONCLUSION

FC is a prevalent problem, especially in young children. Moreover, it ranges from bothersome to having a severe impact on the quality of life of both the child and the family as a whole [38]. At this young age, the children's diet may be changing as an increasing range of solid foods are introduced to the diet, while simultaneously mothers' milks, rich in human milk oligosaccharides (HMOs), or formula milk usually supplemented with prebiotics, are reduced. Such changes are known to impact the bowel habit and the intestinal microbiota; the young child intestinal microbiota is known to be still rather unstable and less diverse [39]. In addition, the young child will be acquiring the skill of using a potty. Prebiotic oligosaccharides might be a more natural approach to treatment of FC in children, or an additional approach to conventional treatment

of FC in children. Moreover, prebiotic oligosaccharides may play an ameliorating role over the long term via the intestinal microbiota. However, large scale, well executed studies are required to investigate this in children [11, 27-29].

The present study assesses if there is value for prebiotic oligosaccharides consumption in young children with FC. Data of this study will help determine whether children with FC may benefit from consuming prebiotic oligosaccharides such as GOS or chicory FOS. Moreover, both prebiotics are already safely applied in foods as well as in infant, follow-on and young child formulas, hence application should be feasible. GOS, FOS and mixtures thereof have shown to have stool softening effects when used in infant, follow-on and young child formulas in healthy infants and in small studies in children with FC [20, 26-28, 30].

Importantly, this is one of the few studies that also gives insight in the intestinal microbiota of functionally constipated children, besides the impact of prebiotic oligosaccharides on the microbiota. Recently the intestinal microbiota has been implicated increasingly in not only bowel habit effects but also systemically on metabolism, immunity and the gut-brain axis [40]. Depending on bowel habit outcome effects, it may give some unique insights into the role of the intestinal microbiota. Moreover, this study will help to further characterize microbial signatures that may be linked to clinical subgroups of children with FC [41]. This might enable us to analyze in detail, which intestinal microbiota profiles and/or specific microbial populations may be predictive of a positive response, or lack thereof, to treatment with GOS or FOS. Besides the direct symptomatic and microbiological effects, the tertiary outcomes in this study of health-related quality of life and the need for escape medication will provide valuable insight into the perceived wellbeing of children with FC.

Our study has several strengths. First, this study covers many of the clinical outcomes, which are of major interest to clinicians and parents and takes into account most outcomes as suggested by the core outcome set for clinical trials in children with constipation [42]. These include stool consistency and frequency, painful defecation, quality of life of patients and parents, side effects of treatment, and if age appropriate fecal incontinence [42]. Another strength is that this study investigates the composition of the intestinal microbiota, as well as its activity in terms of pH and SCFAs, and the potential role of the intestinal microbiota in the treatment of FC in children. Moreover, this study takes into account dietary intake, to also be able to correct for general fiber and fluid intake.

A potential challenge for this study, arising from the nature of the condition, is that many functionally constipated children exert stool withholding behavior around the age of toilet training [43]. This behavior is difficult to address, since the vicious cycle of withholding behavior and consequently the passage of hard and large stools has to be broken. In clinical practice, this cycle is attempted to be broken by giving a higher dose of laxatives, of which the dose is adjusted to the child's need, due to which a child cannot exert this withholding behavior. However, this study uses the same dose for all children to first investigate whether prebiotic oligosaccharides may help in the treatment of FC. Within this study we can differentiate between the children with and without stool withholding behavior. However, in case prebiotic oligosaccharides are

found to be effective in the functionally constipated children, a dose-response study would be a valuable addition to more effectively target those who exert intense stool withholding behavior.

A possible limitation of this study is that during this trial current medication use has to be stopped and this might result in (unsatisfactory) changes in bowel habit. Moreover, stopping current treatment and the fact that there is a chance of being in the placebo group might be a disincentive for parents to include their child in this trial. Secondly, the use of rescue medication, despite being selected to have the least influence on intestinal microbiota composition, will influence bowel habit. However, from the diaries we can evaluate whether the bowel movement was spontaneous or after the use of rescue medication, although it will be impossible to rule-out any potential longer-term influence of the rescue medication on bowel habits. Thirdly, the group of children with FC is very diverse. Therefore, it may be a challenge to correct for potential (confounding) factors such as the differences in stool consistency which is known to influence intestinal microbiota composition, parental child-rearing attitudes and stress factors. [44].

Despite these potential limitations, the results of this study could contribute to the development of novel nutritional strategies to support young children with or at risk of FC. It is one of the first studies to thoroughly check the impact of two established prebiotics on FC in children, covering a broad range of parameters. A novel aspect is the additional microbiota composition and activity analyses performed as part of this study. Findings of this study might have important implications for nutrition and supplementation guidelines for children with FC as well as nutritional management of young children with FC which aims to reduce, prevent or treat the symptoms of this condition.

TRIAL STATUS

The protocol has been approved by the METC of Wageningen University & Research on the 21st of October of 2019 and was registered at ClinicalTrials.gov on the 24 February of 2020 (NCT04282551). The study protocol was transferred on the 28th of January 2021 to the METC of the Amsterdam Medical Centre (METC AMC) due to the termination of the METC of Wageningen University & Research. Study recruitment started March 2020. The Covid-19 pandemic resulted in a delay in recruitment and makes it difficult to predict when recruitment will be completed. This current published protocol which was improved with minor amendments is protocol version 7 (11-05-2021).

DECLARATIONS

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Author contributions: All authors were involved in the conceptualization, design and methodology to develop the study protocol. CAMW is the current coordinating investigator and drafted the manuscript. MHCS, EEV, HS, CB and MAB are involved in supervision/ oversight, were responsible for funding acquisition and were involved in editing and reviewing the manuscript.

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Availability of data and material: Data will be stored in a data repository and human material will be stored for five years after completion of the study at the sponsor's site. Participant data will be stored digitally and kept for 15 years at the sponsor's site and two years at the participating hospital.

Ethics approval and consent to participate: Version 7 of the protocol was approved by the METC of UMC Amsterdam (2019_700#B2021521). This study is conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the WMO. Informed consent has to be signed by, if applicable, both parents before any study-related activities.

Consent for publication: Written consent is obtained from participants to use their anonymized data for reporting and publication purposes.

Competing interests: C.A.M.W. is supported by a grant from TKI (AF17067), FrieslandCampina and Sensus, M.H.C.S. is an employee of FrieslandCampina, E.E.V. is an employee of Sensus (Royal Cosun), M.A.B. has acted as a consultant for Shire, Sucampo, Astrazeneca, Norgine, Allergan, Mallinckrodt, Coloplast, Danone, FrieslandCampina, Sensus, United Pharmaceuticals and Novalac. For the remaining authors, there is no conflict of interest.

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Transanal irrigation in children: treatment success, quality of life, adherence, patient experience and independence

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ABSTRACT

Objectives: To investigate the clinical effectiveness and patient experience of transanal irrigation (TAI) in children with constipation or fecal incontinence.

Methods: Combined retrospective and cross-sectional study including pediatric patients who used a Navina™ TAI system. We retrospectively collected baseline characteristics and data on treatment success at 1- and 6-month follow-up (FU). Treatment success was defined as defecating at least three times per week and having less than one episode of fecal incontinence per week. We cross-sectionally assessed health-related quality of life (HRQoL), treatment adherence, treatment satisfaction (TSQM), illness perceptions, medication beliefs, and patient empowerment with validated questionnaires.

Results: Thirty-four patients were included (median age at start TAI: 11 years old [range 6-18]), 32 in the retrospective review and 26 in the cross-sectional survey (median of 3 years after initiation). Most patients were diagnosed with functional constipation (n=26; 76%) or a neurogenic bowel disorder (n=6; 18%). Treatment success rates significantly improved at each follow-up compared to baseline (baseline: 4/25 [16%]; 1-month FU: 12/16 [75%], p=0.008; 6-month FU: 11/18 [61%], p=0.016; cross-sectional FU: 13/26 [50%], p=0.008). HRQoL scores were high (PedsQL median 73 [IQR 54-85]). Adherence (defined as MARS ≥ 23) was low (36%), while TSQM effectiveness scores were high (median 69 [IQR 47-86]). The majority of children (61%) reported an increase in independence since TAI treatment. Patient empowerment (GYPES) levels were similar to those reported in children with other chronic conditions.

Conclusions: TAI with a Navina system is an effective bowel management system for children with intractable constipation or fecal incontinence.

Keywords: Constipation; Child; Transanal Irrigation; Fecal Incontinence.

WHAT IS KNOWN

- Several systems are available for transanal irrigation (TAI) in children with intractable constipation or fecal incontinence. However, no data have been published on TAI with Navina systems in children.

WHAT IS NEW

- TAI with a Navina system is an effective treatment for children with functional and organic causes of defecation disorders.
- Patients using TAI report relatively high levels of health-related quality of life.
- While patients often use TAI differently than prescribed, satisfaction concerning treatment effectiveness is high.
- TAI treatment may increase patient independence. Patient empowerment levels were similar to those reported in children with other chronic conditions.

INTRODUCTION

Constipation is a common disorder in children and adolescents worldwide [1]. It is characterized by infrequent, painful, hard stools and may be accompanied by fecal incontinence and abdominal pain [2]. In approximately 95% of children, no organic cause is found, and these children are diagnosed with functional constipation (FC) according to the Rome IV criteria [3, 4]. Organic causes of constipation include Hirschsprung disease or neurogenic/neuropathic bowel dysfunction (NBD) [5]. Initial management consists of demystification, education, toilet training, and laxative treatment [6, 7]. If conventional treatment fails, healthcare providers turn to more invasive treatment, such as transanal irrigation (TAI).

TAI entails large-volume water irrigation of the rectum and colon via the anus to prevent accumulation of large quantities of stools [8]. To facilitate TAI, several devices have been developed. To date, all published pediatric studies on TAI have investigated the use of systems (Peristeen® or Alterna®) of one specific manufacturer (Coloplast A/S, Humlebaek, Denmark). These studies show high rates of success, both in clinical outcomes and in improvement of health-related quality of life (HRQoL) [9-15]. These promising findings prompted us to investigate if similar effects are achieved with a Navina TAI system (Wellspect Healthcare, Mölndal, Sweden).

In addition to the evaluation of clinical effectiveness, HRQoL, and treatment adherence, we wanted to investigate patient experience, patient independence, and patient empowerment [16]. These aspects have not been previously studied in children using TAI. We hypothesized that these aspects may affect treatment adherence and quality of life, and may facilitate the parent-to-child transfer of self-management of chronic disease in adolescence.

METHODS

This was a single-center study, which combined a retrospective study and a cross-sectional survey study including children using TAI with a Navina system at our institution, a tertiary hospital for pediatric gastroenterology in the Netherlands. An overview of the questionnaires administered during this cross-sectional survey and the target audience per questionnaire is provided in **Table 1**. Additional details on our methods and a detailed description of the questionnaires and the statistical analyses is available in **Supplemental File 1**.

Table 1 | questionnaires for the cross-sectional survey

Outcomes	Completed by
Clinical effectiveness	All patients
Quality of life	All patients and parents of patients < 18 years of age
Adherence (MARS)	All patients using TAI
Medication beliefs (BMQ)	All patients using TAI
Illness perception (BIPQ)	All patients
Treatment satisfaction (TSQM)	All patients using TAI
Patient empowerment (GYPES)	Patients ≥ 13 years old

CLINICAL EFFECTIVENESS

We collected data on patient's medical history, treatment, symptoms, and treatment success retrospectively. Treatment success was defined in accordance with the Rome IV criteria: defecating at least three times per week and having less than one episode of fecal incontinence per week [17]. Data were retrospectively collected at baseline (before starting Navina), after 1 month of treatment (range 1-3 months), and after 6 months of treatment (range 3-9 months). In the cross-sectional part of the study, we evaluated current symptoms and treatment success.

CROSS-SECTIONAL SURVEY

During the cross-sectional survey several validated questionnaires were completed electronically via Castor EDC [18]. Data are presented separately for children with functional and organic causes of constipation. HRQoL was measured by the PedsQL and PedsQL – GI symptom scales [19-21]. These data were compared with HRQoL data of other studies including Dutch healthy children, Dutch children with FC, American children with FC, and children with functional and organic causes of constipation using TAI from Sweden and Norway [22-26].

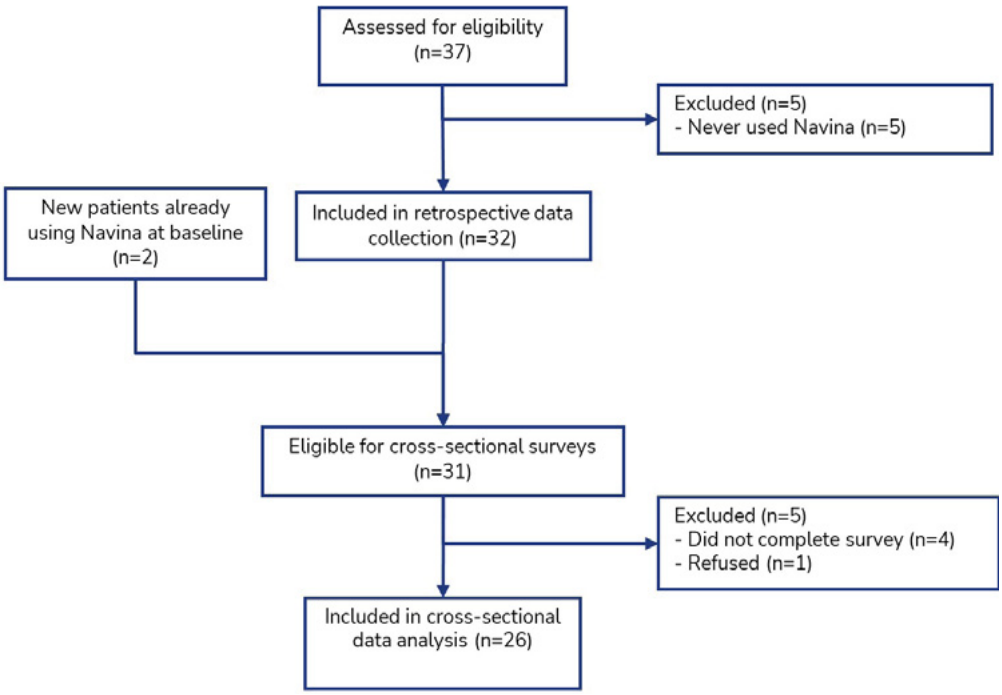

Figure 1 | Patient flow chart

The Medication Adherence Report Scale (MARS was used to assess treatment adherence [27, 28]. The "Beliefs about Medicines Questionnaire (BMQ) – Specific" was used to assess beliefs about the necessity of TAI, and concerns about TAI [29]. The Brief illness perceptions questionnaire (BIPQ) was used to assess cognitive and emotional perceptions of illness [30]. A self-developed questionnaire was used to measure treatment independence and the Gothenburg Young Persons Empowerment Scale (GYPES) was used to assess patient empowerment [31]. The Treatment Satisfaction Questionnaire for Medication (TSQM) was used to assess treatment satisfaction [28, 32]. A self-developed questionnaire was used to evaluate patient experience with the transanal irrigation system and adverse effects.

RESULTS

For the retrospective study, 37 children were invited, none opted out for the use of their data. Five children were excluded because after they received information about TAI with Navina they never initiated TAI treatment. Therefore, 32 children were included in our retrospective data-analysis, see **Figure 1**. Of these 32 children, 29 children were eligible for the cross-sectional part of our study. Two additional patients who could not be included in the retrospective part of the study because they were already using Navina, were invited for the cross-sectional survey only. Of all patients invited for the cross-sectional survey, 26/31 patients (84%) completed all questionnaires (median age 12.6 years, range 7-22, 73% male).

BASELINE CHARACTERISTICS

Baseline characteristics of all patients are shown in **Table 2**. The majority of patients were diagnosed with FC (n=26; 76%) and NBD (n=6; 18%). The majority of patients with FC were using Navina Classic (16/24; 67%), whereas the majority of children with a NBD were using Navina Smart (5/6; 83%). The age at which patients started using Navina systems (median age 11 years old [range 6-18]) did not significantly differ between patients using Navina Classic and Navina Smart (median age 10.4 [IQR 8.3-12.7] and 11.3 [IQR 8.1-12.5] years, respectively).

Table 2 | Baseline characteristics at start with Navina

	All patients (n=34)
Age at start symptoms in years, median (IQR)[a]	3.8 (0.0-4.0)
Age at start TAI with Navina, median (IQR)	11.0 (8.3-12.8)
Duration of symptoms in years, median (IQR) [a]	7.7 (5.3-9.5)
Sex (male), n (%)	26 (77%)
Constipation-related diagnosis and comorbidities	
Functional constipation, n (%)	26 (76%)
Urinary incontinence. n/N (%)	3/26 (12%)

Table 2 | Continued

	All patients (n=34)
ADHD/ADD. n/N (%)	2/26 (8%)
Autism, n/N (%)	1/26 (4%)
History of perianal abscesses, n (%)	1/26 (4%)
Neurogenic bowel dysfunction, n (%)	6 (18%)
Spina bifida, n/N (%)	5/6 (83%)
Traumatic spinal cord injury, n (%)	1/6 (17%)
Hirschsprung disease, n (%)	1 (3%)
Functional non-retentive fecal incontinence, n (%)	1 (3%)
Previous treatment before initiation of TAI (Navina)	
Polyethylene glycol, n/N (%)	17/29 (59%)
Transanal irrigation with other system, n/N (%)	7/29 (24%)
Bisacodyl, n/N (%)	3/29 (10%)
Pharmacological enemas, n/N (%)[b]	5/29 (17%)
Lactulose, n/N (%)	1/29 (3.4%)
Lubiprostone, n/N (%)	1/29 (3.4%)
None, n/N (%)	6/29 (21%)
Reason for initiating TAI (Navina)	
Refractory symptoms, n/N (%)	25/31 (81%)
Dissatisfied with other irrigation system, n/N (%)	5/31 (16%)
Dissatisfied with enema use, n/N (%)	1/31 (3%)

[a]missing n=1

CLINICAL EFFECTIVENESS

Data on clinical effectiveness were available for the majority of patients and are provided in **Table 3**. At baseline, 4/25 children (16%) fulfilled the criteria for treatment success (functional: 3/21 [14%], organic: 1/4 [25%]). At 1-month follow-up, 12/16 children (75%) fulfilled the criteria for treatment success (functional: 10/12 [83%], organic: 2/4 [50%]), this was a statistically significant change compared to baseline (p=0.008). At 6-month follow-up 11/18 children (61%) fulfilled criteria for treatment success (functional: 9/15 [60%], organic: 2/3 [67%]), this was a statistically significant change compared to baseline (p=0.016). At latest follow-up, during the cross-sectional survey, at a median of 3 years after initiation of TAI with Navina systems, 19/26 of children (73%) were still using Navina systems (Navina Smart n=12, Navina Classic n=7). Most patients were using a regular catheter (n=9), followed by a small catheter (n=8), or a cone (n=1), one patient did not answer this question. At this time, 13/26 (50%) fulfilled criteria for treatment success (functional: 11/20 [55%], organic: 2/6 [33%]), this was a statistically significant change compared to baseline (p=0.008). Most children who were not using Navina anymore fulfilled criteria for treatment success (6/7 [86%]), but this was not significantly different compared to children who were still using Navina (7/19 [37%], p=0.073).

Treatment success rates at each follow-up did not differ between system types. Of the 17 children with fecal incontinence at baseline, 11 completed our cross-sectional questionnaire, of whom 4 (36%) were experiencing fecal incontinence on a weekly basis. Of the children using TAI, 14/18 (78%) had spontaneous bowel movements in between irrigations. The seven patients who were no longer using Navina provided the following reasons for cessation: resolution of symptoms (n=4); worsening of symptoms (n=2); or dissatisfaction with the TAI treatment/system (n=1).

Table 3 | Clinical effectiveness

	At start Navina (n=32)	**1 month follow-up (n=25)**	**6 month follow-up (n=20)**	**Latest follow-up (cross-sectional) (n=26)**
Follow-up time in months, median (IQR)	-	1.2 (0.9-1.6)	5.6 (4.5-6.6)	37.8 (27.7-40.4)
Navina use, n (%)	-	25 (100%)	17 (85%)	19 (73%)
Irrigations per week, median (IQR)		7 (7-7)	6 (5-7)	7 (1.5-7)
Symptoms				
Treatment success, n/N (%)	4/25 (16%)	12/16 (75%)*	11/18 (61%)*	13/26 (50%)*
< 3 bowel movements per week, n/N (%)	11/24 (46%)	0/18 (0%)	0/19 (0%)	5/26 (19%)*
Bowel movements per week, a median (IQR)	3.0 (2.0-4.5)	7.0 (7.0-7.0)	7.0 (7.0-7.0)	5.0 (3.0-7.0)*
Weekly fecal incontinence, n/N (%)	17/25 (68%)	4/20 (20%)*	7/19 (39%)	9/26 (35%)*
Fecal incontinence freq/week,[b] median (IQR)	14 (6-28)	6 (4.0-6.0)	6.5 (2.0-7.0)	No data
Fecal incontinence freq/week,[c] median (IQR)	3 (0-15)	0 (0.0-0.0)*	0 (0.0-2.0)*	No data
Large stools, n/N (%)	6/10 (60%)	1/1 (100%)	1/2 (50%)	6/26 (23%)
Painful/hard stools, n/N (%)	11/21 (53%)	0/4 (0%)	0/4 (0%)	7/26 (27%)
Withholding behavior, n/N (%)	8/18 (44%)	0/1 (0%)	No data	8/26 (31%)
Abdominal pain, n/N (%)	16/30 (53%)	5/19 (26%)	7/16 (44%)	No data
Additional treatment[d]				
Oral laxatives, n (%)	18 (62%)	13 (52%)	9 (47%)	13 (72%)
Pharmacological enemas, n (%)	5 (17%)	2 (8%)	2 (11%)	2 (11%)
Loperamide, n (%)	0 (0%)	2 (8%)	2 (11%)	0 (0%)
None, n (%)	6 (21%)	9 (36%)	7 (37%)	5 (28%)

* P Value <0.05 compared to baseline

[a] includes data of 24 children, 18 children, 18 children, and 26 children respectively

[b] includes only data of children with weekly fecal incontinence episodes: 13 children, 4 children, and 6 children respectively

[c] includes all available data on fecal incontinence episodes: 21 children, 20 children, and 17 children respectively

[d] includes data of 29 children, 25 children, and 19 children, and 18 children (active Navina users) respectively

QUALITY OF LIFE

Overall, children with FC in our population report similar PedsQL scores, and even slightly higher PedsQL – GI scores compared to other studies in children with FC [23, 24], see **Table 4** and **Figure 2**.

Table 4 | Health-related quality of life

	Child report (functional)	Child report (organic)	Parent report (functional)	Parent report (organic)
PedsQL, total n	20	6	17	6
Physical functioning, median (IQR)	88 (70-94)	44 (31-88)	88 (59-97)	58 (28-81)
Emotional functioning, median (IQR)	75 (58-85)	60 (50-90)	75 (55-85)	63 (50-90)
Social functioning, median (IQR)	90 (65-93)	75 (65-80)	70 (50-80)	70 (60-75)
School functioning, median (IQR)	68 (55-75)	58 (50-60)	60 (60-70)	58 (50-65)
Total mean score, median (IQR)	79 (64-86)	57 (51-72)	73 (67-84)	60 (51-68)
PedsQL – GI symptom scale, total n	19	6	17	6
Constipation, median (IQR)	68 (54-84)	71 (62-87)	79 (58-88)	71 (28-82)
Total mean score, median (IQR)	74 (61-87)	78 (74-86)	81 (67-89)	79 (77-84)

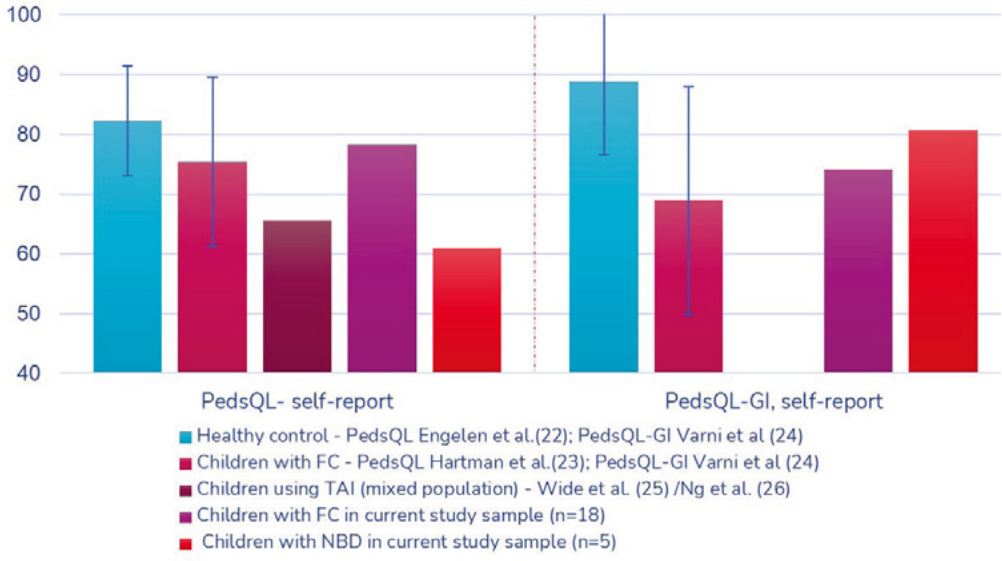

Figure 2 | Health-related quality of life by diagnoses and compared with data of healthy children and children with functional constipation

TREATMENT ADHERENCE, BELIEFS, AND ILLNESS PERCEPTIONS

Data on treatment adherence, beliefs about medication, and illness perceptions are provided in **Table 5**. Fourteen of the 19 patients who were using TAI at time of data collection completed the MARS questionnaire on treatment adherence, median score was 20 (IQR 19-23) and 5 children (36%, FC: n=4; HD: n=1) had a score ≥23 and were considered adherent. Five children (36%) had a score ≥22, and 6 children (43%) had a score ≥21. Low scores were often based on the question about skipping an irrigation, where 7 children (50%) reported to sometimes or often skip an irrigation. Perceived TAI necessity was high, with a median BMQ necessity score of 17.5 (IQR 16–20). TAI concern scores did not exceed necessity scores in any of the children. Results of the illness perception questionnaire showed a wide range in illness perceptions, with relative high scores on the expected duration of constipation, the feeling of control on constipation, and the understanding of constipation.

Table 5 | Patient experience by cause of constipation of patients who completed the cross-sectional survey

	Functional causes (n=20)	**Organic causes (n=6)**
Age in years, median (IQR)	13.4 (11.1-15.8)	11.7 (10.1-14.9)
Sex (male), n (%)	15 (75%)	4 (67%)
Currently using Navina system, n (%)	13 (65%)	6 (100%)
Treatment adherence - MARS, total n	11	3
Median score (IQR)	20 (19-23)	20 (19-22)
Adherent patients (MARS ≥ 23), n (%)	4 (36%)	1 (33%)
Medication beliefs - BMQ, total n	9	5
Necessity score, median (IQR)	17 (16-19)	18 (17-21)
Concerns score, median (IQR	14 (12-15)	11 (8-16)
Differential score, median (IQR)	4 (3-5)	7 (3-12)
Illness perception - BIPQ, total n	20	6
Q1 Consequences, median (IQR)	6.5 (2.3-8.0)	5.5 (4.5-8.5)
Q2 Timeline, median (IQR)	7.0 (2.5-9.8)	9.5 (6.0-10.0)
Q3 Personal control, median (IQR)	4.5 (2.0-6.8)	3.0 (0.0-8.0)
Q4 Treatment control, median (IQR)	7.0 (5.0-8.0)	6.0 (3.0-7.3)
Q5 Identity, median (IQR)	4.5 (2.0-8.0)	3.0 (1.8-8.5)
Q6 Concern, median (IQR)	4.0 (2.3-7.8)	1.5 (0.0-6.3)
Q7 Understanding, median (IQR)	7.0 (5.0-8.0)	6.0 (2.5-8.5)
Q8 Emotional response, median (IQR)	6.5 (2.0-8.8)	4.5 (0.8-10.0)
Treatment satisfaction -TSQM, total n	10	6
Effectiveness, median (IQR)	75 (46-85)	58 (43-89)
Convenience, median (IQR)	58 (43-89)	72 (35-79)
Global satisfaction, median (IQR)	53 (43-83)	61 (56-79)

PATIENT INDEPENDENCE AND EMPOWERMENT

Children reported that since TAI use, their independence greatly increased (n=6; 33%), slightly increased (n=5; 28%), stayed the same (n=5; 28%), slightly decreased (n=1; 6%), or greatly decreased (n=1; 6%). Most children (n=14; 78%) performed TAI with the help of someone else, four of whom needed support during the whole procedure (age range 9-13 years old, FC: n=2, NBD: n=2). Nine children needed support for parts of the procedure including: assembling the system (n=2), filling the water container (n=3), inserting catheter or cone (n=7), removing catheter or cone (n=4), controlling the system (n=3), demounting the system (n=2), cleaning the system (n=5), and using the smart app (n=1). The required amount of time of support ranged between 1-45 minutes (median 3.5 minutes). Four children (age range 10-20 years old, FC: n=2, NBD: n=1, HD: n=1) reported to perform TAI independently. One child reported to be able to perform TAI independently while needing help from a parent with the use of enemas. Patient empowerment was measured using the GYPES (FC: n=11, NBD n=2). The median total empowerment score was 55 (IQR 52-59). The highest empowerment score was reported on the knowledge and understanding domain (median 13, [IQR 12-13]), and the lowest score was reported on the identity domain (median 10, [IQR 8- 11]).

TREATMENT SATISFACTION AND PATIENT EXPERIENCE

Sixteen of the 19 patients who were using TAI at time of data collection completed the TSQM, see **Table 3.** Since only one child reported to have side effects (fatigue), descriptive data of the side effects domain are not included in the table. When combining data of all children, treatment satisfaction effectiveness scores were high (TSQM median 69 [IQR 47- 86]). On a 5-point Likert scale ranging from very dissatisfied to very satisfied, patients were very satisfied (n=13; 72%) or somewhat satisfied (n=5; 28%) with the Navina TAI system. Overall, children found the use of TAI very bothersome (n=4; 22%), somewhat bothersome (n=8; 44%), neutral (n=2; 11%), not really bothersome (n=2; 11%), or not bothersome at all (n=2; 11%). When asked what they found most bothersome responses included: the amount of time it takes (n=12), abdominal pain (n=2), inconvenience (n=1), hassle (n=1), and one parent reported that her child only lets her perform the procedure.

ADVERSE EVENTS

From the participants of the cross-sectional survey, 18/19 children who were using Navina at time of questionnaire completion and reported data on side effects and adverse events. Data are provided per catheter type in **Table 6**. The most common adverse reaction was abdominal pain, which 6 children (33%) reported to experience during the TAI procedure.

Table 6 | Adverse reactions by catheter type and other adverse events

	Catheter type	Never	Rarely	Sometimes	Often	Always
Pain during catheter insertion	Regular	3 (33%)	1 (11%)	3 (33%)	2 (22%)	0 (0%)
	Small	5 (62.5%)	1 (12.5%)	2 (25%)	0 (0%)	0 (0%)
	Cone	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Catheter stays in place	Regular	0 (0%)	1 (11%)	0 (0%)	4 (44%)	4 (44%)
	Small	0 (0%)	0 (0%)	1 (12.5%)	2 (22%)	5 (62.5%)
	Cone	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Abdominal pain during TAI	Regular	1 (11%)	2 (22%)	4 (44%)	2 (22%)	0 (0%)
	Small	1 (12.5%)	1 (12.5%)	3 (37.5%)	3 (37.5%)	0 (0%)
	Cone	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Fluid leaks during infusion of the fluid	Regular	3 (33%)	1 (11%)	3 (33%)	2 (22%)	0 (0%)
	Small	2 (25%)	3 (37.5%)	2 (25%)	1 (12.5%)	0 (0%)
	Cone	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Other responses:

- Incorrect error messages (n=1, Navina Smart-user);
- An error message whilst inflating the balloon which can be prevented by inflating the balloon before insertion (n=1, Navina Smart-user);
- Bursting of the balloon and repetitive expulsion of the balloon which results in repeating the TAI procedure (n=1, Navina Smart-user).

DISCUSSION

This is the first study to assess the clinical application of TAI with Navina systems in the pediatric population, consisting mainly of patients with FC and NBD. Treatment success rates significantly improved compared to baseline at 1-month and 6-month follow-up and improvement persisted at cross-sectional follow-up. Weekly fecal incontinence rates also significantly improved compared to baseline at 1-month follow-up and improvement persisted at cross-sectional follow-up. HRQoL scores were high compared to other studies in similar patient populations. Treatment adherence (defined as MARS≥23) was low (36%), while TSQM effectiveness scores were high. The majority of children (61%) reported an increase in independence since TAI treatment. Patient empowerment levels were similar to those reported in children with other chronic conditions. Based on these results, TAI with Navina systems can be considered to be an effective bowel management treatment for children. Thus far, most studies in children with FC using TAI (with Peristeen®) have used a retrospective study design and various

definitions of effectivity, including partial or complete remission of fecal incontinence episodes, or resolution of constipation/incontinence symptoms [9, 10, 15]. These studies show effectivity rates ranging between 41-73% [9, 10, 15]. In a cross-sectional study in children with FC, fecal incontinence had resolved completely in 41% of all children who still used Peristeen® at the time of survey (n=49) [9]. Studies in children with NBD have also used various study designs and outcome measures, including partial or complete remission of fecal incontinence episodes, or improvement in neurogenic bowel dysfunction scores. These studies have shown TAI with Peristeen® to be effective in this population in 86-91% [12, 15, 33-35]. Treatment success rates in our study are potentially lower due to the prospective setup and more strict definition of treatment success. Therefore, it is difficult to compare our data with data of these other studies.

In our sample, HRQoL scores (PedsQL) were comparable to those of Dutch children with FC and similar compared to data of a population of European children with FC and organic causes of constipation using TAI with Peristeen® [25, 26]. Although the perceived necessity of TAI was high, treatment adherence was low. One study including 78 children with NBD using Peristeen® investigated treatment adherence to TAI by asking if patients were still using TAI after a median duration of 14 months [14]. With this method, the authors reported high levels of adherence (80-92%). When using the same method to measure adherence, the adherence rate in our study sample would also have been high (73%). However, adherence comprises more than confirmation of use. Besides, discontinuation of treatment may be an indication of the treatment being ineffective, or the result of resolution of symptoms. For this reason, we used the validated MARS questionnaire to measure adherence. Another study in children with FC also used the MARS questionnaire to measure adherence to polyethylene glycol [28]. This study reported similar adherences rates compared to our study; a median MARS score of 22 (IQR 20-24) with an adherence rate (MARS ≥23) of 37% [28]. However, the MARS questionnaire may have a limited validity in patients with defecation disorders since deviation of treatment as measured by the MARS (skipping an irrigation, or changing the amount of TAI fluid) may be inherent to the treatment and the underlying disorder. If children defecate spontaneously without the use of TAI, health care providers may even promote to try to skip an irrigation. In our study sample low MARS scores were often related to the question concerning skipping an irrigation, therefore the adherence rate based on the MARS may not be indicative of issues with treatment adherence. In clinical practice, we do not experience issues with treatment adherence on a regular basis in these patients. We think that this might be the consequence of the severity of symptoms in children using TAI and the effectivity of the TAI procedure. Children often experience direct effect of the TAI procedure, which is reflected in the high rates of treatment satisfaction concerning effectiveness in this study cohort which in turn likely positively affects adherence. Most children reported to need help with the TAI procedure. Still, the majority of patients reported an increase in independence since starting TAI treatment. Current rates of independent use of TAI range from 16-79% in the literature [12, 25, 36, 37]. These results are influenced by the definition of independent use, and rely on the age and underlying pathology of children. A previous study in 172 children with NBD showed no difference in the independence of children using TAI or antegrade continence enemas [25]. In addition, they reported that children who always went to the toilet by themselves reported significantly higher

HRQoL scores than those who never went alone. Independence therefore could play a role in the HRQoL of children. The reported increase in independence after initiation of TAI may explain the high treatment satisfaction rates and relatively high levels of HRQoL in our study sample. Perceived effectiveness (based on the TSQM) was high compared to data from a study in children with FC using polyethylene glycol (median of 69 [IQR 47-86] versus median of 48 [IQR 37-62], respectively) [28]. Teens in our study report similar patient empowerment levels as teens with congenital heart disease (mean 54.5 [SD 10.5]) [38] and a slightly lower level of empowerment compared to teens with diabetes (mean 58.9 [SD 7.9]) [31]. However, the clinical consequences of these differences are unclear as to date healthy reference values for the GYPES are unavailable.

Adverse events were uncommon in our study, the most commonly reported adverse event was abdominal pain during the TAI procedure. Patients reported that the most bothersome aspect of TAI is the amount of time it takes. However, we did not collect data on the amount of time they spent on their bowel management before initiation of TAI with Navina. Another study, which investigated the effect of TAI on the time spent on bowel management, reported a significant decrease since initiation of TAI with Peristeen® [12]. In addition, another study comparing the time spent on bowel management between children using antegrade continence enemas and TAI reported that children using TAI spent significantly less time at the toilet for defecation [25]. Thus, although the TAI procedure is time-consuming, it may turn out to be the most time-efficient for children with intractable constipation unresponsive to conventional medical treatment.

Strengths of our study include the use of multiple questionnaires including self-report and parent-report questionnaire in order to gain a clear view of child- and parent perspectives on TAI use. We had a high response rate (84%) and also included participants who were not using TAI anymore.

This study has several limitations inherent to its partial design as a retrospective review. Since part of our study was based on retrospective data, our baseline, 1-month, and 6-month follow-up data rely on data documented in the medical charts. Therefore, the paired measures statistics have a limited reliability due to missing data, as this analysis only takes into account patients with data on both baseline and follow-up time points. In addition, our population originates from a population of only one specialized referral center and may have been at risk of selection bias. Since we had no information on why these children chose to start TAI treatment with Navina, instead of other treatments, or TAI treatment with another system, this could have affected our outcomes and limits the generalizability of our findings. Moreover, we were not able to compare our outcomes with a control group. To further assess the usefulness of TAI with Navina in the management of children with specific indications, prospective studies should be conducted, preferably with validated measurements and standardized symptom-based outcome measures. A randomized controlled trial comparing the efficacy, side effects, and patient/parental satisfaction of different TAI systems or TAI compared to antegrade continence enemas would be of great interest.

To conclude, TAI with Navina systems is an effective bowel management system for children with constipation or fecal incontinence associated with relatively high levels of HRQoL. An increase in independence was reported since TAI use, and perceived effectiveness was high. Although the TAI procedure may be time-consuming and inconvenient, it should be considered as treatment option for children with constipation unresponsive to conventional medical treatment before more invasive surgical treatment is initiated.

SUPPLEMENTAL FILE 1

Patients and their parents were asked to complete several questionnaires, some of these questionnaires were filled out separately (parent-report and self-report). Completing all online questionnaires took approximately 45 minutes and was done at home. After completion of all questionnaires, subjects received a gift card worth €25.00.

Quality of life

The PedsQL 4.0 Generic Core Scales were used to assess HRQoL, and the PedsQL GI symptom scales were used to assess perceptions on gastrointestinal specific symptoms (19-21). The scales used in the current study comprised of parallel child self-report and parent proxy-report formats for children aged 5 to 7 (young child), 8 to 12 (child), and 13 to 18 years old (adolescent). Both questionnaires are comprised of subscales, including a constipation-specific symptom scale. Items are reverse-scored and linearly transformed to a 0 to 100 scale (0=100, 1=75, 2=50, 3=25, 4=0); higher scores indicate less problems or symptoms and, hence, a higher HRQoL. The total score and subscale scores are computed as the sum of the items divided by the number of items answered. The questionnaires were completed by all children, and parents of children under 18 years of age. Data are presented separately for children with NBD and children with FC and compared with HRQoL data of other studies reporting data of Dutch healthy children, Dutch children with FC, and American children with FC (22-24).

Treatment adherence and beliefs, and illness perceptions

The Medication Adherence Report Scale (MARS) questionnaire was used to assess treatment adherence and was completed by all children using Navina systems at time of the survey (27, 28). The questionnaire consists of 5 items scored on a 5-point Likert scale (1=always, 5=never). This resulted in a total score ranging from 5 to 25, with higher scores implying higher adherence. Data are reported both as continuous outcomes as well as the percentage of adherent patients (defined as a MARS score of ≥23) (28). Children using Navina at time of the cross-sectional survey completed the "Beliefs about Medicines Questionnaire (BMQ) – Specific" to assess beliefs about the necessity of, and concerns about TAI (29). Both the necessity and concern subscale scores range from 5 to 25, with higher scores representing stronger necessity perceptions and stronger concerns. All children completed the Brief illness perceptions questionnaire (BIPQ) to assess cognitive and emotional perceptions of illness(30). This questionnaire uses a single-item scale approach with 8 items which are rated on a scale from 0 to 10. Higher scores reflect stronger perceptions of the respective item.

Patient independence and empowerment

A self-developed questionnaire was used to measure treatment independence. Children rated the relative change in independence concerning their bowel management since start TAI on

a 5-point Likert scale ranging from greatly decreased to greatly increased. Children from 13 years of age using Navina at time of the survey completed the Gothenburg Young Persons Empowerment Scale (GYPES) to assess patient empowerment (31). The questionnaire consists of 15 items scored on a 5-point Likert scale (1 = strongly disagree, 5 = strongly agree). Scores were calculated for 5 domains (Knowledge and Understanding, Personal Control, Identity, Decision making, and Enabling others) and a total empowerment score was calculated. This resulted in a total score ranging from 15 to 75, with higher scores corresponding with higher levels of empowerment.

Treatment satisfaction and patient experience

Children using Navina at time of the cross-sectional survey completed the Treatment Satisfaction Questionnaire for Medication (TSQM) to assess treatment satisfaction (28, 32). A self-developed questionnaire was used to evaluate patient experience with the transanal irrigation system and adverse effects. Last, patients no longer using a Navina System were asked about the reason for cessation of treatment.

Statistical Analyses

Statistical analyses were conducted with SPSS for Windows, version 26 (SPSS, Inc., Chicago, IL). Our sample included all patients listed by our nurse practitioner to have ever been scheduled to start TAI with a Navina system in our center, and patients already using Navina whom were followed in clinic. Because of our small patient sample we assumed data were not normally distributed. Therefore, data are presented using medians and interquartile ranges. Differences between groups were either tested with Fisher's Exact test, Mann-Whitney U test, McNemar's test, or Wilcoxon Signed Rank test as appropriate. Data-analysis was performed following a modified intention-to-treat principle, including all children of whom data was available, regardless of their TAI use. A P value of <0.05 was considered statistically significant.

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General discussion & future perspectives

FROM HEALTHY DEFECATION TO FUNCTIONAL CONSTIPATION IN CHILDREN AND ADULTS

The process of defecation involves a wide variety of well-orchestrated, coordinated sensorimotor functions but is also influenced by processes involved in digestion, absorption, secretion and motility, including a broad range of interactions of the intestinal microbiota. Besides these physiological factors, also genetic factors, lifestyle factors, psychological disorders and psychosocial factors such as major life events, educational level, parental child-rearing attitudes and stool withholding behavior in children may influence defecation [1-4]. In this thesis, we discussed defining and measuring healthy defecation patterns in children and the pathophysiology of functional constipation (FC). In addition we delved deeper into FC in children and adults from both a clinical and a microbiological perspective. Also non-pharmacological and intestinal microbiota directed interventions in several functional gastrointestinal disorders (FGIDs), such as functional abdominal pain disorders (FAPD), infant colic and irritable bowel syndrome (IBS) were discussed. In this general discussion, I will elaborate further on defining and measuring healthy defecation patterns in children, provide insight in the role of non-pharmacological and intestinal microbiota directed interventions in several FGIDs including FC and focus on future perspectives in the fields of health-care and microbial ecology in relation to FGIDs such as FC in children and adults.

THE VALIDITY, RELIABILITY AND REPORTING OF A SUBJECTIVE MEASURE

In the field of pediatric gastroenterology, a lot of effort is put into defining clear criteria for a wide variety of FGIDs. However, when it comes to normal defecation, evidence-based data on normal defecation patterns in children is scarce and comes from papers in small cohorts of children [5-7]. With regard to defecation patterns in children many rules-of-thumb exist to 'define' healthy defecation. One example, in the Netherlands, that is frequently used is 'healthy defecation frequencies in human milk-fed children can be once in ten days, or ten times per day' [8]. These rules-of-thumb might differ between countries, but also differ within countries and between primary, secondary and tertiary healthcare [7, 8]. It is, however, important to have consensus as to which defecation patterns can be considered as normal. This is especially the case for functional disorders, where diagnosis and monitoring is often based on, usually validated and reasonably reliable but still, subjective measures. These measures may include, besides the exclusion of organic causes and an evaluation of the medical history, evaluation of symptoms via pain diaries or defecation pattern diaries or by symptom questionnaires such as the validated Rome IV Diagnostic Questionnaire for Pediatric FGIDs (R4PDQ) [9-11]. In the light of FC, where early treatment is associated with better long-term outcomes, such reference values of normal defecation patterns may help clinicians to act sooner and thereby positively influence health outcomes on the longer term [12]. Therefore, we conducted a study (**chapter 4**) to define such reference values in terms of stool consistency and

stool frequency. We concluded that 0-14 week old children had a mean defecation frequency of 21.8 (reference interval (RI): 3.9-35.2) per week, compared to 10.9 (RI: 5.7-16.7) in 15 week to 4 year old children. human milk-fed children had the highest mean defecation frequency of 23.2 per week (RI: 8.8-38.1) followed by mixed-fed children with 20.7 (RI: 7.0-30.2) and formula-fed children with 13.7 (RI: 5.4-23.9). Very few healthy 0-14 week old children were reported to have the hardest stool consistency (1.5%) compared to around one in ten children aged 15 weeks – 4 years (10.5%). Vice versa, many 0-14 week old children were reported to have the softest stools (27.0%) compared to 6.2% of children aged 15 weeks – 4 years. Moreover, although not significant, we found that differences exist between countries. These may be explained by differences in toilet (training) behavior, physical activity and differences in dietary intake, including the amount of fiber and fluid intake which have been associated with stool consistency [13, 14]. However, the majority of the studies included in this systematic review did not use validated stool scales and did not take into account dietary intake. For that reason, we set up a study in healthy children aged 0-4 years to investigate more specifically the stool patterns in the Netherlands, taking into account their dietary intake as well as having parents fill-out the R4PDQ to assess if these children may be at risk for disorders of the gastrointestinal (GI) tract. The results of this study are not presented in this thesis, yet they may contribute to the understanding of healthy defecation in young Dutch children. More than having the reference values on stool frequency and consistency published, it would move the field of well-child care and pediatric gastroenterology forward if these data would be consistently used for national and international guidelines issued by organizations such as, for example, the Dutch pediatrics and general practitioners associations, Nederlandse Vereniging voor Kindergeneeskunde (NVK), Nederlands Huisartsen Genootschap (NHG) and Nederlands Centrum Jeugdgezondheid (NJC) but also international organizations such as North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN).

Another complication in creating such reference values is the manner in which stool consistency is measured, especially in children. In contrast to the adult population, where the Bristol Stool Form Scale is the default and most frequently used stool scale, scales for children come with complications [15]. These complications arise because these scales are often specifically designed and validated for either toilet-trained children or non-toilet-trained children. Moreover, comparisons between studies are impaired due to variation in the numbers of items used; some use a 3-point scale, while others use a 4-, 5-, or 7-point scale [15-18]. In order to conduct research in children one would use a diaper stool scale in non-toilet-trained children, and a different stool scale for toilet-trained children. In addition, even when the same number of items is used for these different scales, a certain type can be differently described and thereby differently interpreted by the person who rates the stool consistency. These problems become even more apparent in the time around toilet-training, which is a period that can be of major interest in the context of defecation issues. Nevertheless, using two scales within one study i.e., one for when the child is toilet trained and another for children who are still using diapers, is not desirable for all above-mentioned reasons. Since in a functional disorder such as FC there is no objective measure, researchers must rely on subjective measures such as questionnaires to assess symptoms

on, in this case, stool consistency. In **chapter 3** we validated the modified Bristol Stool Form Scale (mBSFS) and concluded that this mBSFS, as paper or online version, is reliable, valid and user-friendly to use for Dutch-speaking parents, grandparents and day childcare employees to evaluate defecation parameters in toddlers whether in diapers or toilet-trained [19]. To our knowledge, this is the first scale that has been validated for both toilet and non-toilet-trained children, making it a valuable tool for monitoring disease activity and evaluate the effects of (clinical) interventions in the transition period of toilet-training.

Besides the importance of validated questionnaires or stool scales for a specific target group, **chapter 4** also highlights the need and importance to comply to core outcome sets (COS) for clinical trials. Furthermore, clinical trials should take into account recommendations of scientific bodies such as the Food and Drug Administration (FDA) or European Food and Safety Association (EFSA) for validating such questionnaires [8]. Furthermore, joint committees such as NASPGHAN, ESPGHAN or the Rome foundation should, besides focusing on evaluation and treatment guidelines, be involved in the development of clinical reporting guidelines. Thus, clinical treatment guidelines may be reconsidered with changes in clinical evidence of interventions, based on data of e.g. stool consistency. To make these data comparable, valid and reliable, data collection should be correspondingly comparable, valid and reliable. By having authorities like NASPGHAN, ESPGHAN or the Rome foundation recommend reporting guidelines, we could increase the quality of evidence used for their own evaluation and treatment guidelines. Consequently, there should also be a role for such authorities to either recommend e.g. a stool scale that can be used free of charge and is open access, or to take a stance in making such a stool scale open access and free of charge to use to prevent that such scales are not used due to (high) costs.

In conclusion, reference values of stool consistency and frequency can help to identify children that may be of risk of a functional disorder such as FC at an early stage, in order to start early or offer even prophylactic treatment in children only experiencing some hard stools. Additionally, in a diversity of disorders where subjective measures are currently the only option to diagnose a disorder and evaluate disease activity we should at least aim for valid and reliable tools to do so.

ATTEMPTING TO SOLVE MULTIFACTORIAL DISORDERS WITH A 'ONE SIZE FITS ALL' APPROACH

In **chapter 1** and in **chapter 2** we discuss the epidemiology, (patho)physiology, diagnosis, management, treatment and prognosis of FC in children and adults. The pathophysiology of FC in children and adults is considered to be multifactorial where several overarching factors may be involved, as shown in **Figure 1**. The majority of patients with FC is effectively treated with the use of laxatives [9, 20]. However, adherence to laxative therapy is low, especially in children treated with polyethylene glycol (PEG) that is the first-line treatment. Adherence to PEG in children was reported to be only 37% for which treatment inconvenience, dissatisfaction with treatment and the emotional impact of FC were negative influences on treatment adherence [21]. A similar result was found in an earlier study, which may explain the poor results of treatment in

this young patient population and may be one of the reasons for parents of patients to seek help in the form of alternative or complementary medicine [22, 23].

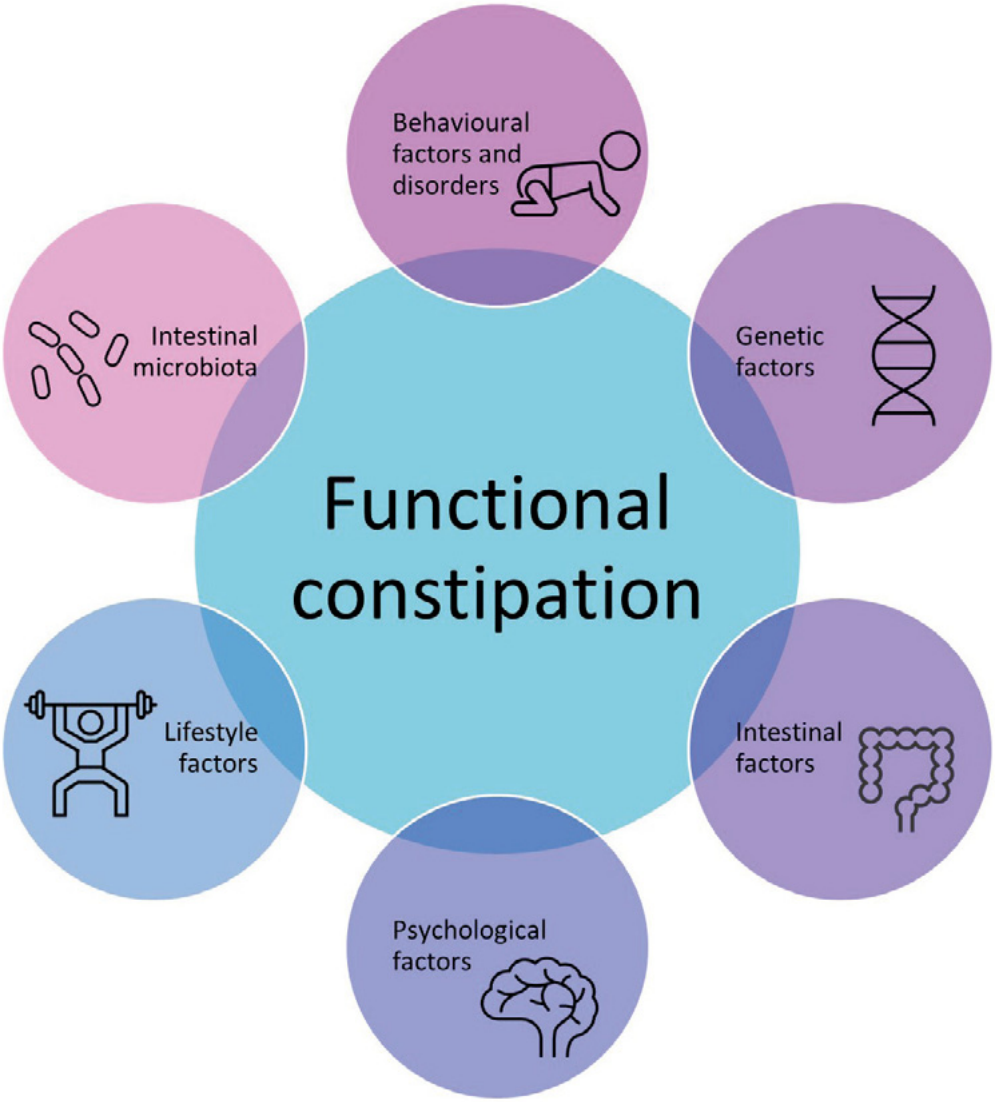

Figure 1 | Pathophysiological factors involved in functional constipation (FC) in children and adults. Clockwise: (1) Behavioural factors and disorders may include autism spectrum disorders (ASD) but also a very important factor in children: stool withholding behavior. (2) Genetic predisposition may have a role in the etiology of FC as it seems to occur more often in certain families. (3) Intestinal factors include the role of deconjungated bile salts, colonic dysmotility and impaired anorectal functioning. (4) Psychological factors may include major life events, socio-economic factors, abuse and trauma. (5) Diet, fluid intake, obesity and physical activity are lifestyle factors that may be of influence. (6) Multiple in vitro and in vivo studies suggest a role of the intestinal microbiota in FC.

In a world where health care becomes 'personalized', multifactorial disorders such as FC may be a great testcase to step away from the 'one size fits all' treatment pyramid, as described in **chapter 1** and **chapter 2**, and explore other means. In general, the treatment pyramid for FC is effective for a majority of patients, despite the fact that the pediatric population is more difficult to treat, and that there are options available within this treatment pyramid to adjust treatment to an individual patient. In contrast, more than a third of parents of patients with FC seek help in complementary or alternative medicine. Therefore, we could conclude that these (parents of) patients are not fully satisfied with the current treatment [23, 24]. In such patients, identification of the main causes of FC should be investigated, and more emphasis should be put on holistic approaches in functional disorders such as FC. And even though I am confident that the large majority of (pediatric) gastroenterologists try to take other factors into account and provide individual and personalized care, there is no time and there are not enough resources to hyper-individualize healthcare for a problem that is considered 'not to be life-threatening'. Nevertheless, this should not stop the field as a whole from trying to move towards more individualized and personalized health-care.

THE ROLE OF THE INTESTINAL MICROBIOME AS HOLY GRAIL IN THE PATHOGENESIS OF FC?

As mentioned in the paragraph above, FC is a multifactorial disorder for which treatment with laxatives is effective in the majority of pediatric patients, but not satisfactory for all [23, 24]. Moreover, since such a large proportion of (parents of) patients look for alternative or complementary treatments, it is worthwhile to investigate the scientific body of such alternative or complementary treatments. The so-called '-biotics', including pre-, pro-, syn,- and postbiotics are among the interventions used by patients with FC. As described in **chapter 5, 6** and **7** there is evidence that differences exist between the intestinal microbiota composition in children with FC compared to that in healthy children. Moreover, several studies have shown that using a '-biotic' can be effective in the treatment of FC [25, 26]. In this context not only the description of the members of the intestinal microbiota, but also the functioning of the microbiome is of interest in health and disease. The microbiome was described in 1988 as a combination of the words "micro" and "biome", naming a "characteristic microbial community in a reasonably well-defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of activity" [27]. This definition is considered the most comprehensive definition since it captures the complexity of the microbiome and considers the many facets of its ecology and evolutionary biology. However, the definition was extended to differentiate the terms microbiome and microbiota and pronouncing its dynamic character. Therefore the definition proposed by Berg *et al.* is: 'The microbiome is defined as a characteristic microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers

to the microorganisms involved but also encompass their theatre of activity, which results in the formation of specific ecological niches. The microbiome, which forms a dynamic and interactive micro-ecosystem prone to change in time and scale, is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health.' [28]. The most important challenge in intestinal microbiome research is exploring and finding causal relationships. This is especially the case for disorders such as FC that are known to have a multifactorial origin [29]. To better understand the role of the intestinal microbiome in the onset of FC, a large prospective longitudinal cohort study in healthy children, including frequent sampling points, would be necessary. This would show how changes in intestinal microbiota composition are related to the onset of FC and other functional disorders or diseases in general. Even with this information it is questionable if we could answer the question whether intestinal microbiota composition change precedes symptoms or whether the change in intestinal microbiota composition is caused by the symptoms. In other words, for example, do hard stools cause a change in intestinal microbiota composition or does the change in intestinal microbiota composition result in hard stools? Besides describing the members of this ecosystem, it is also essential to then look into the function and activity of the intestinal microbiome by making use of the other '-omics' approaches, such as transcriptomics, proteomics and metabolomics [30]. All these pieces of the puzzle will help to shine light on who is there and what they do from a microbiological point of view. Besides that, the interactions of the microbiota and their products with the hosts are another factor to consider. Having said that, because of the multifactorial origin of the disorder, it is unlikely that an intestinal microbiota-directed intervention, even if we would understand all mechanisms and interactions of the microbiome, will work for all subjects due to inter-individual microbiome differences. However, when data of such a large cohort is available, we could specifically aim for those patients that have a certain intestinal microbiota profile, a specific shift in their intestinal microbiota composition or shift in function. This information might help to more effectively treat them with a personalized therapy both in dose as well as in type of '-biotic' intervention. However, what we currently do is shooting with buckshot, hoping to find something based on limited *in vitro* and *in vivo* data, especially in children.

Despite the critical tone of the previous paragraph, there is a sound body of evidence for certain interventions in (subgroups of) patients with FC. One category of such interventions comprises dietary and/or prebiotic fibers. Such fibers are of interest in the treatment of FC due to several reasons: 1) FC is associated with a low fiber intake, which has been identified as risk factor for FC [14, 24, 29, 31-33]; 2) prebiotic fibers are fermented by the intestinal microbiota, which leads to a production of, among others, short chain fatty acids (SCFAs) and tryptophan metabolites. These microbial fermentation products have been shown to alter intestinal motility [34-36]; 3) fermentation of such fibers may lead to an increase in microbial biomass. Subsequently both the increase in osmotic pressure and increase in microbial biomass may lead to an increase in dilation of the intestinal wall which, in turn, can increase in intestinal motility. If we zoom in on the mechanisms behind the effect of SCFAs on intestinal motility, several modes of action have been suggested. Firstly, it was found in animal studies that several SCFAs may affect intestinal motility

by stimulating mucosal receptors and/or the vagus nerve, or that they might act directly on the colonic smooth muscles [37]. It was also suggested that SCFAs may reduce intestinal transit time by increasing concentrations of serotonin in the gut [38]. Evidence from such animal studies shows that specific SCFAs such as butyrate and acetate may influence intestinal motility [39-41]. Moreover, SCFAs as a whole may influence the osmotic pressure in the intestine, causing an increase in water content and pressure and thereby increase intestinal motility [41]. Therefore, supplementation of prebiotic fibers may improve intestinal motility via multiple mechanisms and thereby may influence FC symptoms, see **Figure 2**.

There is, however, another complicating factor in children with FC: withholding behavior. Many children exert withholding behavior by which children end up in a vicious circle: a negative experience with hard stools may lead to fear and withholding behavior, resulting in hard and painful stools [42]. Therefore, current treatment in children exerting extreme withholding behavior is to give a dose of laxatives for which stool withholding is no longer possible, and which can help to break the vicious circle. It is questionable whether inducing such soft stools with intestinal microbiota directed interventions is possible and desirable. It is, however, clear that soft stools at a young age may be beneficial to prevent such negative experiences. It is therefore interesting to see, as found in the explorative part of **chapter 4**, that the development of infant formulae, and especially with the introduction of β-palmitate and prebiotic fibers such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), an increase in range and a slight increase of mean defecation frequencies between and across studies was found. A continuation of this trend to get even closer to the average stool consistency of human milk-fed infants is in the light of FC a positive development. The current experimental formulae might get even closer to human milk not only in terms of clinical effects with respect to e.g. stool frequency and consistency, but also in terms of nutritional composition. An example of this is the inclusion of human milk oligosaccharides (HMOs) as prebiotic ingredients, which is an interesting field that goes beyond the scope of this thesis [43]. Moreover, once talking about human milk and formulae it is important to stress that human milk is still the golden standard in infant feeding [44]. With the data from **chapter 4** it is tempting to speculate that, from a narrow perspective of FC, human milk feeding may also help to keep stools softer and thereby decrease the chance of developing FC while keeping in mind that the onset of FC in children is often multifactorial. This speculation is supported by a study (n=212), indicating that human milk feeding for less than six months was associated with FC in young children [45]. To further investigate whether certain prebiotic fibers may be used in the treatment of FC in young children two studies were designed. The first study, Inside study I, focusses on young children with FC, as described in **chapter 9**. Another study was designed in healthy children that experience hard stools (at least 50% of the time) but do not have FC: Inside study II. This Inside study II, once finished, may provide us with insights on whether an intervention with prebiotic fibers may be effective in treating hard stools in young children. Moreover, it may also provide us with information on whether early treatment in children with hard stools only, i.e. on the borderline of what we defined as healthy in **chapter 4**, may prevent the development of FC in these children.

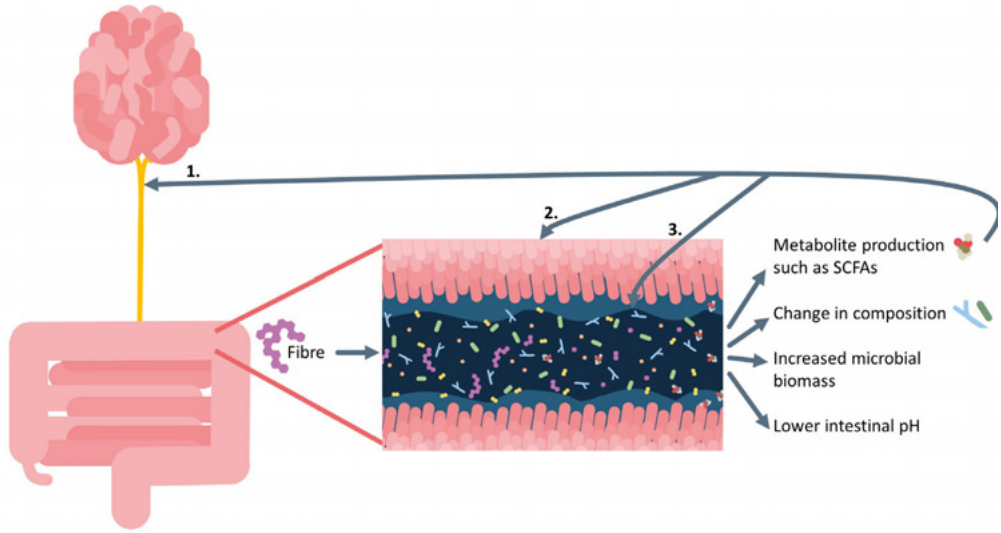

Figure 2 | Potential working mechanisms of prebiotic fibers on intestinal motility. Prebiotic fiber fermentation might result in a change in intestinal microbiota composition, increased microbial biomass, a lowering in intestinal pH and metabolite production such as short chain fatty acids (SFCAs) which in turn might lead to: 1) stimulation of the vagus nerve that may increase intestinal motility. 2) Mucosal effects via stimulation of mucosal receptors and smooth muscle. 3) Intestinal effects via an increase in intestinal serotonin and an increase of osmotic pressure leading to an increase in motility.

With regards to adults, several differences exist of which the absence of stool withholding behavior may be the most interesting with the previous paragraph in mind, as this is not considered as an important factor in adult FC [14]. In **chapter 8** we discussed the clinical effectivity and microbiota changes with a chicory inulin prebiotic fiber intervention in adults with FC. We found an increase in stool frequency after inulin intake compared to placebo (4.0 [2.75, 4.50] vs 2.50 [2.38, 3.50], p=0.046) and stool consistency (2.72 ± 0.22 vs 2.24 ± 0.14; p=0.04). Moreover, quality of life and symptom scores improved above the adopted concept of 'minimally important differences' after inulin, but not after placebo intake, reflected in less rectal tearing and burning (inulin: -0.66 vs placebo -0.47, p=0.036) and improved treatment satisfaction (inulin: -1.23 vs placebo: -0.53, p=0.05). Lastly, several bacterial genera were modulated by inulin intake, but not by placebo (p>0.10). An 1.3-fold increase in relative abundance of bifidobacteria was observed (p=0.02; q=0.36). Furthermore, following inulin intake relative abundance of *Anaerostipes* and *Subdoligranulum* spp. increased with a simultaneous decrease in several genera of the *Ruminococcaeae* family, as compared to the placebo group.

Lastly, in **chapter 10** we looked at clinical effectiveness, health-related quality of life, treatment adherence, patient experience and patient empowerment in children who use transanal irrigation (TAI) [46]. Intestinal microbiota composition was not taken into account in this retrospective and cross-sectional questionnaire based study. It would, however, be interesting to investigate

the effect of regular flushing of the distal part of the colon and potential introduction of oxygen on the intestinal microbiome. To my knowledge, only one study investigated the intestinal microbiota composition in children that use TAI [47]. This specific population was comprised of patients (n=16) with spina bifida. The researchers found significantly increased relative abundances of the bacterial genera *Roseburia* and *Bacteroides* after using TAI, compared to before using TAI. Moreover, the relative abundance of *Roseburia* was positively correlated to the Bristol Stool Form Score. These findings are surprising when having in mind that TAI may also introduce (low levels of) oxygen into the intestinal lumen, since *Roseburia* spp. are known to be highly oxygen sensitive [48]. It would be interesting to extend this research to diverse group of patients that use TAI to further elaborate on the intestinal microbiota composition before and after using TAI, SCFA profiles, intestinal health and the symptoms of these patients.

As shown above, there is an increasing body of evidence suggesting a role for the intestinal microbiome in intestinal motility whereby the intestinal microbiome might influence disorders such as FC. It would be of high value to investigate in more detail via *in vitro*, *ex vivo* and *in vivo* studies if and how a multifactorial disorder such as FC could be approached from a microbiological perspective. Furthermore, one could think of using other microbiome-related interventions beyond prebiotic fibers. Within the group of patients with FC one could think of the influence of the microbiome with (hormonal) pathways that are known to influence GI motility such as the interdigestive hormones motilin and ghrelin or the postprandial hormones and enzymes cholecystokinin (CCK), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). These might be of interest since it is known that certain (products of) microbes can influence such pathways. For example, *Akkermansia muciniphila* was found to secrete the protein P9, which signals to a certain endocrine cell to produce GLP-1 and thereby delays gastric emptying [49]. Although for FC a delay of gastric emptying might not be profitable, there are other examples that might be interesting for the treatment of FC. For example, supernatants of *Bifidobacterium* and *Lactobacillus* spp. cultures were shown to affect ghrelin signaling [49]. Furthermore, physiological concentrations of propionate and butyrate were shown to induce PYY gene expression, leading to an increase in gastric and/or intestinal motility [49]. Since both effects were based on substrates, supernatants and physiological concentrations of SCFAs, I briefly want to touch upon another '-biotic' that has not been explained previously: postbiotics. Postbiotics are functional bioactive compounds, generated in a matrix during microbial fermentation, which may be used to promote health [50]. Since several of the mechanisms for treating FC by intestinal microbiota directed interventions rely on the effect of metabolites of intestinal fermentation, it could be worthwhile to investigate those metabolites or bioactive compounds themselves.

Summarizing, there might be a role for certain interventions targeting the intestinal microbiota in (subgroups) of patients with FC. It is however important to, instead of shooting with buckshot, investigate potential working mechanisms and to investigate how to steer intestinal microbiota composition. But maybe even more important than steering microbiota composition, we should focus on steering intestinal microbiome functioning towards a more favorable state at a personal, dose dependent and type of intervention dependent level.

STUDY DESIGNS AND COMMON PRACTICES IN MICROBIOME RESEARCH

Throughout this thesis I mainly discussed the identification of relative abundances of different bacterial taxa within the intestinal microbiota, while there is also an interesting non-bacterial microbiome that influences the intestinal ecosystem as a whole. Additionally, besides describing any of these relative abundances in relation to specific disorders, it is important to add more layers to this story. These layers include all the '-omics' approaches described briefly in **chapter 1**. These approaches will help understand the potential, function and activity of the intestinal ecosystem in disorders such as FC. While interesting, these approaches and the non-bacterial microbiome were beyond the scope of this thesis. As mentioned before, this thesis remained within the confined area of DNA sequencing where identification of microbes was performed by using only a small section, the V4 region of the 16S rRNA gene, of the microbial DNA which then was mapped to a reference database. This means that results of such analyses are as good as a reference, besides all other technical aspects such as the DNA extraction method, primers that were used and the used bioinformatic pipeline for data processing, analysis and interpretation. Moreover, the importance of positive and negative controls should not be neglected in microbiota composition research. Positive controls are commercially available, but some laboratories including our laboratory created their own, and are defined as synthetic 'communities' [51]. Such mock communities should be used to evaluate whether sample processing went as expected by comparing the expected outcome to the experimental outcome. These mock communities may not be perfect for specific ecosystems, for which more specific custom designed positive controls might be needed, but they are valuable to obtain insight in the accuracy of your data [52]. Beside the positive controls, negative controls are also of essence to identify potential contamination sources. These contamination sources could range from the researcher her- or himself, the used lab equipment or even the extraction kit or other reagents used [52]. It is of importance to identify and describe such inconsistencies to better interpret the data and to safeguard confidence in observed outcomes.

In an ideal world where 1) money would not limit the diversity of '-omics' analyses, 2) no bias would be introduced by any of the laboratory or further downstream analyses and 3) we could go as deep as identifying all members of the microbiota to strain level, there are still several hurdles to overcome. In other words, even if we would have a perfect description of the exact members, their potential, function and activity, we would face a difficult task. First of all, most frequently this data is obtained by analyzing fecal samples, which is a proxy for the large intestine. However, what happens in feces is not representative of all other parts of the GI tract and maybe not even for the majority of the colon. Secondly, case-control studies give us an association, which, in the end, can help in forming hypotheses, but don't provide actual mechanistic understanding. In order to truly understand what such associations mean, studies are needed that bring us closer to causal relationships. This is in humans, and even more so in children, very challenging since it is often not regarded ethical, justly, to expose a child to a condition of which you expect a

causal relationship to a disease. To give a more practical example: assume we would find a certain microbial signature that is highly correlated to FC in children. The best way to prove this would be to expose, in a randomized controlled trial, a healthy child to that exact microbial signature by means of e.g. repeated fecal microbiota transplantation from a child with that signature and FC. The placebo group would then receive an FMT of their own. Even if this would be regarded ethical to do, we cannot rule out other factors such as stressful life events or negative experiences on the toilet of e.g. hard stools that a child would be exposed to and that could influence outcomes. Therefore, there is also great value in *in vitro, ex vivo* and *in vivo* experiments to further deepen the knowledge on such potential causal relationships, but a black-and-white answer will remain very challenging.

In clinical research, randomized controlled trials (RCTs) are considered to provide the most reliable evidence on the effectiveness of interventions where systematic reviews or meta-analysis of such RCTs are the highest in hierarchy of evidence [53]. Such RCTs can be executed in several ways such as a parallel study, where participants are divided into two groups: the intervention and the placebo group. An RCT can also use a cross-over design in which each participant receives both treatments but in a different order: one group starts with an intervention period and then a placebo period and vice versa for the other group. Cross-over studies often have a scheduled gap between treatments, the wash-out period, to reduce carry-over effects from the previous treatment [54]. Both types of study design have their pro's and con's. However, and as we also observed this in our study described in **chapter 8**, a cross-over design in microbiome-directed interventions might not be an ideal study design, despite the advantages that each participant acts as her or his own control and that a smaller number of patients is required. In our study we observed a large carry-over effect, even following a wash-out of four weeks, resulting in a second period that could not be used in the final outcomes. In case a cross-over design is preferred over a parallel study, it is highly recommended to carefully evaluate the wash-out period to prevent such carry-over effects [55].

HOW TO GET A GRASP ON THE FIELD AND WHERE TO START

The fields of pediatric gastroenterology and microbiology are immensely complex. This thesis contains several chapters that are reviews, systematic reviews or meta-analyses. In order to get a first grasp on a topic, it is worthwhile to start with systematically searching the literature for all information available within a certain scope [56]. This does not only help to select for the types of interventions that might be valuable to study in more detail, but it also helps to pinpoint weaknesses of study designs, study executions and ways of reporting.

To illustrate this, **chapter 8**, reported on the effect of a prebiotic fiber compared to a placebo in adults with FC. We found that inulin intake may alleviate FC by improving stool frequency and stool consistency but also quality of life and symptoms scores. Hence, it may improve FC

not only from a clinical point of view, but also from the patient point of view. However, since stool consistency data and symptoms scores showed that the wash-out was not long enough, we had to seriously consider on how to report this while simultaneously learn as much as possible for future bowel habit trials with prebiotics in FC patients. We could not trust the outcome of the full trial, although it is reported in the chapter, due to the remarkable carry-over effect bias introduced into our data due to an insufficient washout, and thereby chose to report the parallel part of the trial only, risking a selection bias. The comprehensive analysis of this study will, however, contribute to not only the knowledge of the clinical and microbiological effects of prebiotic fiber interventions in adults with FC, but it will also contribute to indications of, in case a cross-over is highly preferred over a parallel design, wash-out duration considerations and longer-term effects of an intervention [57].

To further illustrate this, I also want to critically evaluate our Inside study I described in **chapter 9**. This study is an RCT with three parallel arms where participants receive either GOS, FOS or a placebo. During the set-up phase, we critically evaluated all aspects of the study, e.g. study design, the dosage of the intervention, outcomes, feasibility, age group and sample size. Despite this effort, we did not take into account all core outcomes [58]. We did take into account most, but some were not considered due to amendments in the protocol. This study was approved and ready to start in March 2020, when the COVID-19 pandemic occurred. In order to still meet the desired sample size, we decided to change the inclusion criterion for age from one to three years of age to one to five years of age. This change should have been accompanied by adaptations in the outcomes too; where school attendance was not relevant for the age group prior to the amendment, this may be relevant for the four- and five-year old children. This would not change the main outcomes of such trial, but it is something that has been reported as important to parents, and therefore should have been taken into account. For this a role for prospective trial registration organizations and guideline creators such as Consolidated Standards of Reporting Trials (CONSORT) [59] is necessary. One could think of a simple checkbox in a trial registry or on a checklist from CONSORT with the question whether the COS for the respective trial has been used (if available). Moreover, associations such as the International Scientific Association for Probiotics and Prebiotics (ISAPP) may play a role in publishing recommendations on e.g. dosages or durations of trials that study intestinal microbiota directed interventions.

Additionally, when looking at reporting of studies, we found in **chapter 4, 5, 6** and **7**, but especially in **chapter 4,** that reporting of outcomes is a challenge too. This was particularly obvious when trying to combine outcomes from different stool scales into one score on which a meta-analysis could be performed. From a trial perspective it might make sense to compare means or medians and their respective standard deviations or interquartile ranges between groups. However, studies that aim to describe defecation patterns should not only report such means, but should also provide insight in the dispersion of the data by means of e.g. percentages per stool type. Using means only will lead to an unequal weight of soft and frequent stools compared to hard and infrequent stools as the soft and frequent stools will be counted more often in the dataset than hard, infrequent stools. Moreover, if the most frequent stool consistency type is

reported, the average stool consistency of 2 on a 3-point scale could derive from 30 children that always have type 2, but could also derive from 10 children for each stool consistency type; 1,2 and 3. Having said that, taking the most frequent stool frequency or consistency per person in a percentage table per type of stool is also not representative of a normal stool pattern. Examples like these do help to critically reflect on how you could report data. It is also with examples like these in mind that data repositories may be of great value for data that might not be of great importance to the conclusions of a specific paper, but can be for systematic reviews and meta-analyses of such data as the highest in the hierarchy of evidence [60].

In summary, searching the literature systematically, or performing a systematic review and meta-analysis, within a confined scope, will help to better design and report studies while at the same time there might be a role for organizations such as CONSORT, trial registries or scientific associations such as ISAPP in providing better guidance in trial design and reporting.

THE PLACEBO EFFECT OR THE CO-INTERVENTION EFFECT?

In **chapter 8** describing the inulin intervention in adults with FC, we observed a rather large placebo effect when comparing several outcomes versus baseline and also when comparing placebo to the intervention. This placebo effect can be linked to several factors that are known to result in higher placebo effects; 1) the use of a subjective measure, such as questionnaires or diaries and 2) the effect of the patient-doctor or patient-researcher relationship [61, 62]. Besides the placebo effect, several other biases, i.e. systematic distortions, exist that may influence reported outcomes. This may range from selection bias, response bias, attrition bias to co-intervention bias [62].

For co-intervention bias one could think of the following: if you know as participant of a study that the intervention you might receive is a fiber supplement for the treatment of FC, you might be inclined to increase fiber intake via the diet. This may especially be the case if you know that you have e.g. 50% chance to receive a placebo treatment. Unfortunately in the study of **chapter 8**, dietary intake was not recorded, although the participants were requested not to modify their eating or lifestyle habits. Furthermore, the visits to the study nurses may have had influence on the subjects behavior whereby they received attention and were listened to.

Another co-intervention bias that may be introduced in the Inside study I described in **chapter 9** is the amount of attention participants receive compared to the time a medical doctor is allowed to see a patient. For the Inside study I, potential participants are called to see if they want to participate, after which a home visit is scheduled. This home visit takes at least 30 minutes, in which the study is explained. However, from experience we noticed that parents also take this time to go into emotional or practical problems that might not be discussed in such detail in the short time they are in the doctor's office. Moreover, throughout the study parents could reach us as investigators very easily via texting, calling or sending us an email. The co-intervention of the

sole availability of someone that will listen to such problems or concerns, might by itself result in different outcomes. This should level out between groups since it is a double-blind randomized controlled trial, but might result in differences compared to the baseline measurements.

FOCUS ON THE PATIENT

Throughout this thesis there are several chapters where there is emphasis on the perspective of the patient. In **chapter 8** we focused on what patients reported as their most bothersome symptoms. In **chapter 10** we assessed the clinical effectiveness, health-related quality of life, treatment adherence, patient experience and patient empowerment in children using transanal irrigation. These outcomes are subjective and based on questionnaires, but that does not make them less meaningful from the patient perspective. In fact, as mentioned earlier in this discussion, we should listen more carefully to the (parent of the) patient [29, 58, 63]. Sometimes however, the primary outcomes of studies, that form the basis of evidence-based guidelines, are not based on the patient perspective. For example, subjective measures and questionnaire-based research are regarded lower in the hierarchy of evidence, while they might be the most important for the patients themselves. Moreover, with the ever-persisting pressure to publish and the still ongoing trend to only or at least mostly publish positive results, outcomes may be selected to more easily obtainable statistically significant differences instead of clinically or patient-focused relevant outcomes.

It is of major importance that with the development of a COS, the perspective of a (parent of a) patient is taken into account as well [58]. We noticed in the Inside study I (**chapter 9**) that parents of patients are willing to provide us with this parent and patient perspective by filling out questionnaires and diaries. Extrapolating from this experience, one could also think of creating an app to monitor symptoms, quality of life and other important factors for the parents and patients related to for example compliance with medication used. Based on other already existing apps, app-developers should be able to create an app, which translates these data into clinically meaningful summaries for medical doctors too, to better monitor defecation disorders and earlier change treatment when it is not to satisfaction [64-67].

THE IMPACT OF GOOD SCIENCE COMMUNICATION

In a world where everything becomes more complex, information streams bigger and where misinformation rapidly emerges and manifests, it is a challenge to effectively communicate about scientific findings to the wider public. A clear and impactful example is the COVID-19 pandemic, where misinformation, incomprehensible information and an information overload might result in a considerable threat to public health [68]. Approaches, to say in medical terms, of therapeutic and prophylactic methods to counter misinformation have been successful only

to a limited extent [68]. It might therefore be better to focus on preventive approaches. When I think about such preventive measures, the first thing that comes to mind is a platform called '*Kurzgesagt*', the German word for 'in a nutshell'. Kurzgesagt started as a social media channel which uses animations to discuss scientific, technological, political, philosophical, physiological and psychological subjects in a beautiful and educational way. Currently Kurzgesagt has more than 18 million subscribers and close to 2 billion total views. These videos distil complex concepts such as black holes to a 5-10 minute video via engaging narratives to make complex science behind such topics accessible to the general public. Kurzgesagt is in multiple ways an example to rethink science communication and illustrates that the scientific community can learn a lot from their approach; 1) the graphics they use are outstanding, clear and a pleasure to look at; 2) the basis for any video is inspiring and triggering the curiosity of anyone who watches, whether it is within your scientific domain or completely outside of it; 3) metaphors are used in a smart way while at the same time remaining very clear; 4) sources and additional information are well communicated; 5) any conflicts of interest, partnerships or collaborations are clearly stated; 6) the sound effects and music accompanying the videos is smartly chosen to accompany the type of video. Overall, science in general can learn a lot from the science communication as provided by Kurzgesagt by making science entertaining, looking beautiful and freely accessible for everyone [69]. Besides their videos, Philipp Dettmer, CEO of Kurzgesagt, published a book called 'immune', that explains in a very understandable way the complexity and beauty of the immune system - another example of making a highly complex system understandable for the wider public.

SCIENCE IN A WORLD OF LOOPHOLES

The academic world and science in general is a place of amazement, fascination and discoveries. Unfortunately, it is also a place where many loopholes exist. Although, I am not a statistician, or an ethicist, I do feel the obligation to at least touch upon some topics within this general discussion.

First of all, many outcomes in science are evaluated based on 'statistical' significance, without critically reflecting on the clinical significance or relevance. Statistics is a wonderful tool; it allows us to condense a sometimes very complex and elaborate dataset into one dichotomous outcome: $p<0.05$ or $p>0.05$. We then often conclude: it worked or it did not work. However, providing such p-values alone is not enough to value the outcome; the effect size is necessary to provide information on the magnitude of the difference between groups [70]. This effect size is also required to evaluate whether the effect could be clinically relevant. For example, if we would have a population of 100.000 children with FC in a study with two arms and we find a significant improvement $p<0.001$ on stool frequency one could conclude that the trial was successful. This statement is wrong in several ways; finding a $p<0.05$ means that the null hypothesis is rejected, not that the trial was successful [71]. This null hypothesis is by default,

but not necessarily always, that there is no difference between groups. Moreover, since there is no effect size given, the conclusion does not make sense from a clinical perspective. To explain this in more detail it is important to realize that the sample size highly influences the p-value; even a very small difference between both groups of 50.000 children will lead to a statistically significant difference. Only with an effect size, this p-value can be valued properly; if the effect size for stool frequency per week would be 3.21±0.2 in the intervention group versus 3.11±0.2 in the placebo group you could obtain a p-value well below 0.05 with such a big group. However, what does a difference of 0.1 times per week more in the intervention clinically mean? There are many loopholes that can be misused in research to obtain a statistically, but clinically irrelevant difference. This can start with the sample size; if the population is big enough you can keep including participants until you find a significant difference. But also in reporting it is of essence to remain critical when it comes to effect sizes and clinical relevance. In relation to sample sizes, it is important to emphasize the need to perform a well-estimated, clinically relevant sample size calculation based on the primary outcome of the study. This might sound straightforward, but many studies that have a non-clinical primary aim such as an intestinal microbiota composition related outcome do not always perform such power calculations, due to the complexity of the many features that are assessed simultaneously [72]. Other challenges in such sample size calculations for intestinal microbiome outcomes are related to the within-condition variability and the corrections for multiple-testing in such studies. Moreover, noise levels, dynamic ranges and other analysis methods turn out to cause complications to such sample size calculations [73]. Fortunately, Tarazona *et al*. created computational tools to calculate power and sample size for multi-omics applications, among which DNA sequencing methods such as 16S rRNA sequencing [73]. Studies described in this thesis had clinical primary aims, but for studies that do not have such a clinical aim, novel methods such as the one by Tarazona *et al*. should be considered.

Another important pitfall is the way we report all outcomes and get them into the scientific world: we publish. Or at least, that is what we try. However, if the study was successful but the results were negative, i.e. no statistically significant difference and/or clinically relevant effect was found, many journals do not accept your paper. This is understandable from a journal perspective as most people probably do not want to read when something does not work, but utter nonsense from a scientific point of view. This so called publication bias in clinical research may result in unjust conclusions when conducting systematic reviews or meta-analysis, leading to incomplete conclusions and hence on clinical practice or future studies [74]. Fortunately, with the need to register clinical trials, it is possible to publish registered reports. Also, with improved guidelines for systematic reviews and meta-analyses such 'negative' result studies can be found and even the bias can be corrected for despite that they may not be published in peer reviewed journals [75]. Moreover, there is also an increase in journals that accept 'negative' findings and there are even specific journals such as the 'positively negative' collection from PLOS ONE that focusses on negative, null and inconclusive results.

Lastly, even if we have a study that was well executed, where we found statistically significant and clinically meaningful results, researchers have to find means to get their message out:

publish in a high impact journal. This index of journals is based on a matrix that takes into account yearly citations of articles published in the last two years in a given journal. By doing so you can discriminate 'high impact' journals from not so high impact journals. Many words could be devoted to the fairness or unfairness of such system that favors more general science or cross-disciplinary impact compared to specific sub-fields and may be highly skewed by the 80/20 phenomenon: 20% of articles may be responsible for 80% of citations [76]. Despite all its negative sides, it is undoable to read all papers and make a personal evaluation on quality, hence the system stays in place. However, it is essential to stay critical with such systems and indexes [76]. Besides the impact loophole, an even worse loophole exists when it comes to open access publishing. It is a great development that open access publishing is stimulated and sometimes even obliged by universities. It is, however, odd in this transition phase from paying for subscriptions to journals towards all open access that for publishing research, which is funded by public and/or private partners, you have to pay large amounts of money to have it available open access. In current times an institution still pays to have a subscription for the journals and/or papers that are not open access, but also pays to publish [77]. These fees can be more easily be afforded by rich institutes and/or countries, creating even bigger inequalities between those that can and those that cannot afford to pay such fees [78]. Moreover, a proportion of the important work for the journal is done free of charge to maintain the high quality: peer-reviewing [79, 80]. These loopholes in the current transition phase in publishing leads to unclarity and inequality. Therefore, the standing many universities take to force their researchers to publish open access is a good step to choose for one option and not linger for too long in this transition phase. Though, the effect of choosing open access publishing only should be monitored to prevent an even bigger increase in inequalities within the academic community [78].

FUTURE PERSPECTIVES

The study of the intestinal microbiome is, outgrowing its infancy and slowly maturing into its puberty era; the role of the intestinal microbiome in health and disease becomes more and more apparent, but results of studies are often conflicting, frustrating and difficult to interpret. It would, however, bring the field of microbiome research forward if there would be more studies in patient populations compared to healthy controls that take into account (dense) time series to correct for individual and normal fluctuations in clinical outcomes and intestinal microbiota composition outcomes. Moreover, adding more layers to the knowledge with all previously mentioned '-omics' approaches simultaneously will help to understand not only even better who is there, but also the potential, function and activity of the ecological intestinal system and how these layers of information may relate to each other. With an increasing amount of data, an increase in attention should be paid to generating scientific questions, hypotheses, and using appropriate datasets to test those, instead of explorative approaches [81]. It is of essence to define a scientific question and hypothesis that can be tested, especially with such complex and immense datasets, to serve as basis to explain biological phenomena or clinical outcomes.

The main focus of any intestinal microbiota or microbiota directed intervention should become, in contrast to current times, tailored and personalized. The focus should shift more towards studying to what extent the ecosystem of the intestinal lumen and surroundings are disturbed and how. The next step would be to investigate what multifaceted approach of restoration, promotion and/or targeted removal of components of the microbiome could be applied to improve individual health. The use of supervised machine learning and/or data-processing algorithms that could predict the response of an individual to a given substrate, whether that is food, supplements or other microbes, might be used to guide this transition. This and the growing understanding of the role of the intestinal microbiome and the interactions with the individual harboring this ecosystem will enable medical doctors to provide better care in the prevention of diseases and will help to more effectively treat diseases that have been linked with differences in the intestinal microbiome. Inter- and transdisciplinary projects that may include microbiologists, data scientists, nutritionists, and medical doctors should bridge the gap between pathogenesis in the host and alterations in the intestinal microbiota and its function. Ideally this information should be used to specifically design individual recommendations in terms of personalized food and/or '-biotic' interventions to promote favorable health outcomes.

In the end all types of interventions, improvements and advances in the medical field should be focused on the patient's perspective; is the patient doing clinically better? And even more importantly - is the patient feeling better? Moreover, when age appropriate, is the patient satisfied with the treatment plan and does the patient feel in control of their disease? In medical science, especially in functional disorders, it is good to critically reflect on why such a large percentage of (parents of) patients looks for alternative or complementary medicine. This might also help to improve treatment quality in regular medicine. Moreover, 'there is a child surrounding the intestine' is something that is especially relevant in FC in children. Withholding behavior is an important factor in FC in children, which could be the result of many other factors such as a history of hard and painful bowel movements, toilets in school that are not clean and stressful life events. Therefore, we should not forget these factors, especially in children that are unresponsive to current therapeutic strategies.

We live in the digital era, where it is of essence to keep up with the rapid development in digitalization of health with e.g. wearables and of healthcare with viewing your own medical files online. This will probably result in an even bigger increase of digital health solutions, apps and wearables that could monitor health. Such means could also be embedded in the current healthcare system to improve adherence. However, one could also think of creating an app to provide a better and easier resource for parents that contains the first information about healthy defecation, education on toilet training and which factors to think of in case the going gets tough, such as dietary and lifestyle recommendations. Moreover, this could also become a monitoring app for children diagnosed with FC or other defecation disorders to monitor the symptoms and increase therapy adherence by e.g. reminders or tips and tricks. Additionally, this app could also include a reward system for the children when defecating and/or toilet training goes well. Efforts should be made to develop and test such app to improve patient care in children with FC or other defecation disorders.

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'Our painful experiences aren't a liability—they're a gift. They give us perspective and meaning, an opportunity to find our unique purpose and our strength.'

THE CHOICE - EDITH EVA EGER

CHAPTER α, APPENDICES

Englisch summary

Dutch summary

ENGLISH SUMMARY

The process of defecating involves a wide variety of well-orchestrated, coordinated sensorimotor functions. Additionally, defecation is influenced by, amongst others, genetic, lifestyle, behavioral and psychosocial factors. Moreover, all processes involved in digestion, absorption, secretion and motility, including interactions of the intestinal microbiota, may be of influence and therefore of interest for further investigations, particularly when the going gets tough. This is the case for functional constipation (FC), a common and bothersome condition with symptoms of hard and/or infrequent bowel movements with a pooled worldwide prevalence in children of 9.5% and a prevalence of 14% in adults. Moreover, about a quarter of children who have received treatment for FC as a child still experience symptoms of constipation as adult. Treatment options range from education, dietary and lifestyle recommendations to osmotic laxatives, stimulant laxatives, enemas, botox and transanal irrigation, all the way to surgical interventions. Despite these treatment options, about one third of parents of a child with FC seek help in the form of alternative and/or complementary medicine.

Part I of this thesis describes the physiology, pathophysiology, evaluation, management, treatment and prognosis of functional constipation in children and adults and highlights the potential role of the intestinal microbiota in this disorder. The general introduction of **chapter 1** gives an overview of the seemingly simple, but complex and well-orchestrated system involved in normal defecation. We then describe what can go wrong and describe the wide range of functional gastrointestinal disorders (FGIDs) and zoom in on FC in children and adults. This chapter gives an overview of epidemiology, pathophysiology, diagnosis, management, treatment and prognosis in adults with FC. Not only the description of the members of the intestinal microbiota, but also the microbiome as a whole is of interest in health and disease. The role of the intestinal microbiome in FC in children and adults is of major interest, since it is clear that differences can be found in the intestinal microbiota composition of children and adults with FC compared to healthy individuals. We come back to the evidence for intestinal microbiota directed interventions and clinical studies investigating the intestinal microbiota in FGIDs in part III and part IV. **Chapter 2** gives a more clinical overview of FC in children from epidemiology, physiology, pathophysiology, evaluation, management, treatment to prognosis and future perspectives.

One factor that is of major influence on treatment success in FC is the time between the first symptoms and effective treatment. In order to effectively treat children and adults, FC has to be diagnosed rapidly, and treatment has to start early. The diagnosis for FC is a clinical diagnosis, based on typical history and physical examination. The Rome IV criteria are symptom-based criteria where, amongst other symptoms, stool frequency and stool consistency are among the important symptoms. While focusing on what is considered an unhealthy defecation pattern, as is the case for FC, definitions of a healthy defecation pattern in children is mostly based on clinical experience and not on hard data. Moreover, measuring defecation patterns in children may also come with challenges, as described in **part II**. Defecation patterns are often

described in terms of stool frequency and stool consistency, while some also take into account smell, color and quantity. In **chapter 3** we investigated what could be a good tool to measure stool consistency in children. We validated the modified Bristol Stool Form Scale (mBSFS) for children. We concluded that our mBSFS, as paper or online version, is reliable, valid and user-friendly to use for Dutch-speaking parents, grandparents and day childcare employees to evaluate defecation parameters in toddlers whether in diapers or toilet trained. This is, to our knowledge, the first stool scale that was validated for both toilet-trained and non-toilet trained children. This is of importance since scales are generally specifically designed and validated for either potty trained children or non-potty trained children. Moreover, some use a 3-point scale, while others use a 4-, 5-, or 7-point scale. This makes comparisons between studies very difficult, if not impossible. These challenges in terms of reporting became even more apparent in **chapter 4**. Besides evaluating defecation patterns in terms of consistency, it is also of essence to understand what normal defecation patterns entail. The need for such evidence-based reference values is evident considering the fact that symptoms of infrequent and hard bowel movements are important in the diagnosis of functional disorders such as FC and other FGIDs. Currently, evidence-based data on normal defecation patterns in children is scarce and comes from old papers in small samples of children. To get better insight in the actual ranges, a systematic review and meta-analysis was performed as described in **chapter 4**. We found weighted mean defecation frequencies in 0-14 week old children are 21.8 times per week compared to 10.9 times per week in 15 week-4 year old children.

As mentioned before, many (parents of) patients seek help in the form of alternative or complementary treatments. Therefore, we investigated in **part III** the evidence for non-pharmacological and intestinal microbiota directed interventions in several FGIDs. In **chapter 5** we investigated the effectiveness of probiotics in children with functional abdominal pain disorders (FAPD) and FC. We found that the use of probiotics in both conditions is safe, but that there is no evidence for the use of probiotics in the treatment of FAPD or FC. It is, however, likely that *Lactobacillus rhamnosus* GG can reduce frequency and intensity of abdominal pain but only in children with IBS. In **chapter 6** we investigated the wide range of non-pharmacological treatments in the treatment of FC in children. Overall, there is a need for high quality multicenter trials that follow trial recommendations and use the core outcome set outcomes. Such trials may focus on the most promising interventions found in this systematic review and meta-analysis: specific prebiotic and fiber mixtures, abdominal electrical stimulation, *Cassia Fistula* emulsion, and Xiao'er Biantong granules. Future studies may also investigate interventions of interest of which no trials were found like personalized gut-microbiota interventions, exercise, (electro)acupuncture, other non-invasive neuromodulating therapies like posterior tibial nerve stimulation, and virtual and digital interventions. In **chapter 7** we investigated in more detail the role and effect of fiber and prebiotics on a variety of gastrointestinal disorders and the role of the intestinal microbiome in these disorders. In this review we found indications for the presence of a specific intestinal microbial signature in infants with colic and differences in intestinal microbiota composition in children with IBS compared to healthy controls, albeit based on

limited data. In contrast, the data for the microbiota composition of constipated children in comparison with healthy controls was found to be inconsistent. Moreover, we concluded a lack of large, randomized placebo controlled trials evaluating the effect of different interventions such as fibers and prebiotics in children with FGIDs. That brings us to the next part of this thesis.

Part IV of this thesis includes (protocols of) clinical studies in FC and other defecation disorders. First of all, **chapter 8** describes a study conducted in adults with FC. In this study, patients received either chicory inulin or a placebo for four weeks. Inulin intake resulted in an improvement in stool frequency, stool consistency, quality of life and symptom scores compared to placebo. Moreover, after inulin intake an 1.3-fold increase in relative abundance levels of bifidobacteria was observed and relative abundances of *Anaerostipes* and *Subdoligranulum* spp. increased with a simultaneous decrease in several genera of the *Ruminococcaeae* family, as compared to the placebo group. **Chapter 9** shows the protocol for the so called Inside Study I. This study aims to investigate the effect of fructo-oligosaccharides or galacto-oligosaccharides versus a placebo in young children with FC. This trial is still ongoing at the time of writing of this thesis and will, once finished, provide us not only with insights on clinical effects of such prebiotic interventions, but will also shine a light on the role of the intestinal microbiome before, during and after such an intervention in children with FC. In **chapter 10** we investigated a patient population for which earlier treatment was not sufficient and who had to start transanal irrigation to better manage their symptoms. The patient population consisted of children with both functional and organic causes of constipation. We found that transanal irrigation with the system used was an effective bowel management system for children with intractable constipation or fecal incontinence. Last but not least, **chapter 11** provides an overall discussion of the findings described in this thesis and future perspectives.


A

DUTCH SUMMARY

Het proces van ontlasten omvat een grote variëteit aan goed afgestemde, gecoördineerde sensomotorische systemen. Daarnaast zijn er veel andere factoren van invloed op ontlasten zoals genen, levensstijl, gedrag en psychosociale factoren. Ook hebben processen zoals vertering, absorptie, secretie en darmmotiliteit, met daarbij de interacties van onze darmmicrobiota, een grote invloed. Hierdoor is dit een interessant onderzoeksgebied als ontlasten niet meer zo vanzelfsprekend is. Dit is het geval bij functionele obstipatie (FO), een veelvoorkomende en hinderlijke aandoening die gekenmerkt wordt door symptomen als harde en/of infrequente ontlasting. FO heeft een gepoolde wereldwijde prevalentie van 9.5% in kinderen en 14% in volwassenen. Een kwart van de kinderen die behandeling hebben gehad voor FO blijven symptomen houden als volwassene. De behandeling voor FO begint met voorlichting over ontlasten, leefstijl en dieet advies. Als dat niet werkt kunnen osmotische of stimulerende laxantia worden gegeven, klysma's, anale botox injecties of een behandeling door middel van darmspoelen. Uiteindelijk kan er soms ook worden gekozen voor chirurgische ingrepen. Ondanks deze vele behandelopties zoekt ongeveer 1/3 van de ouders van een kind met FO hulp in de vorm van alternatieve en/of complementaire geneeswijzen.

Deel I van dit proefschrift beschrijft de fysiologie, pathofysiologie, evaluatie, behandeling en prognose van FO in kinderen en volwassenen en gaat dieper in op de potentiële rol van de darmmicrobiota in deze aandoening. De algemene introductie van **hoofdstuk 1** geeft een overzicht van de ogenschijnlijk simpele maar in werkelijkheid complexe en goed afgestemde systemen betrokken bij normale ontlasting. Daarna beschrijven we wat er mis kan gaan in het geheel aan functionele gastro-intestinale aandoeningen en zoomen we daarna in op FO in kinderen en volwassenen. Dit hoofdstuk geeft een overzicht van de epidemiologie, pathofysiologie, diagnose, behandeling en prognose in volwassenen met FO. Daarnaast hebben we gekeken naar de darmmicrobiota. Hierbij zijn niet alleen de darmmicrobiota zelf interessant voor gezondheid en ziekte, maar ook het zogenaamde darmmicrobioom als geheel. De rol van dit darmmicrobioom in kinderen en volwassenen met FO is interessant omdat het duidelijk is dat er verschillen te vinden zijn in de samenstelling van de bacteriën van kinderen en volwassenen met FO ten opzichte van gezonde individuen. Wij komen terug op het bewijs voor interventies die gericht zijn op de darmmicrobiota en klinische interventie studies in de behandeling van verschillende functionele gastro-intestinale aandoeningen in Deel II en Deel III. **Hoofdstuk 2** geeft een overzicht vanuit de klinische kant van FO in kinderen waarin we hebben gekeken naar de epidemiologie, fysiologie, pathofysiologie, evaluatie, behandeling, prognose en het toekomst perspectief voor deze groep.

Een belangrijke factor die van invloed is op het succes van de behandeling van FO is de tijd tussen de eerste symptomen en effectieve behandeling. Om FO in kinderen en volwassene effectief te behandelen is het van groot belang dat de diagnose snel wordt gesteld en behandeling eveneens snel begint. De diagnose FO is een klinische diagnose die wordt gesteld aan de hand van de anamnese, symptomen en het lichamelijk onderzoek. De Rome IV criteria zijn symptoom-gebaseerde

criteria waar, naast andere symptomen, de ontlastingsfrequentie en -consistentie belangrijke symptomen zijn. Wat wij als een ongezond ontlastingspatroon zien is duidelijk, echter zijn definities of beschrijvingen van een normaal ontlastingspatroon gebaseerd op klinische ervaring en bevindingen, niet op duidelijke data. Daarnaast zijn er ook veel uitdagingen in het meten van het ontlastingspatronen van kinderen, zoals beschreven in **deel II**. Ontlastingspatronen worden vaak beschreven in ontlastingsfrequentie en -consistentie. Soms worden ook de geur, kleur en hoeveelheid meegenomen. In **hoofdstuk 3** hebben we onderzocht wat een goede tool zou kunnen zijn om de consistentie van ontlasting te kunnen meten in kinderen. Om die reden hebben we de zogenaamde gemodificeerde Bristol Stoelgangenkaart (mBSFS) voor kinderen gevalideerd. Uit ons onderzoek konden we concluderen dat de mBSFS, op papier of online ingevuld, betrouwbaar, valide en gebruiksvriendelijk is voor Nederlandssprekende ouders, grootouders en kinderdagverblijf medewerkers om de consistentie van ontlasting van kinderen te kunnen scoren onafhankelijk van of dit in een luier of op een potje/het toilet was. Dit is, naar ons weten, de eerste ontlastingsschaal die gevalideerd is voor zindelijke en niet-zindelijke kinderen. Dit is van groot belang aangezien deze schalen normaal gezien worden gemaakt en gevalideerd voor zindelijke óf voor niet zindelijke kinderen. Daarnaast zit er veel verschil in het aantal items dat gebruikt wordt voor zo'n schaal: sommige gebruiken een 3,- 4-, 5,- of 7-puntsschaal. Dit maakt de vergelijking tussen studies ook erg lastig, dan niet onmogelijk. Deze uitdagingen en verschillen werden des te meer duidelijk in **hoofdstuk 4**. Naast dat het belangrijk is om ontlastingspatronen te beschrijven aan de hand van de consistentie is het dus ook erg belangrijk om te begrijpen wat we verstaan onder een normaal ontlastingspatroon. De noodzaak voor zulke evidence-based referentie waarden of normaalwaarden is met name van belang als je in gedachten houdt dat harde en infrequente ontlasting belangrijke symptomen zijn in de diagnose van aandoeningen zoals FO en andere functionele gastro-intestinale aandoeningen. Op dit moment zijn er geen evidence-based normaalwaarden voor ontlastingspatronen in kinderen en komt de informatie die er wel is uit oude studies in kleine groepen kinderen. Om een beter inzicht te krijgen in de daadwerkelijke normaalwaarden en uitersten hebben wij een systematisch review en een meta-analyse uitgevoerd, zoals beschreven in **hoofdstuk 4**. Wij vonden voor kinderen van 0-14 weken oud een gewogen gemiddelde frequentie van 21.8 keer ontlasten per week in vergelijking met 10.9 keer per week in kinderen van 15 weken tot en met 4 jaar.

Zoals eerder genoemd zoeken veel (ouders van) patiënten met FO hulp in de vorm van alternatieve of complementaire geneeswijzen. Om die reden hebben we in **deel III** gekeken naar het wetenschappelijke bewijs voor deze niet-farmacologische en darmmicrobiota gerichte interventies in een aantal functionele gastro-intestinale aandoeningen. In **hoofdstuk 5** hebben we onderzocht hoe effectief probiotica interventies zijn in kinderen met functionele buikpijn aandoeningen en FO. We vonden dat probiotica gebruik veilig is voor deze aandoeningen maar dat er geen bewijs is voor het gebruik van probiotica in de behandeling van functionele buikpijn aandoeningen of FO. Het is echter wel aannemelijk dat *Lactobacillus rhamnosus* GG de frequentie en intensiteit van buikpijn verlaagt, maar alleen in kinderen met het prikkelbare darm syndroom. In **hoofdstuk 6** hebben we breder gekeken naar allerlei niet-farmacologische

behandelingen bij FO in kinderen. Over het algemeen zagen we dat er behoefte is aan goede kwaliteit multicenter studies die de richtlijnen voor zulke studies volgen en uitkomsten rapporteren uit de zogenaamde core outcome set. Zulke studies zouden zich kunnen richten op de meest hoopgevende interventies gevonden in ons systematisch review en de meta-analyse, namelijk: specifieke prebiotica en vezel mixen, elektrische stimulatie van het abdomen, *Cassia Fistula* emulsie en Xiao'er Biantong granules. Zulke toekomstige studies zouden zich ook nog kunnen richten op interventies waar geen studies voor gevonden werden zoals gepersonaliseerde darmmicrobiota interventies, beweging, (elektro)acupunctuur en niet-invasieve neuromodulatie therapieën zoals stimulatie van de *nervus tibialis* posterior of virtuele en digitale interventies. In **hoofdstuk 7** hebben we in meer detail onderzocht wat de rol en het effect van vezels en prebiotica is op een aantal gastro-intestinale aandoeningen en de rol die het darmmicrobioom hier in speelt. In dit review vonden wij indicaties dat er mogelijk een specifiek verschil zit tussen gezonde baby's en baby's met koliek en gezonde kinderen en kinderen met prikkelbare darm syndroom in de samenstelling van de darmmicrobiota, ondanks dat er maar weinig data over te vinden was. Voor FO vonden wij echter geen duidelijk, consistent beeld voor mogelijke verschillen tussen gezonde kinderen en kinderen met FO. Daarnaast vonden we ook hier weer dat er te weinig grote, gerandomiseerde en placebo gecontroleerde studies waren die het effect onderzoeken van vezels en prebiotica in kinderen met functionele gastro-intestinale aandoeningen, wat ons breng bij het volgende deel van dit proefschrift.

Deel IV van dit proefschrift omvat (protocollen) van studies in FO en andere ontlastingsstoornissen. In **hoofdstuk 8** beschrijven wij een studie in volwassenen met FO waarbij deelnemers cichorei inuline of een placebo kregen voor vier weken. Inuline inname resulteerde in een hogere ontlasting frequentie, zachtere ontlasting en een verbetering van de kwaliteit van leven en symptoom scores in vergelijking met de placebo. Daarnaast observeerden we ook een 1.3-voudige verhoging van de relatieve abundantie van bifidobacteriën en een verhoging van de relatieve abundantie van *Anaerostipes* en *Subdoligranulum* spp. terwijl een aantal genera van de *Ruminococcaeae* familie juist verlaagde in vergelijking met de placebo groep. In **hoofdstuk 9** beschrijven wij een protocol van de Inside Study I. Deze studie heeft het doel om te onderzoeken wat het effect is van fructo-oligosacchariden of galacto-oligosacchariden versus een placebo in jonge kinderen met FO. Deze studie loopt op het moment van dit schrijven nog en zal, zodra deze klaar is, ons niet alleen informatie verschaffen over de klinische effecten van deze prebiotische interventies maar zal ook zijn licht doen schijnen op de rol van het darmmicrobioom voorafgaand, tijdens en na zo'n interventie in kinderen met FO. In **hoofdstuk 10** hebben we onderzoek gedaan naar een patiëntenpopulatie waarvoor eerdere behandelingen niet effectief waren en daarom met darmspoelen zijn gestart om hun symptomen beter onder controle te krijgen. Deze patiëntenpopulatie bestond uit kinderen met functionele en organische oorzaken voor hun obstipatie. Wij vonden dat darmspoelen met het gebruikte systeem effectief was voor kinderen met moeilijk te behandelen obstipatie of fecale incontinentie. Als laatste is in **hoofdstuk 11** een algemene discussie te vinden van de bevindingen in dit proefschrift aangevuld met een toekomst perspectief.


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Hauke, relatively late you became actively involved in my PhD project, but I am very grateful and happy that you did! You are a fantastic person and working with you felt like a privilege. For every struggle I shared with you, you tried to come up with solutions and tried to share the load where possible. I am grateful you stepped in and helped me out with all the noob questions and mistakes I made (and still make) when it comes to the miraculous world of microbial ecology. But more than only teaching me about content, you were there for the personal development part of a PhD: you showed me how much fun science can be, despite the struggles. Moreover, you are an example to me that shows what some attention to the person as a whole can do; spending some extra time to talk about silly things, beers, kids (in crop-tops) and growing older, but also important things like Star Wars, created for me the environment in which I thrive. I also want to thank you for sometimes slowing me down and make me re-consider taking that extra (super interesting) load. Your support and little check-ups to see how I was doing (well... you know the running joke for our live meetings) meant the world to me in the challenge that conducting a PhD is. Seems you have mastered the Force ('and a powerful ally it is') to sense what someone needs and 'still much to learn I have' when it comes to your knowledge content-wise but also situational leadership-wise.

Marc, where to start. Ben Witteman directed me to you because I was interested in doing an internship in the field of pediatrics with a link to nutrition and health. I came to the AMC and what I mostly remember is that we spoke for an hour about sports and a little bit about research. Not long after, I started my internship which 'had the potential to be turned into a PhD'. Well, here we are. It was not a very smooth process and Murphey's law seemed to be very applicable to this project. But I am very grateful that you invested so much time and energy to turn the Inside project into a PhD project. When the Inside project was seriously delayed you also helped me to get involved in different projects and you were always thinking along in how to help and how to keep me busy with projects that would deepen my knowledge. Even in times that were very difficult for you, you always tried to be there for your PhDs. Moreover, I am extremely thankful for the group of people you managed to gather around you. Every yearly diner I am

surprised by the warm memories all former PhD's of you still have of their 'bunker-time'. The Monday meetings were (often) a lot of fun and it brings a smile on my face thinking about all times you started singing (especially limericks), made poo-jokes ('wat fijn dat het gaat lopen, nu de ontlasting nog'), did the Sally-challenge against Klaartje (and lost), shared inspiring and beautiful stories, or recommendations for books and movies, and hear you ventilate about silly rules, money and bureaucratic struggles. Your door is always open, you respond faster to mails than anyone I know and feedback on manuscripts are back without even having one day of rest. Thank you Marc, for giving me this opportunity, the amazing group you gathered around you but most of all, for being there with your full support when I needed you most.

Clara, thank you for giving me the opportunity to help and turn the Inside studies into a PhD project. When things did not go as planned, you gave me the chance to work on different projects. It has not always been the easiest path, but I admire your perseverance in the bumpy road being in tenure track must be. Thank you for the nice group of people you have gathered around you and the efforts you make to create a close group with breakfast meetings and microbial bake-offs.

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Dearest **Jannie**, I cannot imagine doing a PhD without you. It has been a privilege to be your colleague, but even more to have you as a friend. There is no-one I rather wanted to have next to me as paranymph. Heavy moments became less heavy because of you, and fun moments became even more fun (with countless instances we were laugh-crying). I know I can always count on you, and I am confident that that will remain, even though we are no longer close colleagues. While typing this, my eyes become watery because it reminds me of all great, fun, amazing, sad, special, extraordinary and beautiful memories that I will cherish the rest of my life. I know no one with whom I have so many inside jokes. Your knowledge of microbial ecology research is admirable, but your knowledge on internetgekkies, memes, gifs and insta-movies is out of this world. The whole covid-situation was tough for the both of us, but our deal to work at either of our homes together weekly (with amazing jackfruit sandwiches, salads, soups or fruithapje lunch), and many more walking coffees made this time a lot more bearable and fun. There is so much I want to thank you for, but I guess that will become a manuscript by itself so I'd rather hug you and cry a little after the ceremony and tell you once again (sober or not) how grateful I am to have you as a friend.

Dearest **Marie**, we go way back (about 13!! years), and have we been not only friends but also housemates and colleagues and I am delighted that you are my paranymph (du Igelschnäuzchen). It is difficult to summarize what these 13 years have meant for me, and what you have meant for me during this PhD. You were always there for me and my heart is filled with love when I think back of all things we have shared (sweating from my eyes while typing this). You are an amazing woman, the best hugger I know and I admire your way of life where you just sparkle and take everyone along in this vibrant, fun and positive mood ('ohh my, defecation is so exciting!'). Life

is not always rainbows and butterflies, unless you draw them yourself. And that is what you do. I loved working with you, talking about the best ways to bring a message, digging deep into a dataset, learn a lot from you and be able to ask all the silly questions without actually feeling stupid. It meant the world to me that you would work with me until late in the evening and night to finish a manuscript in time. I sincerely hope we can share many more special moments and I am grateful that I met you all those years ago!

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All people of the PhD-trip 2019: thank you! This was such an extraordinary trip and I am very grateful that I could spend short of longer moments to get to know each other better. First of all, those that organized: I know how much work it is and I want to thank you all for managing it all so well, even in times of crisis: **Giannis**, **Caifang**, **Catarina**, **Costas**, **Enrique**, **Ivette**, **Lot**, **Nong**, **Ran**. But of course also all other participants in this trip: **Janneke**, **Menia**, **Sharon**, **Catarina**, **Despoina**, **Taojun**, **Max**, **Thijs**, **Joep** (I have a coffee for Joe, Joe P.), **Mamou**, **Patrick**, **Costas**, **Wen**, **Prokopis**, **Jannie**, **Hugo** (let's make a gif), **Wasin**, **Christos**, **Rik**, **Martha**, **Lyon** (karaoke-master), and of course the ones being responsible (and acting accordingly) **Diana** and **Raymond**.

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The first time I entered the bunker I saw the only two boys and a drawing saying 'you are my best friend'. The resemblance was good enough to recognize these two guys; **Ilan** and **Kay**. **Ilan**, I mostly saw you when you were close to finishing your 20kg thesis with 298 chapters which set the standard for all PhDs that would do something with functional constipation. I am happy that I could also work together with you later on; you are super smart, critical in a good way and lift manuscripts to the next level. I admire your perseverance and passion for this field but I guess we could have expected this since your phenotype seems to resemble that of the Boss. **Kay**, the not-fat, tall and better looking guy in the drawing (guess who drew). Fellow-Limburg buddy. You also pretty soon left after I entered the bunker, but we managed to still spend some nice evenings with good food together and it is amazing to see that you are well on your way to become a pediatrician! **Nina**, we grabbed a cup of coffee immediately after I first entered the bunker which was a very warm welcome. Thank you for that and it was amazing to see that you manged to finish your PhD! **Maartje**, thank you for you warm welcome and all great times, talks, food and drinks! I admire how you chose your own path and how you make time for the things that are important to you and how you fully commit yourself to it. I look forward to join a yoga session and share more amazing food with you! **Pam**, you are the most calm and reflective but on the other hand fierce woman I know. You dare to stand up and speak up for what you think is important and I admire that. I also admire how you combine being Olivier's mom and partner to Pepijn with becoming a GP. I am sure your patients will be blessed to have you as their GP. **Juud**, by just writing your name I hear your contagious laugh in my mind which I think is typical for what you meant for me and probably many more; you always tried to cheer everyone up. I will never-ever forget the way you talked about handling your own sample; it still makes me laugh out loud. But...you are also always there for serious conversations. You also had a big role in the organization of the weekend in Limburg and managed to open-up conversations about deeper feelings and improve the working environment. I admire that you dare to speak up and your beautiful Frenkie is blessed to have such a beautiful and kind power-woman as mother! **Lau**,

gekke-Lau, you are the best to share good wine and good food with! And the best thing is that such evenings also come with great conversations. Some serious, many resulting in laughing hard. I hope you will find your viking or boat-owner one day, but till then I hope we can still share many more food- and wine-evenings! **Hil**, thank you so much for all the nice (wine) moments, chats, good food and moments in corona times to share an online cup of coffee. Sometimes it takes 3 months to find another date to hang out, but it is always great to catch up again! **Rob**, thank you for all the help when it came to protocols, forms, etc. But also thank you for showing that you can be fierce without being unkind to arrange things faster. Also, I am super impressed by how you managed to do a PhD simultaneously with finishing an MSc. **Char**, my fellow microbiology-buddy. Thank you for all nice lunch moments, parties, and help to maybe stay in academia. **Maan**, fellow constipation buddy; thank you for tagging me in messages about animals that poo cube-shaped. But mostly thanks for answering all my questions that were constipation-related. I admire that you chose your own path and I am confident that you will become an amazing GP. **Els**, thanks a lot for the days and evenings during conferences or just relaxed moments to share good food and great wine! I admire how you seemed to always be relaxed and in control despite all tasks and responsibilities. **Marin**, I am always inspired by the way you seem to go through life; always positive, happy and extremely patient with patients. I cannot wait to share a 'pintje' with you! **Klaar**, maatie, the best tapdancer in purple sandals, winner of the Sally challenge and super talented athlete (not only in hockey). Thank you so much for all the good times, talks and moments we have shared. And let's not forget to thank you for hooking me up with an opponent to ride on the Felyx with... Dear **Sjoerd**, our coffee mornings dragged me through the boring and difficult times. I cannot express how nice it was to have short chats in the morning about life and to set some goals for that day. You are the most interested person I know and I think your PhD party made it clear that you are that to not only your bunker-colleagues but to so many more. You share and multiply love and warmth. I miss our coffee-mornings but am mostly super grateful that we could share struggles and celebrate achievements. I wish you, Joos and Murph all the best and I am sure you will follow the footsteps of your father and brother and become an amazing pediatrician or other type of medical doctor! **Desiree**, my SR buddy! You are a very driven, smart and ambitious woman and I have learned a lot from you. Without you my thesis would not have been the same. On a personal level we clicked and share an interest in many things outside of science, which is a lot of fun to talk about. I hope we can keep having lunch together every now and then and I hope you can take a good rest after finishing your own PhD. **Hannah**, **Jasmijn**, **Jalina**, **Carlijn**, **Michelle**, **Aysenur**, **Koen** and **Anna**; the new (or not very new anymore) inhabitants of the bunker. You all have fierce predecessors which set the standards high, but I am confident that you will do great too. But mostly I wish you all the amazing time that I had being a part-time member of the bunker. Enjoy these years, celebrate the ups and support each other in the downs in this rollercoaster.

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pediatrician and scientist and your view on systematic reviews helped a lot to lift manuscripts to a higher level. I hope we can share another glass of wine together and if needed book you an uber home. Of course also a big thank you to all other members of the department of pediatric gastroenterology for your input and help.

Jannie en **Ruud**, (Ruuuuudddd), laat ik niet nog een epistel schrijven maar jullie zijn het meest sportieve, open, en fijne stel wat ik ken. Ik voel me altijd thuis bij jullie en hoop dat we, zodra al het verhuis- en verbouwzand is neergestreken, veel fijne weekenden tegemoet gaan met zeilen, suppen, lekker eten, drinken en lachen. Ruud, je bent een soort broer; bij tijden irritant maar vooral iemand waar ik veel om ben gaan geven en ik ben dankbaar voor alle support die je me gegeven hebt. **Tim** en **Tanya**, ik kan niet genoeg zeggen hoe fijn het is om jullie zo dicht in de buurt te hebben. Ik geniet altijd enorm van samen zijn met jullie; hoe lekker het is om echt jezelf te kunnen zijn in gezelschap. Samenzijn met jullie is een garantie voor hard lachen. Daarnaast kan je niet met iedereen op vakantie, maar met jullie kan dat zeker wel! Fijn om te weten dat er ergens altijd een deur open staat waar er altijd een lekkere lunch, wokje, pizza of uitgebreider op tafel wordt getoverd. Jullie zijn voor mij de meest fantastische mix van enorm slimme mensen wat in een mengkom is gedaan met relaxte all-star skaters, LoTR en anime geeks met een vleugje kleurrijkheid. Het is wonderlijk om te observeren hoe gesprekken over diepgaande economische problemen toch subtiel overvloeien in LoTR en visa versa. **Gijs** en **Miriam** (en Tarzan). Dank voor alle fijne avonden met heerlijk eten, jullie open deur en de katers na al onze 30e verjaardagen. Elke avond is een genot met jullie en ik kijk uit naar de volgende oester en champagne dubbel-date! Elk weekend met jullie in binnen- en buitenland waren een feest. En dank dat jullie mijn thesis mede mogelijk hebben willen maken (die 30 cent plus 2,50 tijdens oud en nieuw 2021/2022). Jullie zijn me enorm dierbaar (mijn portemonnee niet) en ik ben erg dankbaar voor alles wat we hebben mogen delen en kijk uit naar nog vele fijne momenten samen. Lieve **Patrick** en **Margo** het is heerlijk om met jullie samen te zijn en ik geniet altijd erg van de relaxte dagen van werken, strand, zee, zon, Luna en gezelligheid. Eigenlijk moet ik vooral zeggen lieve Margo want Patrick heeft al een lofzang gehad. Margo, ik ben dankbaar dat ik je via Patrick heb leren kennen. Je bent een super fijn, getalenteerd en attent mens en ik kan niet zeggen hoe blij en dankbaar ik ben dat jij mijn omslag en binnenwerk hebt willen maken. Ik zal jullie beide erg missen als jullie in de USA zijn maar hoop dat we toch manieren vinden om slechte filmavonden te spenderen! **Cornelia** en **Marco** (ja bèèst), dank dat ik een kind aan huis mocht zijn en ik altijd (gewenst of iets minder gewenst) mee mocht eten, drinken en borrelen (thuis of als +1). Fijn dat Mathijs ook hier gebruik van mocht maken na die tijd. Ik hoop dat we nog vaak kunnen borrelen (met zelfgemaakte worst, dat vind ik *lekkàr* of misschien moet ik tegenwoordig lekkâh zeggen), buikspieroefeningen kunnen doen zonder dat je kin je borst raakt, dineren, klimmen en in de hottub zitten. En ook dank dat ik in de laatste fase bij jullie mocht logeren; ik kan niet uitdrukken hoe fijn het is om je ergens thuis te voelen in zo'n intense periode. **Anne**, **Peter** en **Esmee**, dank voor alle lieve kaartjes, home deliveries en andere uitingen van jullie zorg in tijden die even lastiger waren. Jullie zijn het meest gevende stel wat ik ken en ik ben enorm dankbaar dat ik jullie al zo lang ken. Anne, onze vriendschap heeft geen regelmaat nodig, we vinden elkaar

altijd weer en telefoontjes binnen 30 minuten houden is gewoonweg onmogelijk. **Hanne** en **Lim**, dank voor de leuke online catan en ticket-to-ride avonden en gezelligheid! Het was altijd fijn jullie te zien en ik hoop dat we dat snel in 3D kunnen gaan doen! **Noud** en **Ivanka**, dank voor fijne avonden met lekker eten, leuke gesprekken en imitaties. Ivanka, je bent een bijzonder, lief en getalenteerd mens. Dankje dat je de moeite nam, na het jammerlijk verpesten van de eerste, toch weer spontaan een prachtig gedicht voor te dragen. **Yahya** and **Enrique**, thank you so much for the many fun evenings in your house with amazing food! **Alejandro**, **Sergio** and **Burak**, thank you for being such kind neighbours, I had a lot of fun on many shared activities such as using a wok as sledge in the snow. **Pim** and **Jovana**, you two are amazing, talented and kind people. I am thankful that I met the two of you and I wish you all the best as newly-weds! **André** en **Jorien**, dank voor alle fijne momenten, de beste knuffels en de vele momenten van onbedaarlijk lachen. Ik hoop dat jullie een geweldige tijd tegemoet gaan in Italië en ik hoop dat we daar ooit gezamenlijk kunnen genieten van truffels, een goed glas wij en la dolce vita. **Giovanni** en **Sanne**, dank voor de fijne avonden, momenten en gesprekken, vaak inclusief schuddebuiken van jou Gio en uitzonderlijke beschrijvingen van geuren en smaken van wiskey van jou Sanne. Ik hoop dat er nog veel van dit soort momenten zullen volgen! Lieve **Lux Aeterna,** dank dat ik altijd welkom was bij menig feest en zelfs een mini-vakantie en altijd met open armen word ontvangen (ondanks dat ik me echt niet zou opdringen bij Mathijs zijn vrienden). **Anne** en **Lars**, dank voor al het lekkere eten en dat ik altijd bij jullie mocht komen zitten onafhankelijk van of DenB open was of niet. **Josephine**, thank you for all the nice conversations, food and the piece of chocolate cake when needed. **Frans**, dankjewel voor alle gesprekken en realisatie momenten om te luisteren en handelen naar mijn grenzen. **Arjen**, dankjewel voor de vele fijne (wandel-) gesprekken, inzichten en openheid. **Robbert**, **Laura**, **Anna** en **Femke**, beter een goede buur dan een verre vriend is maar weer bewezen: wat fijn om jullie als buren te hebben, ondanks puzzel-steel-taferelen. Lieve **Lotte** en **Mignon**, ondertussen kennen we elkaar 17 jaar en in al die tijd is er veel lief en leed gedeeld. Ik ben dankbaar dat we, ondanks alle drukte, nog steeds momenten vinden om elkaar te zien of te spreken. Met zo veel geschiedenis voelt het altijd als vanouds. Dank voor al jullie steun toen en nu. Daarnaast ook dank aan de vele schrijvers van de 162 boeken die ik heb gelezen of geluisterd om even te ontsnappen en me onder te dompelen in een andere prachtige wereld.

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I hope that my memory did not fail me to remember all that were important to me in this PhD-cycle, but forgive me if I did and feel free to add your name here: _____. Thank you!

With love and gratitude,

Carrie

ABOUT THE AUTHOR

Carrie Wegh was born on the 25th of March in 1991 in Venray, the Netherlands. In the same year the release of the first website that would later on lead to the world wide web, and the launch of the first open-access repository for pre- and postprints of scientific papers appeared. Carrie was raised in Leunen and later moved to Arnhem to combine her BSc in Nutrition and Health with her professional archery career at the national training center Papendal. In 2012 Carrie decided to quit her professional archery career and fully focus on finishing her BSc and MSc Nutrition and Health. She moved to the birthplace of her father: Wageningen. She developed a keen interest in the interplay between nutrition, health and the intestinal microbiota in gastroenterology, especially in pediatrics. For her first MSc thesis, which was later published, she worked on a trial in adults with ulcerative colitis under the supervision of dr. Nicole de Roos and prof. dr. Ben Witteman. For her second MSc thesis she set-up a trial in patients with irritable bowel syndrome (IBS) to investigate the effect of chewing gum containing cannabidiol on IBS symptoms under the supervision of prof. dr. Renger Witkamp and prof. dr. Ben Witteman. During this time Carrie also worked as a student-assistant to several BSc and MSc courses and practicals and was location coordinator for the BSc Open Days. These activities made Carrie realize that she loved to transfer knowledge and take a coordinating role. During her MSc internship at the University Medical Centre Amsterdam she worked on a proposal for a large randomized controlled trial investigating the effect of prebiotics in children with functional constipation. After finishing her MSc *cum laude* she worked as research assistant at the Laboratory of Microbiology under the supervision of dr. Clara Belzer. In 2018 she started as a PhD candidate at the Laboratory of Microbiology at Wageningen University and Research and Pediatric Gastroenterology at University Medical Centers Amsterdam, AMC under the supervision of prof. dr. Hauke Smidt, prof. dr. Marc Benninga and dr. Clara Belzer to set-up and carry-out two large randomized controlled clinical trials in children with functional constipation and healthy children with hard stools. Being a PhD candidate in this project brought everything together; multi-disciplinarity with pediactrics, gastroenterology, nutrition and microbiology, coordinating two big trials, and being involved in knowledge transfer via courses and by supervising thesis students. The research described in this thesis is a product of collaboration between two universities, but also of a public-private partnership with Sensus B.V. (Royal Cosun, Roosendaal, The Netherlands) and FrieslandCampina (Amersfoort, The Netherlands). Besides her research activities Carrie was involved in setting-up a thesis ring for BSc and MSc students and was involved in an advisory project from the Dutch ministry of

health, wellbeing and sports (VWS) on how to better support researchers conducting clinical research. Carrie was also an active member of the Laboratory of Microbiology PhD board, the graduate school PhD board (VLAG) and the Wageningen PhD council. In her role as external secretary she was in close contact with the Wageningen Graduate Schools, WUR council, and the national PhD candidate network (PNN) to think along, discuss and solve problems when needed. Currently, Carrie works as program manager at ZonMw (The Hague) and lives together with her partner Mathijs in Voorburg.

LIST OF PUBLICATIONS

Wegh, C. A. M., Schoterman, M. H., Vaughan, E. E., Smidt, H., Belzer, C., & Benninga, M. A. (2022). Effect of prebiotic oligosaccharides on bowel habit and the gut microbiota in children with functional constipation (Inside study): study protocol for a randomised, placebo-controlled, multi-centre trial. Research Square. preprint.

Baaleman, D. F.*, **Wegh, C. A. M.***, Hoogveld, M. T., Benninga, M. A., & Koppen, I. J. (2021). Transanal Irrigation in Children: Treatment Success, Quality of Life, Adherence, Patient Experience and Independence. Journal of Pediatric Gastroenterology and Nutrition, 10-1097.

Wegh, C. A.M.*, Baaleman, D. F.*, Tabbers, M. M., Smidt, H., & Benninga, M. A. (2021). Nonpharmacologic treatment for children with functional constipation: a systematic review and meta-analysis. The Journal of Pediatrics.

Wegh, C. A.M., Hermes, G. D., Schoterman, M. H., Vaughan, E. E., Smidt, H., Belzer, C., & Benninga, M. A. (2021). The Modified Bristol Stool Form Scale: A reliable and valid tool to score stool consistency in Dutch (Non) toilet-trained toddlers. Journal of Pediatric Gastroenterology and Nutrition, 73(2), 210-216.

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Wegh, C. A. M., Benninga, M. A., & Tabbers, M. M. (2018). Effectiveness of probiotics in children with functional abdominal pain disorders and functional constipation: a systematic review. Journal of clinical gastroenterology, 52, S10-S26.

Wegh, C. A. M., Schoterman, M. H., Vaughan, E. E., Belzer, C., & Benninga, M. A. (2017). The effect of fiber and prebiotics on children's gastrointestinal disorders and microbiome. Expert Review of Gastroenterology & Hepatology, 11(11), 1031-1045.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities	Organizing institute(s)	Year
Consortium meetings	WUR/AmsterdamUMC	2018-2022
Amsterdam kinder symposium	EPGS	2018
BROK course	NFU	2018
20th Gut Day	AmsterdamUMC/WUR/ Microbiota Center	2018
Meest gestelde vragen aan de kindergastroenteroloog	EPGS	2018
Castor course	Castor EDC	2019
Meest gestelde vragen aan de kindergastroenteroloog	EPGS	2019
Data exploration and analysis in gut microbiome profiling studies	Radboud UMC	2019
21st Gut Day	AmsterdamUMC/WUR/ Microbiota Center	2019
Nutritional management of pediatric Crohn's Disease	ESPGHAN/Nestlé	2020
VLAG lecture series	VLAG	2020
AGEM retreat	AGEM	2020
In vitro studies of the human microbiota symposium	MIB – VLAG	2020
Amsterdam kinder symposium	AmsterdamUMC	2021
WCPGHAN/ESPGHAN 2021	ESPGHAN	2021
UEG week 2021	UEG	2021

General courses	Organizing institute(s)	Year
Research data management	WGS	2018
How to keep the editor happy	VLAG	2018
PhD workshop carousel	WGS	2018
Big data	AFSG	2018
PhD week	VLAG	2018
Start to supervise BSc and MSc thesis students	ESD	2018
Supervising BSc and MSc thesis students	ESD	2018
Scientific publishing	WGS	2018
Competence assessment	WGS	2019
Scientific writing	WGS	2019
Verwachtingen managen	MyDevelopment WUR	2020
Datacamp R	DatacampR	2020

Scientific integrity	WGS	2020
Reviewing a scientific manuscript	WGS	2020
Start to teach	ESD	2020
Career perspectives	VLAG	2021

Assisting in teaching and supervision activities	Year
Teaching MSc course MIB-10306/CBI-50806/FHM-22306/HAP-31806	2018-2021
Supervising students	2019-2021

Other activities	Organizing institute(s)	Year
Peer review MBC gastroenterology, neurogastroenterology & motility, pediatrics and child health, and future foods		2018
Workgroup meetings MolEco	WUR (MIB)	2018-2022
Journal Club	WUR (MIB)	2018-2020
PhD meeting	WUR (MIB)	2018-2022
VLAG PhD council	VLAG	2018-2020
Siam meeting/health and disease meeting	WUR (MIB)	2018-2019
Preparation of research proposal	VLAG	2018
Research bespreking AmsterdamUMC	AmsterdamUMC	2018-2022
PhD trip	WUR (MIB)	2019
Wageningen PhD council	VLAG	2019-2020
Young KLV 'PhD, pleasure or burden?'	WUR (youngKLV)	2018, 2020
Article baby24.nl 'Obstipatie bij je kind: wat je kan eraan doen?'		2019

ABOUT THE COVER

The cover-design is based on the work of Yoyo Sena, an artist based in Spain. Carrie and Yoyo met in Madrid by coincidence and the idea was born to have art on the cover of the PhD thesis, instead of a more typical graphical representation of the thesis content. The reader may appreciate this seeing how much of this thesis is poo-related. Yoyo Sena creates bright, vibrant, colourful and joyous paintings and made the artworks on the cover and on the inside of this thesis upon request. The colors used for the cover are similar to those used for the logo and style of the project that was supposed to be the main topic of this thesis: the Inside studies. The artwork was adapted by the talented Margo Togni, a graphical designer and author specialized in cover designs. She used the artwork as a basis to create a coherent style throughout the thesis. The cover may not be the typical, but to quote Will Durant: 'Every science begins as philosophy and ends as art; it arises in hypothesis and flows into achievement'.


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COLOPHON

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