Constipation in infancy and childhood: New insights into pathophysiological aspects and treatment

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Constipation in Infancy and Childhood
New Insights into Pathophysiological Aspects and Treatment

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het college voor promoties
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LOVE-LIFE

The head in the air
Everything in the world seems to be fair
Feeling the wind in the hair
Makes one feel life is really there

Happy as you could ever be
Everything seems pink, you see?

The world is there not only for me
But also for thee
For thee who also see
What a beautiful persons we are to be

Love is about you and me
This is how it is supposed to be

We feel free
What life will bring us, we will see....

-Curly Me-
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General introduction
1. DEVELOPMENT, STRUCTURE & FUNCTION OF THE COLON

Development of the gut & colonic motility

The neuromuscular development of the human gut largely occurs during the first trimester of pregnancy. Circular and longitudinal muscles are detected in the large intestine in a foetus of 10 weeks of age. Auerbachs and Meissners plexuses are recognized at 13 weeks and cranio-caudal migration of ganglion cells is complete at 20 weeks after conception. Neuro-endocrine cells are present at 10 weeks of gestation. The smooth musculature and enteric neuro-endocrine system necessary for propulsive motor activity are present and differentiated long before the initiation of enteral feeding, but mature innervation and contractile activity is not achieved until near or after birth.(1) In contrast, colonic mechanisms responsible for defecation are poorly developed until close to term. This is illustrated by the fact that even severe stress is unlikely to cause in utero passage of meconium in the premature born infant, whereas in the term fetus, passage of meconium commonly occurs as a response to stress such as fetal hypoxia.(2;3) Colonic motility requires coordinated contractions of colonic muscles which subsequently result in aboral propulsion of luminal contents through the rectum for expulsion from the anus. Ninety five percent of healthy term infants pass their first stools, meconium, within 24 hours after birth and 99% within 48 hours.(4;5) This percentage however drastically drops to 66% in preterm born infants. Recently, De Lorijn et al showed that preterm infants (GA 28-32 weeks) with delayed first passage of meconium exhibited a normal rectoanal inhibitory reflex. Moreover, all other anorectal motility parameters were similar to preterm infants of the same age who passed meconium within 48 hours.(6;7) These latter studies suggest that either mechanisms of rectal propulsion are impaired, leading to failure of normal expulsion of the meconium plug, or that the meconium plug itself is too difficult to expel as a result of its consistency. Indeed, it is well-known that meconium of preterm infants differs in composition (glycoprotein, saccharides, calcium, copper, iron and phosphorus) from that of term infants, making it thicker in consistency and more difficult to expel.(8) But whether total duration of passing meconium before its transit to non-meconium stools, is also prolonged in premature born infants has yet to be evaluated.

Colon: mucosa, serosa and chloride channels

The colon, except for the rectum and anal canal, is characterized by a mucosa with goblet cells, gland cells and absorptive cells. The colon maintains important functions to 1) absorb short chain fatty acids and bacterial metabolites, 2) slowly propel luminal contents in a caudal direction, 3) hold residual faecal material
in the distal colon until defecation, and 4) move faecal content rapidly in a caudal direction during mass movements initiating defecation. But it also plays an important role in the homeostasis of water and electrolytes, and exhibits considerable segmental heterogeneity with regard to ion transport mechanisms along its longitudinal axis. With respect to Na⁺ absorption, electroneutral NaCl absorption predominates by luminal Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange in the surface epithelium of the proximal colon, and electrogenic absorption occurs via the electrogenic sodium channel (ENaC) in the distal colon of humans as well as in other species.(9-12) Those Na⁺ channels located mainly in the apical membrane of the colon regulate sodium absorption.(13) But the colon also has secretory capacities.(14;15) Although secretion of bicarbonate and potassium occurs along the entire intestine, the predominant electrolyte driving fluid secretion is chloride. (16) Chloride secretion is activated by the cAMP or intracellular Ca²⁺-dependent stimulation of luminal cystic fibrosis transmembrane regulator (CFTR) chloride channels and is paralleled by the secretion of K⁺, which takes place via luminal K⁺ channels and Na⁺ transport through the paracellular shunt.(17) Water transport is believed to follow the osmotic gradient, which is set by the direction of net ion transport, and is then transported via specialized aquaporin water channels.(18) On the basolateral membranes, secretory cells containing Na⁺K⁺2Cl⁻ cotransporters take up chloride from the serosal side of the epithelium together with Na⁺ and K⁺ (figure 1). Basolateral K⁺ channels also allow for K⁺ recycling via the basolateral membrane, thus hyperpolarizing the epithelial cells, and maintaining the electrical driving force for chloride secretion.(13;19) Therefore, the coordinated action of these apical and basolateral ion channels is essential during the process of secretion.

Figure 1. Apical and basolateral membrane in the human colon.
(figure 1). Congenital deficiencies in intestinal chloride secretion, occurring in cystic fibrosis, are accompanied by intestinal malabsorption and obstruction as well as constipation. Therefore small changes in net fluid transport are believed to lead to diarrhoea or constipation. However, the question whether patients with constipation have disturbed sodium absorption or chloride secretion remains to be clarified.

2. BOWEL HABITS

Normal bowel habits

Available data describe an average of 3-4 bowel movements per day in the first two weeks of life in term newborn infants. At the age of 4-6 months, bowel movement frequency drops further to a frequency of once to twice per day. In children younger than 2 years, a defecation frequency of 1-2 per day is described by several studies worldwide. Another study evaluated the bowel habits in children aged 1-4 years without complaints of constipation and reported a bowel frequency of once per day. With aging, up to the age of 8 years bowel movement frequencies of once per day or every other day is reported in 95% of healthy children. Interestingly, in preterm born infants comparable bowel movement frequencies (3-4/ day) are found in the first 4 weeks of life provided that ingestion of milk is more than 150/mg/kg per day; infants receiving parenteral nutrition open their bowels only once daily. Studies reporting bowel habits of preterm versus term born infants in a prospective manner for a more extended period (> 4 weeks after birth) are however lacking.

So far, information concerning bowel habits is not only scarce in the younger group of children but particularly refers to normal bowel movement frequencies. The necessity for more data from infants and young children is mandatory not only to define normal bowel habits but also to diagnose abnormalities such as found in constipation. To reach this, in addition to bowel movement frequency, stool characteristics such as consistency, amount and colour as well as gastrointestinal symptoms need to be evaluated. For stool characteristics for example, adults use the Bristol stool form scale as a reliable tool serving for stool appearance classification. In infants such a stool form scale has yet to be developed.

Constipation

Pathophysiology

The pathophysiology of childhood constipation consists of multiple factors where in the vast majority of patients, no organic cause can be found, and, therefore, these patients are considered to have a functional disorder. In less than 5% of children
presenting with constipation, organic causes such as Hirschsprung’s disease (HD) are well described. However, the role of other organic factors such as lower spine abnormalities and histopathological changes remain poorly understood. In infants cow’s milk allergy is also suggested to play a role in this condition. Furthermore, novel insights advocate disturbance in colonic homeostasis of water and electrolytes as well as colonic flora in constipation.

Organic causes

Congenital causes

Anorectal malformations, HD, endocrine disorders and spinal cord anomalies

A well recognized group of organic disorders is known to cause chronic constipation which can be excluded by careful examination and adequate investigation.(34) Those organic causes include congenital anatomical or structural defects resulting in obstruction of the distal colon, which are recognized in the newborn as anorectal malformations (imperforate anus, anteriorly displaced anus).(34) Hirschsprung’s disease is a developmental disorder of the enteric nervous system characterized by absence of ganglion cells in the myenteric and submucosal plexuses along a variable proportion of the distal colon. Therefore, rectal suction biopsies (RSBs) are of importance in diagnosing HD.(35) Moreover, RSBs are the most accurate test in the diagnostic work-up of HD.(36) Interestingly, evaluation of the RSBs often shows hypertrophied and disorganised rectal mucosal muscle only in children with severe functional constipation. In solitary rectal ulcer syndrome, mainly presenting in adults, the rectal mucosal muscle is also characterized by hypertrophy and disorganisation.(37;38) In the latter condition, recommendations comparable to those given to constipated patients are applied; avoidance of straining, high fluid and fibre intake in combination with enemas may relief symptoms in 58-83%. (38;39) A change in muscle thickness, such as hypertrophy, might therefore be associated with constipation symptoms. Nonetheless, studies evaluating the rectal mucosal muscle and its possible association with constipation symptom duration have yet to be performed.

Other organic causes such as metabolic and endocrine disorders (diabetes insipidus, hypercalcaemia, hypothyroidism) may also cause (secondary) constipation. In addition, neurological disease related to damage of the spinal cord in conditions such as meningomyelocele, trauma, surgery, tumours, cauda equina and tethered cord might also cause constipation. So far, only one American retrospective study found spinal cord abnormalities (n=8) in 88 children with intractable constipation.(40) In the latter study 85% recovered from constipation symptoms after neurosurgical treatment. However, due to the retrospective design, it remains unclear what the effect of conservative therapy (laxative treatment) would be without neurosurgical
treatment. A prospective study should be performed in constipated children to better evaluate the prevalence of spinal cord abnormalities and facilitate follow-up to assess the clinical course of constipation when adequately treated with conservative approach prior to neurosurgery.

_Cow’s milk allergy_

Children with cow’s milk (CM) intolerance might also present with symptoms of constipation. The majority of these children presented with a low defecation frequency in combination with anal fissures and perianal erythema or edema. (34) This can be diagnosed by eosinophilic infiltration of the submucosa on rectal biopsy. (41) In one study 27 infants with idiopathic constipation (mean age 20.6 months) showed that constipation resolved within 1 month following a CM protein free diet. (42) However, in a recent study in constipated children with atopic signs (n=12), CM protein elimination neither improved defecation frequency nor stool consistency. (43) Therefore, the role of CM intolerance in constipation remains to be elucidated in larger studies.

_Miscellaneous causes: novel insights_

Other causes such as chloride secretion disturbance and gut flora imbalance are also suggested to play a role in chronic constipation. (44-48) Dysbiosis of intestinal flora, alteration in the relative proportions of intestinal bacterial species, has been reported in chronic constipation. (49) For example, faeces of constipated children contain more bifidobacteria and clostridia compared to controls. (49) While bifidobacteria counts are elevated in faeces of constipated children, probiotics intake (including bifidobacteria) potentially reverse this dysbiosis (50) and results in positive effects on constipation symptoms. (51-54) The effect of probiotics, such as bifidobacteria and lactobacilli, is stimulating peristalsis by lowering the pH in the colon as a result of lactic, acetic and other acids production by probiotics. (45) A lower pH enhances peristalsis and subsequently decreases colonic transit time which is beneficial in the treatment of constipation. (45;46) But it is unknown whether a mixture of different probiotics strains can also alter this dysbiosis. Another proposed aetiological factor in constipation is disturbance in fluid secretion that might result in chronic constipation. It is evident that pathological consequences will follow when chloride secretion is reduced as occurs in cystic fibrosis. (55) Interestingly, chloride secretion agonists showed an increase in bowel movements and improved stool consistency in adults with constipation. (56) Conversely, a disturbance in chloride secretion in colonic mucosa of constipated subjects has yet to be evaluated in both, constipated adults and children.
Functional constipation

Epidemiology & Cost

A median prevalence of 10.4% is found for functional childhood constipation in Western and Asian countries, when defined as defecation frequencies below three times per week and without evidence for organic disease.(57) In our tertiary centre, with a specific outpatient clinic for children with functional gastrointestinal disorders, approximately 45% of the referrals are due to defecation disorders. (58) Although these numbers are not based on proper designed epidemiological studies, they are indicative for the great impact of childhood constipation on health care. Indeed, Liem et al. showed that American children with constipation use more health care services than children without constipation. This amounts to an additional cost of $3.9 billion/year for children with constipation.(59)

Definition: Rome III criteria

In 1999 a group of experts in the field of paediatric gastroenterology made the first attempt to set criteria for functional gastro-intestinal disorders such as constipation, leading to the first paediatric Rome II criteria.(60) In 2006 the Rome II criteria were updated when several studies showed that those criteria were too restrictive for clinical use.(61-64) To fulfil the new Rome III criteria for functional constipation, table 1, children > 4 years should have 2 or more of the following symptoms: 1) two or fewer defecations per week, 2) at least one episode of faecal incontinence per

Table 1. The Rome III criteria for paediatric functional constipation

Function constipation, neonate/ toddler

Must include one month of at least two of the following in infants up to 4 years of age:

1. Two or fewer defecations per week
2. At least one episode/week of faecal incontinence after acquisition of toileting skills
3. History of excessive stool retention
4. History of painful or hard bowel movements
5. Presence of a large faecal mass in the rectum
6. History of large diameter stools which may obstruct the toilet

Accompanying symptoms may include irritability, decreased appetite and/or early satiety.

The accompanying symptoms disappear immediately following passage of a large stool.

Function constipation, child/ adolescent

Must include all of the following, in a child with a developmental age of at least 4 years, for at least two months:

1. Two or fewer defecations per week
2. At least one episode of faecal 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 faecal mass in the rectum
6. History of large diameter stools which may obstruct the toilet

Criteria fulfilled at least once per week for at least 2 months prior to diagnosis
week, 3) stool retentive posturing, 4) painful or hard bowel movements, 5) presence of a large faecal mass in the rectum or 6) large diameter stools that may obstruct the toilet, without objective evidence of a pathological condition.(32)

In infants less than 6 months of age, the Rome III criteria described infant dyschezia as a common functional defecation disorder.(62) Infants with infant dyschezia present with at least 10 minutes of straining and crying before the successful passage of soft stools. Symptoms usually begin in the first months of life and resolve spontaneously after a few weeks. While constipated children usually present with painful infrequent defecation, in infant dyschezia infants open their bowels daily to expel soft stools. Therefore reassurance of the parents is the treatment of choice in these infants. To date, exact criteria for constipation in the new born and children < 2 years of age are lacking. (61;62) The Rome III criteria described functional constipation for the age group younger than 4 years without distinguishing symptoms found in younger infants (table 1). As bowel habits significantly change in the first months of life, it is necessary to define symptoms categorized per age group, e.g. 12 months 24 and 24-48 months, so that changes associated with different age, and (accordingly) nutritional intake, can be distinguished between health and disease.

**Symptoms**
Defecation frequency below 3 times per week is found in 64-88% of children with chronic functional constipation. (65;66) Moreover, faecal incontinence is reported in 78-81% of these patients.(65;67) A recent trial showed that stool withholding behaviour (68%) and painful defecation (65%) are important features of constipation as well. (68) A rectal scybalus found either by abdominal palpation or rectal examination, and defined as a large faecal mass, unlikely to be passed on demand is present in 30-75% of the constipated toddlers and older children.(69-71) Expulsion of these large stools are, reported in 65-70% of constipated children.(67;72)

**Onset**
In approximately 17-40% of children, the onset of constipation occurs in the first year of life.(73-75) In toddlers, withholding behaviour plays an important role in the development and/ or persistence of functional constipation. Furthermore, the time of toilet training is thought to be a critical period when constipation may occur as a consequence of struggle between child and parents.(76) Interestingly, Borowitz et al. found no association between timing, style or techniques used for toilet training and developing early childhood constipation.(77) On the other hand, Burkett et al. showed that constipated children, especially between 2-3 years, were perceived by their parents as more stubborn, specifically regarding toilet behaviour,
than children without constipation. This stubbornness might play a role in developing constipation or influence treatment response at a younger age. Several other factors might also lead to stool withholding behaviour: 1) previous experience with painful or hard stools, 2) anal fissures, 3) lack of time for regular toileting, 4) resistance to use toilets other than the child’s own, 5) stressful events, and 6) intercurrent illness. Retained stools become progressively more painful and difficult to evacuate leading to even more fear and avoidance of defecation. Therefore, consequential retentive posturing is believed to be the main cause for development and/or persistence of functional childhood constipation.

TREATMENT

As the exact pathophysiologic mechanism of functional childhood constipation remains unknown, treatment is based on a symptom relieving approach where education of the child and parents, behavioural modifications and laxative therapy are applied.

Education & demystification

Treatment of children with constipation begins with demystification, education and a non-accusatory approach by care giver and parents. Information concerning prevalence and symptoms of constipation might help parents understand and hence control this condition. The reason why children have faecal incontinence, which is present in the majority (85%) of children with constipation, should be explained. It should be emphasized that faecal incontinence is due to overflow of loose stools in an impacted rectum and not the result of the child’s behaviour. Furthermore, it is essential to accentuate that treatment is often a gradual and irregular progress with periods of improvement and deterioration. A follow-up study in 403 constipated children, who were initially treated successfully with either medical or behavioural treatment, showed that 50% of the children had at least one relapse during the first five years. Surprisingly, relapse was more common in males.

Behavioural approach

It has been suggested that an acquired behaviour after experiencing painful defecation is the most common cause of constipation in infants and toddlers with chronic constipation. Retained stools become progressively more difficult and painful to evacuate, leading to fear and avoidance of defecation. This vicious circle is described as learned behaviour. Therefore laxatives are needed to breakthrough this circle by facilitating expulsion of softer stools and, thus, preventing stool-withholding behaviour sufficiently. Paediatric psychologists
showed that the adjunct of behavioural interventions to laxative therapy, rather than laxative therapy alone, improves incontinence in children with functional constipation. (83-85) Nonetheless, a recent randomized controlled trial including 134 constipated children showed no difference in clinical defecation symptoms between those treated with conventional therapy with laxatives alone, versus those who received additional behavioural therapy by a paediatric psychologist.(68) Interestingly, in the latter study, the proportion of children with behaviour problems was significantly lower at follow-up, in children receiving additional behaviour therapy compared to children receiving conventional therapy.(68) Therefore, when behaviour problems are present in constipated children, behavioural therapy or referral to mental health services should be considered.

Medical approach
The medical treatment consists of disimpaction of rectal faecal mass followed by maintenance therapy with oral and/ or rectal laxatives.(79)

Disimpaction
As described above rectal faecal impaction is a common symptom in functional constipation. It is assumed that initial treatment with oral laxatives might paradoxically result in an increase in faecal incontinence. In our tertiary clinic, we therefore start with rectal disimpaction using rectal enemas. Many paediatricians, however, consider that uncomfortable, embarrassing, or painful anal manipulations such as rectal examination and rectal enemas should be avoided. These care takers stress that adequate doses of oral laxatives is in the majority of cases sufficient to treat these children. To date however, only one group reported successful disimpaction with high doses (1.5 g/kg/day) of oral polyethylene glycol (PEG) in constipated children.(86;87) They demonstrated that after only three days, seventy-five percent of these children were successfully disimpacted. A drawback of this study was that the frequency of faecal incontinence during treatment was not documented.(86) In our clinical experience, administration of oral laxatives in children with severe faecal rectal impaction is often not effective. Rectal enemas are repeatedly necessary to remove the large faecal mass before oral laxatives can reach their effect. Randomised controlled trials evaluating the effect of enemas versus oral laxatives are needed to assess difference in successful disimpaction as well as anxiety scores between both treatments. The latter is especially important since treatment with enemas is suggested to be disliked by children.

Maintenance treatment
Following disimpaction, maintenance therapy is started aiming to prevent re-accumulation of faeces. For maintenance therapy several oral laxatives can be
prescribed consisting of osmotic laxatives, such as lactulose and PEG, or stimulant laxatives, such as bisacodyl or senna.(58) However, PEGs, long linear polymers with a high molecular weight of 3500 or 4000 Daltons on which water molecules bind through hydrogen-binding, now account for 47% of most commonly chosen laxatives.(88) As a result PEG helps increasing the water content of stools in constipated patients.(89-91) When treatment with PEG is compared to treatment with lactulose, a favourable outcome for those treated with PEG is found.(66;92-94) But a comparison between different products of PEG, for example PEGs containing extra electrolytes and those without, has never been evaluated in children for an extended period of time. It remains therefore unclear what the additional effect is when adding electrolytes to PEG laxatives during the total treatment period.

**Alternative management**

*Electrical therapy*

Various forms of electrical therapy, direct electrical stimulation of sacral nerves, transcutaneous nerve stimulation (TENS) and interferential therapy (IFT), are applied aiming to increase colonic motility. Direct electrical stimulation proved to increase bowel movement in women with constipation and in patients with spinal cord injuries.(95) Transcutaneous nerve stimulation has also been used successfully, in conjunction with biofeedback therapy, in children with faecal incontinence following anorectal malformation.(96) Furthermore, in a recent pilot study IFT improved colonic motility in 7 out of 8 patients. After three months 4 children still sustained improvement in bowel movements compared to 5 with sustained reduction in faecal incontinence episodes.(97) The mechanism underlying those positive effects is reflex relaxation of the pelvic floor following with evacuation of the rectum.(98)

*Probiotics*

There is growing interest in the use of probiotics in organic and functional gastrointestinal disorders. Probiotics are live microbial microorganisms, that when administered in adequate amounts, confer a health benefit on the host.(99) Food ingredients containing probiotics are reported to be valuable in the treatment of inflammatory bowel disease, travellers’ diarrhoea and constipation.(53;100-102) To date, several studies have been performed, mainly in adults, in order to determine the effects of probiotics on symptoms of constipation.(45;46;52;103-105) It has been shown that probiotic strains, such as Lactobacillus shirota and the Bifidobactreium infantis, increase defecation frequency and soften stools in adults with constipation and irritable bowel syndrome.(51;52) A recent study in children with constipation showed an increase in defecation frequency and a decrease in abdominal pain using the strain Lactobacillus rhamnosus. (53) In contrast to the latter study however,
the probiotic strain *Lactobacillus GG* did not have a positive effect on constipation symptoms, when used as an adjunctive therapy with lactulose (104). The effect of treatment with a mixture of probiotic strains rather than only one strain has yet to be evaluated in constipated children.

**Novel compounds**

*Lubiprostone*

Lubiprostone is an oral bicyclic fatty acid that selectively activates chloride channels in the apical membrane of the gastrointestinal epithelium, resulting in luminal chloride secretion and consequently resulting in water movement by which it reaches its laxative properties. (106;107) In adults, this newly developed drug acting on stimulation of chloride secretion in the gut proved to be valuable in treating constipation.(47;48;56) In a recent multicenter, parallel-group double-blind controlled trial (n=242), higher mean number of spontaneous bowel movements at week 1 was found in the lubiprostone group compared to placebo in constipated adults (5.69 vs. 3.46, p= 0.0001). A greater frequency of bowel movements was also reported at weeks 2, 3, and 4 (p<or= 0.002).(56) In constipated children no clinical trials have been performed.

*Tegaserod*

Tegaserod (5-hydroxytryptamine; 5-HT) is a selective serotonin receptor agonist that acts at 5-HT₄ receptors in the gut wall.(108) The central role of serotonin in modulating motility, visceral perception, and intraluminal secretion in the gastrointestinal tract makes the serotonergic system an important therapeutic target. It increases stool frequency, improves stool consistency, stimulates the peristaltic reflex and intestinal secretions, and inhibits visceral sensitivity.(109;110) In children with constipation, tegaserod increased defecation frequency and decreased faecal incontinence episodes. Therefore, tegaserod was increasingly prescribed by paediatric gastroenterologists in the United States.(111) However, marketing of tegaserod was suspended in March 2007 after retrospective analysis of data from clinical studies showing a statistically significant difference in cardiovascular ischemic events in adult patients taking tegaserod compared with those receiving placebo (incidence of 0.11% vs 0.01%, respectively).

*Prucalopride*

Prucalopride, a 5-HT₄ receptor agonist, is a newly developed highly potent and selective compound to enhance colonic motility. In several animal models, it has been demonstrated that prucalopride is a highly effective prokinetic agent that can stimulate GI motility and transit throughout the length of the GI tract. (112;113) A single oral dose of prucalopride (1 or 2 mg) was able to shorten oro-
coecal and whole-gut transit time in healthy volunteers. (114) Furthermore, this dosage significantly increased stool frequency and decreased stool consistency. More importantly, the same positive result was found in adults with chronic idiopathic constipation. Prucalopride was not only able to improve colonic transit in the subset of patients with slow transit but also increased stool frequency in all patients, irrespective of their transit status at baseline.(114;115) In children, studies with this newly developed agent have yet to be performed.

**Follow-up and prognosis**

Initial treatment with lactulose or PEG laxatives results in complete remission of symptoms in 40% and 70% of constipated children, respectively, after 2 weeks of treatment.(116) After 2 months of treatment with PEG, 56% of patients were successfully treated according to strict criteria.(66) Furthermore, at one year follow-up treatment success was reported in 62% of children with chronic constipation. (117) Two studies, including 62 and 137 children with constipation reported that 50-66% of the patients were symptom free at 5 years after diagnosis with intensive medical and behavioural therapy. (118;119) In a larger study population (n=401) with 11 years follow-up, 25% continued to have symptoms of constipation at adult age.(120) These data illustrate that development of other treatment options, new compounds, probiotics and other alternative therapies, are required.(121) The importance of treating functional constipation adequately is especially emphasized by the finding that a quarter of those children continue to have symptoms at adult age. Also, poor clinical outcome is associated with longer delay between age of onset and age of first visit to a specialized centre.(120) Therefore, referral to a specialized clinic should be considered at an early stage for children unresponsive to first line treatment. Furthermore, in a long-term follow-up cohort, a cross-sectional study showed that compared to controls, quality and course of life were delayed in adults with a history of childhood constipation. These patients achieved fewer milestones with respect to autonomy and social development, had lower educational levels and a higher unemployment rate. (120) In general, functional constipation is often regarded as mild but new insights now illustrate the contrary and make clear that this condition is not a self-limiting entity.
SUMMARY AND AIM OF THE THESIS

Functional constipation is a common problem where research is still challenging. Especially since multiple aetiological aspects such as chloride secretion, need to be further elucidated. In addition, new treatment options such as probiotics, should be revised not only to improve symptoms but also to improve overall prognosis. In this thesis multiple pathophysiological aspects as well as treatment options are further studied in children presenting with chronic functional constipation.
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General introduction


General introduction


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Outline of the thesis
INTRODUCTION

Ninety five percent of healthy term born infants pass meconium stool within 24 hours after birth. Delayed first meconium stool passage is an important symptom to differentiate functional constipation from Hirschsprung’s disease. The first part of this thesis focuses on first meconium stool passage as well as stool characteristics and bowel habits in otherwise healthy term and preterm born infants.

The pathophysiology of functional constipation is undoubtedly multi-factorial, and not well understood. In more than 90% of all age groups no obvious cause can be identified. The second part of the thesis discusses new insights into pathophysiological mechanisms of constipation.

The treatment of children with constipation is mainly based on clinical experience and usually consists of a behavioural strategy with oral and sometimes rectal laxatives. The treatment of chronic constipation in childhood is based on 4 important phases: 1) education, 2) disimpaction, 3) prevention of re-accumulation of faeces and 4) follow up. However, studies comparing initial disimpaction with oral laxatives or with rectal enemas are lacking. In the third part of this thesis the outcome of such comparison will be described. It is well-know that 50% of children with constipation still have symptoms, such as infrequent defecation and faecal incontinence, after 5 years of intensive medical or behavioural treatment. The duration of studies evaluating the efficacy of laxatives in these children however is only 8 weeks. Therefore we designed a prospective randomized controlled trial evaluating the long term efficacy (1 year), tolerability and safety of two different polyethylene glycol compounds. In the second part of part 3 the results of this maintenance study are discussed.

AIM

Part I - stool characteristics and bowel habits- aims to describe factors responsible for different bowel habit aspects, such as stool frequency and consistency, between preterm and term born infants. Part II -pathophysiology- aims at elucidating possible underlying pathophysiological mechanisms for children presenting with functional constipation whereas part III -therapy- discusses therapeutic options.

Part I  Stool characteristics and bowel habits

Meconium, the first stool excreted, is a dark viscous substance composed of different minerals. It is known that first passage of meconium is often delayed in premature
born infants. Difference in duration of meconium stool passage between preterm and term born infants had yet to be determined. We therefore performed a study, in chapter 1, in which we prospectively collected data about duration (in days) of meconium stool passage. Furthermore, we evaluated the effects of other factors with respect to duration of meconium stool passage, such as birth weight and morphine therapy. During this study, photographs of stools of premature and term born infants were taken in order to create a stool form scale which was lacking in infants. In adults such a stool form is available, entitled “the Bristol stool form scale” and utilized as a clinical tool to evaluate stool consistency and form. This easy-to-use, accessible system to quantify stool consistency and form is an important asset, particularly one allowing an understanding of the relationship between stool characteristics and defecation-related patient complaints. Furthermore the Bristol scale has been valuable in epidemiological studies and clinical trials. Because in infants and children such a scale was lacking, we developed a new infant stool form scale including stool amount, consistency and colour in chapter 2. In order to prospectively compare bowel habits such as defecation frequency and gastrointestinal complaints between preterm and term born infants, this cohort of children was followed and evaluated at regular time points starting at the first day of life until they were 2 years of age, chapter 3.

Part II Pathophysiology

In children with constipation no organic aetiological factors are found in more than 90% and therefore constipation is considered to be a functional disorder. Nevertheless, experiencing painful defecation is suggested to be the most common cause of childhood constipation. Also, different factors such as lumbar spine disorders, morphological changes in colonic epithelium and homeostasis of water and electrolytes by chloride secretion are suggested to play a role in developing constipation. Indeed, the chloride channel stimulator lubiprostone was effective in adults with constipation, significantly increasing defecation frequency and improving consistency of stools. However, so far no studies were performed to assess whether disturbance in chloride secretion in the colonic epithelium plays a role in the development of chronic constipation. In order to answer this question, chapter 4, describes the results of a study in which we evaluated chloride secretion in the colonic mucosa of constipated children and controls.

In patients suspected of Hirschsprung’s disease (HD) and in children with treatment resistant constipation colonic mucosa and submucosa are often investigated. Interestingly, only in children with therapy resistant constipation the rectal mucosal muscle is frequently reported as hypertrophied and disorganised. Whether there is a difference in histological aspect of the rectal mucosa between HD and therapy resistant constipated children is unknown. Therefore, we evaluated the difference
in thickness and organisation of the rectal mucosal muscle in patients with HD, constipated children and controls in chapter 5. Another aetiological factor associated with constipation is lumbar spinal disease. One study showed high prevalence (9%) of lumbar spinal cord abnormalities in children with intractable constipation. Moreover, neurosurgery relieved symptoms in 86% of these children. However, the latter study was retrospectively conducted in only those who were refractory to laxative treatment and opening their bowels < 3 times per week.

To assess the prevalence of lumbar spinal cord abnormalities in a prospective manner in children presenting with functional defecation disorders, such as functional non-retentive faecal incontinence and functional constipation, we conducted a clinical study. The results of the latter study are described in chapter 6. Additionally, to evaluate the effect of conservative treatment prior to neurosurgery, in all participating children with and without lumbar spinal cord abnormalities follow-up was carried out for each patient up to 12 weeks.

Part III Therapy

For more than 20 years, we initially treat children with faecal impaction with enemas aiming to clear the rectal faecal mass and to subsequently reduce faecal incontinence episodes. One study showed that high doses of oral polyethylene glycol (PEG) 1.5 g/kg/day results in a 100% disimpaction rate. Studies comparing disimpaction with oral PEG versus enemas are not performed and therefore the question whether oral therapy is superior to treatment with enemas is to be answered. To do so, we performed a randomised controlled trial, in chapter 7, comparing disimpaction with enemas to disimpaction with high doses of oral PEG. After disimpaction, maintenance therapy with oral laxatives is mandatory. An alternative to oral laxatives is treatment with probiotics. Probiotics are live microorganisms which confer a beneficial health effect on the host, which are described to be beneficial in gastrointestinal disease such as constipation. In children, one study showed no adjunctive effect of the probiotic strain lactobacillus GG when added to lactulose. To date, no studies are performed comparing a mixture of probiotics as a treatment compound for constipated children. Therefore, we assessed the effect of a mixture of probiotics containing lactobacilli and bifidobacteria on constipation symptoms during 4 weeks of treatment in chapter 8.
PART

Stool characteristics and bowel habits
CHAPTER 1

Duration of Meconium Passage in Pre- and Term Born Infants

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ABSTRACT

Background
First passage of stool after birth, meconium, is delayed in preterm infants compared to term infants. The difference in duration of meconium passage until transition to normal stools has however never been assessed in preterm and term infants.

Hypothesis
Preterm infants have prolonged duration of passage of meconium (PoM) compared to term infants.

Methods
Between August and November 2006, all infants born in an academic and a non-academic hospital with gestational age (GA) 25 - 42 weeks and without metabolic, congenital diseases or gastrointestinal disorders, were included. Infants were divided into four groups: A) GA ≤ 30 wks; B) 31 ≥ GA ≤ 34; C) 35 ≥ GA ≤ 36; D) GA ≥ 37 (term born).

Results
A total of 198 infants (102 males); 32, 62, 33 and 71 infants in group A, B, C and D, respectively, were included. With decreasing gestation a trend was found for delayed first PoM (\(p<0.001\)). Compared to term infants 79\% (56/71), less preterm infants passed their first stool within 24 hours after birth; group A; 44\% (14/32), B; 68\% (42/62) and C; 73\% (24/33).

With decreasing gestation a trend for prolonged PoM was found (\(p<0.001\)). The mean PoM duration was prolonged in group A; 7.8 days (±2.5), B; 4.3 days (±2.4) and C; 2.9 days (±1.3) compared to term infants. Furthermore, PoM was associated with birth weights ≤ 2500 grams (\(p=0.03\)) and morphine therapy (\(p=0.03\)). Duration of PoM was not associated with type of feeding, SGA, LGA or need for respiratory support.

Conclusion
PoM was not only delayed but also prolonged in preterm infants. Duration of PoM was associated with gestational age, birth weight and morphine therapy.
INTRODUCTION

More than 99% of term infants pass their first stool, meconium, within 48 hours after birth.\textsuperscript{1, 2} It is well-known, however, that preterm infants have a delayed passage of their first stools.\textsuperscript{1, 2} Most likely, this delay is due to ongoing developmental maturation of bowel function which results in intestinal hypomotility.\textsuperscript{3} We therefore hypothesise that the phase of passing meconium stools before its transit to normal stools is prolonged in preterm infants.

METHODS

Between August and November 2006, all infants born in the departments of obstetrics of the Emma Children’s Hospital of the Academic Medical Centre of Amsterdam and a non-academic hospital (OLVG) in Amsterdam with a gestational age (GA) between 25 - 42 weeks and without metabolic, congenital diseases or gastrointestinal disorders requiring surgery were eligible for this study. Infants who died during their hospital stay and infants transferred to other hospitals were excluded. Using standardised daily bowel diaries, bowel habits were recorded from the first day of life until at least the first 2 weeks after birth. Stools were classified as meconium or not-meconium, based on consistency and colour. Meconium is characterised as thick, sticky, and greenish-black in colour. Type of feeding, morphine therapy as well as need for respiratory support were documented for each infant. Infants were divided into 4 groups, based on their GA: A) GA ≤ 30 wks; B) GA between 31-34 weeks; C) GA between 35-36 weeks; D) GA ≥ 37 weeks (term born). Infants were small for gestational age (SGA) if birth weight was lower than 10th percentile and large for gestational age (LGA) if their birth weight exceeded the 90th percentile. Birth weights between the 10th and 90th percentile were considered normal. Birth weights were divided into four categories from the 25th to the 75th percentile of birth weights of this study group. Passage of meconium (PoM) stools in days exceeding the 75th percentile in term infants was considered as prolonged.

STATISTICAL ANALYSES

All data were collected in an SPSS file (Inc 14.0.1). Baseline characteristics were given in mean values (SD). A chi-square test for trend was used to examine whether a relation existed between gestational age (four groups) and the proportion of patients having their first meconium passage within 24 and 48 hours respectively. Differences in mean PoM time between gestational age groups were analyzed using an ANOVA test for linear trend.
In a multivariable linear regression model we examined the independent effect of the following factors on duration of PoM: gestational age (continuous), birth weight (categorical), SGA, LGA, administration of morphine in the first week of life (yes/no) and the need for respiratory support (yes/no). Values of \( p < 0.05 \) were considered statistically significant.

**RESULTS**

A total of 198 infants (102 males) were included with the following mean gestational age distribution: group A (n=32) with mean GA 38.0 (±1.4) weeks; group B (n=62) 32.6 (±1.1) weeks; group C (n=33) 35.4 (±0.5) weeks and group D (n=71) 39.3 (±1.5) weeks.

With decreasing gestation a trend was found for delayed first PoM within 24 hours (\( p < 0.001 \)) as well as within 48 hours (\( p = 0.003 \)). Passage of meconium within 24 hours after birth occurred in 79% (56/71) of the term infants (table 1). First PoM within 48 hours occurred in 83% (59/71) of term infants. Furthermore, first PoM was delayed in LGA infants but not in SGA infants compared to infants with a normal birth weight (\( p = 0.03 \)).

A significant trend (\( p < 0.001 \)) was found between decreasing gestation and prolonged PoM (figure 1). A total of 26 infants in group A, 17 in group B, 2 in group C and 2 in group D had prolonged PoM.

A total of 25 infants received morphine, 13, 9, 2 and 1 infant(s) from respectively group A, B, C and D. Mean administration of morphine was 1.41 (±2.3) days in group A,

*Table 1:* Baseline characteristics and first & duration of passage of meconium (PoM). TPN: total parenteral nutrition

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>( p )-value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age</td>
<td>≤ 30</td>
<td>31-34</td>
<td>35-36</td>
<td>≥ 37</td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>32</td>
<td>62</td>
<td>33</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Median Birth weight (min.-max.)</td>
<td>1015 (660-1500)</td>
<td>1863 (1100-3560)</td>
<td>2552 (1550-3670)</td>
<td>3474 (1875-4900)</td>
<td></td>
</tr>
<tr>
<td>Type of feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- number breastfed</td>
<td>20</td>
<td>33</td>
<td>14</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>- number formula fed</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>- number combination</td>
<td>4</td>
<td>17</td>
<td>8</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>- number TPN</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>First PoM within 24 hrs</td>
<td>44% (14/32)</td>
<td>67% (42/62)</td>
<td>73% (24/33)</td>
<td>78% (56/71)</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>First PoM within 48 hrs</td>
<td>75% (24/32)</td>
<td>84% (52/62)</td>
<td>76% (31/41)</td>
<td>83% (59/71)</td>
<td>( p = 0.003 )</td>
</tr>
<tr>
<td>Mean duration PoM (days, SD)</td>
<td>7.8 (2.5)</td>
<td>4.3 (2.4)</td>
<td>2.9 (1.3)</td>
<td>2.0 (1.3)</td>
<td>( p &lt; 0.001 )</td>
</tr>
</tbody>
</table>

*\( p \)-values for trend between gestational age and first (<24 hours & <48 hours) and duration of PoM.
0.52 (±1.50) in group B, 0.15 (±0.61) days in group C and 0.04 (±0.36) days in group D. Morphine therapy prolonged duration of PoM by 3.5 days (p<0.001; table 2).

All infants in group A needed respiratory support for 5.7 (±1.7) days in the first week of life. Thirty, 10 and 3 infants from respectively group B, C and D needed respiratory support for respectively 1.2 (±1.8), 0.5 (±1.0) and 0.1 (±0.4) days. Duration of PoM was delayed by 3.0 days for every day of respiratory support (p<0.001; table 2).

Type of feeding was not different between the groups (table 1). Breastfeeding and formula feeding were not associated with duration of PoM (table 2). Total parenteral nutrition, however delayed PoM by 2.2 days (p=0.004).

The prescription of enemas was significantly higher in group A and B compared to group C (p<0.04) and D (p<0.001). In the period of PoM, 16, 11 and 1 infant(s) from respectively group A, B and C and none from group D were prescribed enemas. Infants with longer duration of PoM received more enemas (p<0.001).

**Figure 1.** Correlation between gestational age in weeks and mean (±SEM) duration of meconium passage (PoM) in days.
Chapter 1

We applied a multivariate analysis to describe the effect of factors described above. On average, duration of PoM was delayed by 0.35 days for each week of prematurity, by 1.2 days when birth weights ≤2500 grams and 1.0 day if having received morphine therapy. Furthermore, the duration of PoM was prolonged by 1.36 days in infants small for gestational age (table 2).

DISCUSSION

This study shows that prematurity is associated with prolonged passage of meconium (PoM) when compared to term infants. Furthermore our data show that duration of PoM was influenced not only by GA but also by birth weight, SGA and the number of days needing respiratory support.

In accordance with earlier studies, more than 98% of term infants pass their first meconium stool within 48 hours.\textsuperscript{1,2} Immature motility of the gastrointestinal tract of premature infants may play a role in delaying first PoM as well as in prolonging PoM stools. Earlier studies in preterm infants showed impaired gastric electrical activity and gastric emptying, characterised by more frequent clustered phasic contractions.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Change in PoM time univariate model (95%CI)</th>
<th>p-value</th>
<th>Change in PoM time multivariate model (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (per week)</td>
<td>-0.42 (-0.49 to -0.35)</td>
<td>&lt;0.001</td>
<td>-0.35 (-0.50 to -0.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weights, 4 categories</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1: ≤1500 grams</td>
<td>reference</td>
<td></td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>- 2: 1500-2500 grams</td>
<td>-4.19 (-5.10 to -3.27)</td>
<td>&lt;0.001</td>
<td>-1.17 (-2.19 to -1.15)</td>
<td>0.03</td>
</tr>
<tr>
<td>- 3: 2500-3500 grams</td>
<td>-4.52 (-5.36 to -3.67)</td>
<td>&lt;0.001</td>
<td>-0.59 (-2.13 to 0.95)</td>
<td>0.45</td>
</tr>
<tr>
<td>- 4: &gt;3500 grams</td>
<td>-4.19 (-5.10 to -3.27)</td>
<td>&lt;0.001</td>
<td>0.93 (-1.15 to 3.00)</td>
<td>0.38</td>
</tr>
<tr>
<td>Birth weight adjusted for GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Normal for GA</td>
<td>reference</td>
<td></td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>- SGA</td>
<td>1.26 (0.39 to 2.14)</td>
<td>0.01</td>
<td>0.38 (-0.19 to 1.30)</td>
<td>0.14</td>
</tr>
<tr>
<td>- LGA</td>
<td>-0.82 (-2.01 to 0.37)</td>
<td>0.18</td>
<td>0.56 (-1.58 to 0.64)</td>
<td>0.40</td>
</tr>
<tr>
<td>Type of feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Breastfeeding</td>
<td>-0.88 (-1.97 to 0.20)</td>
<td>0.11</td>
<td>-0.32 (-0.99 to 0.36)</td>
<td>0.35</td>
</tr>
<tr>
<td>- Formula feeding</td>
<td>-0.30 (-1.21 to 0.61)</td>
<td>0.51</td>
<td>-0.17 (-1.04 to 0.69)</td>
<td>0.69</td>
</tr>
<tr>
<td>- Combination breast &amp; formula</td>
<td>1.91 (0.53 to 3.29)</td>
<td>0.01</td>
<td>0.37 (-0.75 to 1.49)</td>
<td>0.51</td>
</tr>
<tr>
<td>- TPN</td>
<td>reference</td>
<td></td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>Morphine therapy (yes/no)</td>
<td>-3.54 (-2.53 to -4.55)</td>
<td>&lt;0.001</td>
<td>-1.01 (0.11 to 1.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Respiratory support (yes/no)</td>
<td>-3.01 (-3.66 to -2.37)</td>
<td>&lt;0.001</td>
<td>0.70 (-1.46 to 0.52)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

GA, gestational age; LGA, large for gestational age; PoM, passage of meconium; SGA, small for gestational age; TPN, total parenteral nutrition
but of shorter duration and lower amplitude.\textsuperscript{4-7} Furthermore, manometric investigations of small intestinal motility demonstrated that duodenal clusters were less common and antroduodenal coordination was more immature in preterm infants compared to term infants.\textsuperscript{5, 8, 9} Little information is available regarding the prenatal development of colonic motility. One study using amniography, showed that progression of contrast from mouth to colon took 9 hours at 32 weeks of gestational age but only half that time at term.\textsuperscript{10} Recently, de Lorijn et al showed that preterm infants (GA 28-32 weeks) with delayed first PoM all exhibited a normal rectoanal inhibitory reflex. Moreover, all other anorectal motility parameters were similar to preterm infants of the same age who passed meconium within 48 hours.\textsuperscript{11, 12} These latter studies suggest that either mechanisms of rectal propulsion are impaired, leading to failure of normal expulsion of the meconium plug, or that the meconium plug itself is too difficult to expel as a result of its consistency. Indeed it is well-known that meconium of preterm infants differs in composition (glycoprotein, saccharides, calcium, copper, iron and phosphorus) from that of term infants, making it thicker in consistency and more difficult to expel.\textsuperscript{13}

In our study duration of PoM was associated with gestational age, birth weights <2500 grams and with SGA. In contrast to other studies in which SGA infants had delayed first PoM, we only found prolonged PoM (univariate model) but not delayed first PoM in those infants.\textsuperscript{1, 2} LGA infants, however had delayed first passage of PoM compared to infants with normal birth weight, but duration of PoM was not more prolonged in these infants. A possible explanation is that 80% of LGA infants born from mothers without diabetes suffer from hypoglycaemia 1 hour after birth.\textsuperscript{14} This may suggest higher circulating glucose levels during the prenatal period of LGA infants with consequences for gastro-intestinal tract motility. For the colon, hyperglycaemia blunts mechanoreceptor-mediated gastrocolonic responses and ascending contractions of the peristaltic reflex, not caused by hyperinsulinaemia or direct muscle actions.\textsuperscript{15} As we only found delayed first passage but not prolonged PoM, the effect of prenatal hyperglycaemia which disappears after birth, could be well applied for explaining a delayed but not prolonged PoM in LGA infants. Nevertheless, other studies show a rather enhanced or equal colonic motility in respectively diabetic rat models or humans.\textsuperscript{16, 17}

As expected morphine therapy was associated with prolonged duration of PoM. In the central nervous system, opiates, such as morphine act at opioid receptors slowing intestinal transit and inhibiting secretion of fluids in the intestinal lumen.\textsuperscript{18-21} This may explain prolonged PoM in infants receiving morphine therapy as we found in our study.

Interestingly, more enemas were prescribed for those with longer duration of PoM. However, the observational character of this study makes it impossible to draw firm conclusions of this finding. A randomised controlled trial is needed to evaluate the effect of enemas on PoM. Based on the findings of this study, it
remains questionable whether therapeutic intervention is necessary. Moreover, it is unclear whether intervention can indeed hasten passage of meconium stools in infants with delayed PoM.

In summary, passage of meconium stools was delayed and prolonged in preterm infants compared to term infants. Prolonged passage of meconium was associated with gestational age, birth weight ≤ 2500 grams and morphine therapy. Type of feeding, small for gestational age and the number of days needing respiratory support did not delay passage of meconium stools.
REFERENCE LIST

Infant Stool Form Scale: development and results

Noor Bakkali
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Letty van Toledo
Marc Benninga
ABSTRACT

Background
For infants an instrument describing stool characteristics has yet to be developed.

Aim
To develop an infant stool scale describing consistency, amount and colour and test its usefulness by assessing the differences between term and preterm infants, between breastfed and formula fed infants and examining interobserver and intraobserver variability.

Methods
Information about gestational age, postnatal age, and feeding type was collected in relation to each photograph taken. An infant stool form scale describing consistency (4-point scale), amount (4-point scale) and colour (6 categories) was developed. All photographs were scored twice with the newly developed scale to assess interobserver and intraobserver variability. Consensus database describing stool characteristics was developed.

Results
A total of 555 photographs of infant stools were analysed. Sixty (11%) of the infants were term and 495 (89%) were born prematurely. No differences were found in stool characteristics between preterm and term infants. Breastfed infants had smaller amounts of stools compared with formula-fed infants (p<0.001).

The interobserver weighed Κ value (95% CI) was good for consistency and amount; simple Κ value was good for colour. For observers I and II, intraobserver Κ values were excellent.

Conclusion
This “Amsterdam” stool form scale is useful to assess defecation patterns in both premature and term born infants.
INTRODUCTION

To date, a stool form scale describing stool characteristics such as consistency, amount and colour of faeces in infants has yet to be developed. In adults, the Bristol Stool Form Scale (BSFS) appears to be a reliable tool for describing and classifying stool appearance. It consists of a 7-point scale in which stool consistency together with form of stool are described for every point in this scale. For example, score 1 describes stools that are hard lumps, like nuts (hard to pass) while score 4 describes stools like a sausage or snake, smooth and soft. The BSFS is used in clinical practice to monitor change in intestinal function. Higher stool water content was associated with more rapid gastrointestinal transit and higher scores on the BSFS. The converse was true for stool with less water content. More importantly the BSFS has proved acceptable both to subjects in epidemiological surveys and to patients attending gastrointestinal clinics for measuring their stool form. Furthermore, it has been suggested to use this scale in research to prospectively assess stool form to discriminate between patients with functional defecation disorders such as irritable bowel syndrome, diarrhoea and constipation.

A comparable scale for infants is lacking, but it would help both parents and clinicians in describing and differentiating between physiological and pathological stool appearance. Therefore the aim of this study was to develop an infant stool form scale and test its usefulness by assessing the difference in stool characteristics between term and preterm born infants and between breastfed (BF) and formula-fed (FF) infants and examining interobserver and intraobserver variability.

METHODS

Scale development

Daily digital photographs were taken from all stools of preterm and term infants during their hospital stay in an academic and nonacademic hospital in Amsterdam the Netherlands, between August and October 2006. Stools of otherwise healthy infants without metabolic, congenital diseases or gastrointestinal disorders requiring surgery were photographed. Photographs were taken at daytime by 2 researchers with a digital camera (zoom lens, original magnification x4 and 7.2 megapixels) while positioning the diaper a distance of 20 cm from the digital camera. The macro function of the digital camera was applied for each photograph. To be able to take pictures of fresh stool, nurses informed researchers every 4 hours about the production of faeces in diapers of all admitted infants during daytime. This pool of stool photographs was evaluated by 2 observers, a medical student (I) and a medical doctor (II). The choice of characteristics to include in the stool
scale was determined by face validity (by examining the items, the scale should measure what it should measure). In a face-to-face meeting the observers reached consensus on 4 typical photographs for describing the consistency as watery, soft, formed and hard. For describing the amount of stool each diaper was divided into 9 areas; the middle area (99cm²) was the reference surface area (Figure 1). Then 4 typical photographs were chosen upon consensus by the 2 observers to describe the amount into: ‘smear’, <25%, 25-50% and >50% of faeces in the reference area. To classify colour, 6 photographs were chosen illustrating the colours yellow, orange, green, brown, meconium and clay-coloured. These typical photographs (n=14 pictures) describing the different categories of consistency, amount and colour were used as visual anchor points in the newly developed infant stool form scale (Figure 2).

**Interobserver and intraobserver variation**

With this newly developed stool scale, all photographs were then scored in random order to assess interobserver variability for the items consistency, amount and colour. The 2 observers scored the pictures independently from each other. Photographs scored by the observers contained information about the age of the infant (gestational age [GA] and age in days after birth) and type of feeding. This information was used to further analyse stool characteristics in relation to age and type of feeding. After 3 months, the same photographs were scored a second time by the same 2 observers to assess intraobserver variability.

**Differences in stool characteristics by gestational age and type of feeding**

For these analyses, a consensus reading for all stool aspects was constructed based on consensus classification by the 2 observers and described in Table 1.
Table 1. The scored items consistency, amount and color in relation to GA

<table>
<thead>
<tr>
<th>Stool characteristics</th>
<th>GA&lt;=28</th>
<th>29=&gt;GA&lt;34</th>
<th>34=&gt;GA&lt;37</th>
<th>GA&gt;=37</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watery</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Soft</td>
<td>98</td>
<td>134</td>
<td>67</td>
<td>37</td>
<td>336</td>
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<tr>
<td>Formed</td>
<td>55</td>
<td>71</td>
<td>33</td>
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<td>179</td>
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<tr>
<td>Hard</td>
<td>9</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>Amount</td>
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<td></td>
</tr>
<tr>
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<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Up to 25%</td>
<td>72</td>
<td>87</td>
<td>55</td>
<td>25</td>
<td>239</td>
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<tr>
<td>25-50%</td>
<td>73</td>
<td>99</td>
<td>38</td>
<td>27</td>
<td>237</td>
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<tr>
<td>&gt;50% of reference area</td>
<td>18</td>
<td>27</td>
<td>19</td>
<td>8</td>
<td>72</td>
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<tr>
<td>Colour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yellow</td>
<td>81</td>
<td>96</td>
<td>45</td>
<td>21</td>
<td>243</td>
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<tr>
<td>Brown</td>
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</tr>
<tr>
<td>Green</td>
<td>10</td>
<td>9</td>
<td>3</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>Orange</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>Meconium</td>
<td>26</td>
<td>28</td>
<td>13</td>
<td>4</td>
<td>71</td>
</tr>
<tr>
<td>Clay coloured</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

Premature infants were divided into 3 groups according to their GA in weeks; group 1: GA ≤ 28 weeks; group 2: 29³GA≤32 and group 3: 33³GA≤36. For evaluation of differences in stool characteristics between young and older infants three age groups were selected based on the percentiles of the total age (in days after birth) of all participating infants. Group I were infants of 15 days and younger (25th percentile), group II consisted of infants older than 15 days and younger than 30 days (25-75th percentile) and age groups III (≥75th percentile) were infants 30 days and older.

The study was approved by the Medical Ethical Committee of the Academic Medical Centre of Amsterdam.

STATISTICAL ANALYSES

The number of photographs of stools and infant characteristics like gestational age, age in days after birth and type of feeding were analysed in a descriptive way. The interobserver and intraobserver variability were evaluated by calculating the proportion of exact agreement and the kappa statistics for nominal data (colour) and weighted kappa’s (Fleiss & Cohen) for items in which there is a natural ordering of categories (consistency and amount). Agreement, based on the value of kappa (k), was categorized, as described by Altman, as poor (k≤0.2), fair (0.21≤k≤0.40), moderate (0.41≤k≤0.60), good (0.61≤k≤0.80) or excellent (0.81≤k≤1.00).
The Mann-Whitney test was used to examine differences in stool consistency, amount and colour between premature and term born infants and between breastfed (BF) and formula fed (FF) infants. Ordinal regression analyses were performed to evaluate the difference in stool consistency; amount and colour (when ranged ordinal from dark to light colours) with time divided over 3 age groups. All analyses were performed using SPSS statistical software (SPSS Inc.14.0.2). All p-values less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics
A total of 907 digital photographs of infant stools were taken of which 555 could be analysed. The other photographs (n=442) were duplicates or of poor quality and were therefore not useful for further analysis. Images of 555 stools of infants with a median GA of 31 weeks (range: 25-42) and with a median age of 15 days after birth (range: 1-120) were analysed (figure 3 and 4). Sixty of the participating infants (11%) were term born. Of the premature born infants 166 (30%) were born ≤ 28 weeks of gestation; 217 (39%) between 29 and 32 weeks and 112 (20%) between 33 and 36 weeks. One hundred and six infants (19%) received BF and 237 (43%) were FF. Two hundred and five infants (37%) were simultaneously breastfed and formula fed. Seven infants (1%) did not receive enteral feeding.

Infant Stool Form Scale
The final infant stool form scale describing consistency (ordered categories), amount (ordered categories) and colour together with typical photographs illustrating each category is given in figure 2.

Interobserver and intraobserver variation
The proportion of photographs in which the exact same category was assigned by the 2 observers was 78% for consistency, 71% for amount and 68% for colour. The proportion of photographs in which the category assignment differed in more than 2 categories between the observers was 0% for consistency and amount; and 0.35% for colour.

The interobserver weighed k value (95% CI) was 0.68 (0.62 to 0.74) for consistency, 0.74 (0.69 to 0.78) for amount and simple k value of 0.75 (0.70 to 0.79) for colour. After 3 months, the observers scored the same photographs again showing an excellent intraobserver agreement. The intraobserver weighed k value was 0.84 (0.75 to 0.93) and 0.89 (0.83 to 0.94) for respectively consistency and amount; and
Figure 2. The newly developed infant stool form scale
simple k value of 0.85 (0.79 to 0.91) for colour for observer I, whereas observer II had a weighed k value of 0.90 (0.87 to 0.93) and 0.94 (0.92 to 0.96) for respectively consistency and amount; and simple k value 0.92 (0.90 to 0.95) for colour.

**Stool characteristics in relation to gestational age**

No differences in consistency (p=0.27), amount (p=0.68) and colour (p=0.25) were found between preterm and term born infants. Furthermore, no difference in stool characteristics was found between the premature infants from different gestational age groups (table 1).

**Stool characteristics in relation to type of feeding**

Stool consistency differences between BF and FF infants did not reach statistical significance (p=0.07). However, amount of stools produced by FF infants was

**Table 2. Amount of stools of BF infants and FF infants**

<table>
<thead>
<tr>
<th>Consistency</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>BF</td>
<td>6 (6)</td>
</tr>
<tr>
<td>FF</td>
<td>4 (2)</td>
</tr>
<tr>
<td>BF+FF</td>
<td>3 (2)</td>
</tr>
<tr>
<td>NEF</td>
<td>1 (14)</td>
</tr>
</tbody>
</table>

**Figure 3.** Number of stool images in relation to the age in days after birth.

**Figure 4.** Distribution of GA of participating infants.
significantly larger compared to the BF infants (p<0.001) (table 2). Colour was not different between BF and FF infants (p=0.43).

**Impact of ageing on stool characteristics**

With increasing age, consistency of stools changed into harder stools (p<0.001). Sixty six percent of infants younger than 16 days (group I) had soft stools compared to 54% from group II and 58% from group III. Formed stools were found in only 26% of infants from age group I compared to 41% and 35% in respectively age groups II and III.

Furthermore, with increasing age the amount of stools alters into larger stools (p<0.001). Larger stools (amounts III and IV) were found in 47% of infants from group I compared with 60% and 71% of infants in age group III and IV (p<0.001).

In addition, the colour of stool changed with age (p<0.001) as well. The most common colour found in age group I was brown (38%) and meconium (28%), compared to yellow (29%) and brown (60%) in group II and yellow (50%) and brown (37%) in group III.

**DISCUSSION**

On the basis of the analysis of more than 500 digital infant stool photographs, we were able to develop the first stool form scale for premature and term born infants aged up to 120 days after birth. Validity of this scale was supported by the good to excellent interobserver and intraobserver agreement scores. With this stool scale, no difference was found in stool characteristics between preterm and term born infants. Significant differences in stool amount were found between BF and FF infants, but not in consistency and colour.

A Japanese chart for infant and children’s stools describing stools in gastrointestinal conditions such as diarrhoea and biliary atresia exists. However, these photographs were mainly based on only 1 patient per condition. Furthermore, we evaluated the amount of stool by determining the percentage diaper surface filled
An infant stool form scale should at least include consistency, amount and colour. For consistency, we chose watery, soft, formed and hard as physicians and parents describe infant stool consistencies in these terms. For describing amount, we chose for example smear (score 1) as indeed parents usually describe this phenomenon in their constipated child who is failing to defecate. By only describing amount and consistency the scale would still lack information about stool colours. Stool colours such as green might concern parents and therefore visualising those colours in the scales would reassure them. By coverage of those 3 stool aspects (consistency, amount & colour), we aimed for content validity of our stool scale. Content validity means whether the scale covers all aspects which have to be measured. Furthermore, we found good interobserver and excellent intraobserver agreement using this infant stool form scale. The agreement between the 2 observers was good for all items. In several studies from other disciplines evaluating interobserver variability, in clinically applicable tools, for example, interpretation of mammograms or assessment of carotid plaques, moderate to good agreement findings are found acceptable. Davies et al reported a close correlation between subject reported and an independent observer reported stool form evaluation \( (r = 0.93) \). These preliminary results indicate that this scale could be a useful addition in daily practice to monitor changes in stool characteristics.

In contrast to other studies, in which breastfed infants had mainly pasty and larger stools compared to stools of formula fed infants, we did not find significant differences in stool consistency between those 2 groups. Because 90% of the infants were premature born, an immature colon with yet lower water holding capacity may have contributed to similar consistencies of stools of both BF and FF infants. Another possible explanation might be the supplementation of galacto- and fructo-oligosaccharides in formula feeding which are currently commonly added to infant formula. Supplementation of the latter products may result in looser stool consistency comparable to BF infants. Unfortunately, we did not collect data concerning the exact type of formula feeding.
In contrast to the findings of Weaver et al., we found that BF infants passed more frequent and smaller amounts of stools compared to FF infants. A possible explanation might be that we defined amount by using an infant stool form scale based on pictures of infant stools in a diaper rather than the commonly used 3-dimensional illustration model. Furthermore, Weaver et al. used a 3-points scale recorded by nurses while we used a 4-points scale scored from diaper pictures making those results difficult to compare. Because BF infants defecate more than FF infants one would expect those groups to produce comparable total amounts of stool per day as they ingest comparable amount of feeding (ml) per day. Consequently, BF infants would produce smaller amounts of stools as they defecate more frequently.

Comparable to other studies, with aging the frequency of harder stools increased, reflecting maturation of the water conserving capacity of the gut. This is illustrated by rat models where permeability of the colon, not only to water and electrolytes, is increased in the weaning compared to the adult rats.

One limitation of our study is that our newly developed scale does not have criterion validity for consistency and amount as we described those characteristics upon consent between 2 observers. The observers chose typical pictures for each type of consistency based on appearance. Furthermore, and in contrast with adult studies we were not able to correlate colonic transit time with our infant stool form scale. The medical ethical board of our hospital, however, gave no permission to use carmine red to evaluate colonic transit times in premature infants. Further studies using this new tool are needed to confirm our findings and to relate colonic transit times to stool characteristics in infants.

In conclusion, the newly developed “Amsterdam” infant stool scale enables parents and clinicians to reliably rate different aspects of stools, such as consistency, amount and colour of premature and term infants. This scale might be helpful in differentiating between normal and abnormal defecation patterns in infants. Therefore, future studies are necessary validating the applicability and validity of this scale for practical and research purposes.

ACKNOWLEDGMENTS

The authors would like to thank Paolo Valerio, MD, who assisted in the acquisition of data and Chris Bor, who made possible the realisation of the layout of the newly developed stool scale.
REFERENCE LIST


Bowel habits in the first 24 months of life: Preterm versus term born infants

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Fleur Moesker
Letty van Toledo
Johannes Reitsma
Sofie Hamers
Paolo Valerio
Marc Benninga

Submitted
Chapter 3

ABSTRACT

Background
Prospective studies concerning feeding and bowel habits of term and preterm infants from birth up to 24 months of life are lacking.

Aim
The aim of this study was to describe and compare 1) feeding habits and 2) bowel habits between term and preterm born infants starting from birth up to the age of 24 months.

Methods
Between August and November 2006 all infants admitted to an academic and non-academic neonatal care unit, with gestational age 25-42 weeks participated. Bowel diaries were recorded 1 and 2 weeks, and 3, 6, 12 and 24 months after birth. Infants with gastrointestinal surgery, neurological diseases, metabolic diseases or congenital abnormalities were excluded.

Results
A total of 199 (126 preterm) infants were eligible; 153 gave consent for participation. While feeding frequency was higher in the first 3 months in the preterm born, overall feeding frequency decreased between the first 3 follow-up periods (p<0.001) in both groups. In the first and second week, breastfed infants had 2.41 more episodes of defecation per week compared to the formula-fed (p=0.017 and p=0.021, respectively). Higher median (10th-90th percentile) defecation frequency was only found in week 1 in the term compared to preterm group (24 (9.4-31.6) vs. (16 (6.5-31); p=0.002). Overall median defecation frequency was 16 (7-30) per week from birth up to the age of 24 months over the total group of participating infants.

Conclusion
Term and preterm infants have a comparable defecation frequency from the second week of life up to the age of 24 months.
BACKGROUND

Bowel patterns in infants are rarely described and therefore little is known about normal bowel habits in the first years of life.\textsuperscript{1-8} These cited studies mainly report defecation frequency and consistency whereas information concerning amount and colour of stools are usually lacking. The latter information might be helpful since young parents are often uncertain about these specific aspects of stool. We recently published the Amsterdam infant stool form scale which provides information concerning stool amount, consistency and colour. This stool form scale is however only a ‘snap-shot’ of the situation and gives therefore no information about the changes in stool characteristics during a longer period of time.\textsuperscript{9}

The necessity for more prospectively obtained data concerning infants and young children is mandatory, not only to define normal bowel habits but also to recognise abnormal bowel habits such as constipation. Although the Rome III criteria defined functional constipation for children younger than 4 years, well-defined criteria for the youngest infants are lacking. New data with respect to symptomatology might be helpful to set new criteria in order to differentiate between normal bowel habits and constipation in specifically this age group. The latter is particularly important as bowel habits change in the first months of life due to ageing but also as a result of change in feeding habits.\textsuperscript{1-8,10}

Therefore the aim of the current study was to describe and compare feeding and bowel habits in term and preterm born infants including feeding and defecation frequency, consistency, amount and colour of stool in a cohort of newborn infants up to the age 24 months.

METHODS

Patients

Between August and November 2006 all infants with gestational age (GA) 25-42 weeks were eligible for participation. Infants were admitted to the neonatal care units of an academic (Emma Children’s hospital/AMC) and a non-academic hospital (OLVG) in Amsterdam, the Netherlands. Infants were divided into two groups: 1) term born: GA ≥ 37 weeks and 2) preterm born: GA ≤ 36 weeks. Infants with history of gastrointestinal surgery (anal atresia, Hirschsprung’s disease), neurological disease (spinal bifida), metabolic disease (e.g. hypothyroidism) or congenital abnormalities (e.g. cystic fibrosis, Down’s syndrome) were excluded from the study.
Bowel diaries

Bowel habits were recorded by using standardized bowel diaries which included information about defecation frequency per week, stool consistency, amount and colour of stool, pain during defecation and the presence of gastro-intestinal symptoms such as flatulence, vomiting, abdominal cramps and abdominal distension. Use of laxatives was also recorded in the diaries. Bowel diaries were daily recorded in the first two weeks of life by the researchers during hospital admission of the infant (passive participation) or by parents (active participation). At the age of 3, 6, 12 and 24 months daily bowel diaries were recorded for 7 consecutive days by the care-takers.

At the age of 24 months additional information was gathered with respect to toilet training.

Informed consent & follow-up

The hospital’s medical ethics committee approved the research protocol. Parents gave written consent. At follow-up new bowel diaries were sent by post (or email) including a covering envelope. A second bowel diary was sent when the bowel diaries were not sent back in time. Hereafter, parents were called by telephone to verify the reason for the lost to follow-up.

Data analysis and interpretation

Patients’ characteristics were documented descriptively. Amount and frequency of feeding were analysed using the Student’s t-test. In a univariate linear regression model (Unianova) the independent effect of type of feeding on defecation frequency was examined. We compared the defecation frequency across babies with different types of feeding (categorical). A log transformation (base 10) was performed on the defecation frequency because of its skewed distribution. Results therefore are expressed as geometric means.

Difference in defecation frequency between preterm and term born infants was expressed in median values (10th- 90th percentiles) and analysed using the Wilcoxon rank test. Amount, consistency and colour were documented descriptively. Total number of symptoms was described in median values (min-max) and analysed using the Kruskall-Wallis test. Difference in presence (yes/no) of gastrointestinal symptoms was tested using (Yates’ continuity corrected) $\chi^2$ statistics or Fisher’s exact test, depending on cell frequencies.

All other values were expressed in means ±SD or median (10th-90th percentile). Statistical significance was defined as $p< 0.05$. All analyses were performed using the statistical software package SPSS (version 14.0; Inc, Chicago, IL).
RESULTS

Baseline
A total of 199 infants (126 premature) were eligible during the study period with mean gestational age of 35±4 weeks; 153 parents gave consent for participation during hospital admission (figure 1). During admission, passive participation into the study was refused by 25 and 21 parents of the term and the preterm born infants, respectively. After 24 months of follow-up, bowel diaries were recorded by 33% (50/153) of parents from start up to the end of the study. The lost to follow-up rate was highest at the age of 3 months (34%) and lowest at 24 months (17%). Reasons for lost to follow-up varied from drug addicted mothers (n=2), psychological problems parent (n=1), emigration (n=3), GI surgery (n=2), language problems (n=6), too busy (n=37; of whom n=18 due to having twins) while all others gave no specific reason for non-compliance to the study diaries.

Figure 1. Flowchart participating infants

Feeding

Feeding frequency
Feeding frequency was different between the two groups in the first 3 months of life. Term born infants had lower feeding frequency per day in the first week (6.9±1.9 vs. 9.2±3.7; p<0.001), the second week (7.7±1.5 vs. 10.2±3.7; p=0.001) and the third month of life (6.4±0.9 vs. 7.9±2.2; p=0.002) compared to preterm born infants.

Comparing the feeding frequency between the follow-up periods, a significant difference in feeding frequency was found between week 1 and week 2 (8.9±3.2 and 9.7±3.2; p<0.001), between week 2 and month 3 (9.7±3.2 and 7.2±1.8) and between month 3 and 6 (7.2±1.8 and 4.8±0.8; p<0.001) in both groups.
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Amount of feeding

As expected the total amount of enteral feeding (fluid) 1 week after birth was higher in the term born group compared to the preterm group (table 1). However, total amount of feeding per kg weight was comparable between both groups during the first week of life (p=0.12). During the third month, infants who were term born were fed higher amounts of feeding (568±45 vs. 381±137; p=0.02). The weight of infants was not recorded in this follow-up period. Thereafter up to the age of 24 months no differences were found in total amount of enteral feeding between both groups (table 1) neither in the total amount of feeding per kg weight at 12 and 24 months of age.

Bowel habits

Defecation frequency

As shown in table 1 and figure 2 in week 1, the median defecation frequency per week was higher in the term born group (24 (9.4-31.6)) compared to the preterm group (16 (6.5-31); p=0.002). This difference remained after excluding infants receiving morphine and/ or no enteral feeding (18 (9-38.4); p=0.04) in week 1. Thereafter, up to the age of 24 months no differences were found in defecation frequency between both groups (table 1) neither in the total amount of feeding per kg weight at 12 and 24 months of age.

Type of feeding and defecation

During week 1 and week 2, median defecation frequency was higher in the breast fed infants. In the first and second week of life, breast fed infants had 2.41 more episodes of defecation per week compared to the formula fed (p=0.017 and p=0.021, respectively).

In the term born infants feeding frequency and defecation were correlated in week 1 (r=0.75; p<0.001) and week 2 (r=0.29; p=0.02). Whereas in the preterm group feeding frequency and defecation were correlated in week 1 and month 3 (r=-0.21; p=0.04 and r=0.35; p=0.03 respectively).

---

**Table 1.** Median \(_{10}^{90}\)th percentile defecation frequency per week (DFW) from birth to the age of 24 months.

<table>
<thead>
<tr>
<th></th>
<th>Term Median DFW (_{10}^{90})</th>
<th>Premature Median DFW (_{10}^{90})</th>
<th>P-value</th>
<th>Term Mean ±SD ml feeding/day</th>
<th>Premature Mean ±SD ml feeding/day</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>24 (9.4-31.6)</td>
<td>16 (6.5-31)</td>
<td>0.002</td>
<td>285±153</td>
<td>146±145</td>
<td>0.001</td>
</tr>
<tr>
<td>Week 2</td>
<td>24 (10-36)</td>
<td>24 (12.7-33)</td>
<td>0.93</td>
<td>287±114</td>
<td>197±143</td>
<td>0.17</td>
</tr>
<tr>
<td>Month 3</td>
<td>8.5 (3.7-27.6)</td>
<td>16 (3-34)</td>
<td>0.06</td>
<td>568±45</td>
<td>381±137</td>
<td>0.02</td>
</tr>
<tr>
<td>Month 6</td>
<td>12 (7-26.2)</td>
<td>12 (3-25)</td>
<td>0.69</td>
<td>180±40</td>
<td>204±110</td>
<td>0.67</td>
</tr>
<tr>
<td>Month 12</td>
<td>14 (6.6-23.8)</td>
<td>16 (8-21)</td>
<td>0.59</td>
<td>288±236</td>
<td>380±287</td>
<td>0.33</td>
</tr>
<tr>
<td>Month 24</td>
<td>9.9 (5-14.7)</td>
<td>9 (5.8-15.4)</td>
<td>0.78</td>
<td>763±474</td>
<td>633±375</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Bowel habits in preterm and term born infants

CHAPTER 3

Stool characteristics

Stool characteristics of both groups are depicted in table 2. With aging, amounts (volume) of stools in term born infants remained much or normal whereas preterm infants start with little amounts of stools (72%) in week 1 changing into much or normal (80%) at later age. Stool consistency was normal/soft during the entire period of follow-up in both groups. Colour of stools changed from mainly yellow and green into brown at later age in both groups.

Gastrointestinal symptoms

As depicted in table 3 the total number of gastro-intestinal symptoms was higher in the term born group (2 (0-7) compared to the preterm group (1 (0-5) at 2 weeks follow-up, p=0.001. Thereafter up to the age of 24 months no differences were found in total number of gastrointestinal symptoms between both groups.

Constipation & laxative treatment

Despite the lack of a clear definition for constipation for young infants, a total of 7 term born infants versus 31 preterm born infants had a history of treatment with laxatives (once or more) during the first 24 months of life; p=0.04 (figure 3). The term born infants were treated for constipation at the age of 2 weeks (n=1), 6 months (n=1), 12 months (n=5) and 24 months (n=1). In the first week of life

Figure 2. Median (10th-90th percentile) defecation frequency per week for the ages of 1 & 2 weeks and 3, 6, 12 & 24 months after birth, respectively.

Figure 3. Median defecation frequency per week for the ages of 1 & 2 weeks and 3, 6, 12 & 24 months after birth.
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15 infants, all preterm with median GA 31 (25-35) weeks and birth weight 1370 (690-2920) grams, were treated for constipation. Thereafter, they were treated for constipation at the age of 2 weeks (n=10), 3 months (n=10), 6 months (n=1), 12 months (n=5) and 24 months (n=1). Only 2 preterm infants initially treated with laxatives continued laxatives at week 2 and the third month of follow-up. One infant was treated with laxatives at week 1 and month 3. All other 12 infants

| Table 2. Stool characteristics from birth to the age of 24 months. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Week 1          | Week 2          | Month 3         |
|                 | Term | Preterm | Term | Preterm | Term | Preterm |
| Amount          |      |         |      |         |      |         |
| - Much          | 6%   | 7%      | 16%  | 12%     | 37%  | 22%     |
| - Normal        | 84%  | 15%     | 70%  | 74%     | 50%  | 57%     |
| - Little        | 6%   | 72%     | 8%   | 12%     | 6%   | 9%      |
| - Smear         | 2%   | 5%      | 0%   | 2%      | 6%   | 3%      |
| - Unknown       | 2%   | 1%      | 6%   | -       | 1%   | 9%      |
| Consistency     |      |         |      |         |      |         |
| - Meconium      | 26%  | 53%     | 1%   | 11%     | -    | -       |
| - Hard          | 0%   | 5%      | 5%   | 8%      | 1%   | 4%      |
| - Normal/soft   | 67%  | 40%     | 85%  | 79%     | 90%  | 89%     |
| - Watery        | 4%   | 1%      | 9%   | 2%      | 9%   | 7%      |
| - Unknown       | 3%   | 1%      | -    | -       | -    | -       |
| Color           |      |         |      |         |      |         |
| - Yellow        | 54%  | 26%     | 90%  | 68%     | 76%  | 89%     |
| - Green         | 40%  | 67%     | 9%   | 24%     | 21%  | 9%      |
| - Brown         | 3%   | 7%      | 1%   | 5%      | 3%   | 2%      |
| - Orange        | 0%   | 0%      | 0%   | 0%      | 0%   | 0%      |
| - Unknown       | 3%   | 0%      | 0%   | 3%      | 0%   | 0%      |

| Table 3. Median number of total gastro-intestinal (GI) symptoms and total number of infants with specified GI-symptoms at each follow-up period. *Significant difference (p=0.001) between the two groups in total number of symptoms. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Wk 1 n=43       | Wk 1 n=110      | *Wk 2 n=29      | Wk 2 n=82 |
| Median no. of symptoms (min-max) | 0 (0-6)         | 1 (0-8)         | 2 (0-7)         | 1 (0-5)   |
| Total no. GI symptoms | 36              | 27              | 66              | 54         |
| Abdominal pain, no. (%) | 9 (25%)         | 0               | 17 (26%)        | 8 (15%)   |
| Diarrhea, no. (%) | 1 (3%)          | 0               | 8 (12%)         | 16 (30%)  |
| Flatulence, no. (%) | 8 (22%)         | 0               | 15 (23%)        | 3 (5%)    |
| Vomiting, no. (%) | 5 (14%)         | 8 (30%)         | 8 (12%)         | 5 (9%)    |
| Crying at defecation, no. (%) | 3 (8%)          | 0               | 5 (7%)          | 2 (4%)    |
| Red face at defecation, no. (%) | 6 (17%)         | 0               | 7 (11%)         | 2 (4%)    |
| Distended abdomen, no. (%) | 4 (11%)         | 19 (70%)        | 6 (9%)          | 18 (33%)  |
| Alarms, no. (%) (apneas, bradycardia) | 2 (5%)          | 53              | 0               | 20         |
stopped laxative treatment after the first week of life. Initial laxative treatment at week 2 (n=7) was continued in 2 infants at month 3. Infants who started with laxatives at the age of 12 months discontinued treatment at follow-up. Interestingly, no difference was found in type of feeding between those infants.

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<th>Month 6</th>
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<table>
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<td>Mo. 3</td>
<td>Mo. 3 n=26 Mo. 6 n=26 Mo. 12 n=25 Mo. 24 n=29</td>
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<td>Mo. 12</td>
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<td>63</td>
<td>80 54 62 47 91 40 47</td>
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<tr>
<td>15 (24%)</td>
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<td>5 (8%)</td>
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<td>6 (9%)</td>
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<td>5 (8%)</td>
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<td>9 (14%)</td>
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<tr>
<td>8 (13%)</td>
<td>10 (12%) 10 (18%) 10 (16%) 7 (15%) 13 (14%) 9 (23%) 7 (15%)</td>
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</table>
The reason starting treatment for constipation varied from unsatisfying stool characteristics (hard or small amounts), low defecation frequency or symptoms such as straining and abdominal distension (figure 3).

**Toilet training**

At the last follow-up period, 7 (33%) and 9 (31%) infants from the term and premature born group started toilet training at the age of 20.7±2.8 and 20.7±7.4 months respectively (p=0.99). None of these infants was toilet trained by the age of 24 months.

**DISCUSSION**

In this prospective study we demonstrated that breast feeding was positively correlated with defecation frequency in only the first two weeks of life in both term and preterm born infants. The frequency of feeding was statistically higher in the term compared to preterm born infants in the first 3 months of life. Furthermore, term born infants and preterm born infants had comparable defecation frequency from the second week of life up to the age of 24 months.

In contrast to other studies, we demonstrated that breast feeding, in contrast to formula feeding, was associated with significantly higher defecation frequency in only the first two weeks of life. The landmark study by Weaver et al, showed however higher defecation frequency in the first eight weeks of life of breast fed...
Bowel habits in preterm and term born infants

Breast fed infants opened their bowels four times per day compared to 2.75 in the formula fed infant. A possible explanation for our observation might be the change in the composition of infant formula which is currently more resembling breast feeding. Indeed, in accordance with our results more recent studies showed comparable defecation frequency between infants who were formula or breast fed.

Breast feeding containing more bifidobacteria, resembling breast feeding, is associated with higher defecation frequency and fewer incidences of constipation than standard formula feeding.

Comparison of term born infants with preterm born infants revealed higher defecation frequency in the term born in the first week of life. Possible explanations are twofold: the preterm group consisted of infants younger than 34 weeks of gestation with a yet to further develop motility pattern. For example, at 32 weeks of gestation McLain observed that progression of contrast material from proximal to the distal colon took as long as 9 hours, taking only half of that time at the term age. Furthermore, in preterm piglets dysfunctional enteric neurons in combination with inappropriate gut hormone release such as motilin and gastrin is decreased. In infants however, increase in gut regulatory hormones in response to feeding is comparable between term and preterm infants with similar responses occurring for breastfeeding and formula feeding. But this does not preclude the possibility that a functional immaturity of such cells and their receptors contribute to a disturbed gastrointestinal motility response to enteral feeding in preterm neonates. Secondly, the higher volumes of enteral feeding consumed by the term born infants increase defecation frequency as a result of the gastrocolonic response to gastric distension by feeding.

Comparable to other studies which describe a defecation frequency of 1-2 per day in infants < 2 years, we found median defecation frequency of 16 (P_{10}-P_{90}: 7-30) per week over the total follow-up period of 24 months. Based on those latter studies and our data we might conclude that infants younger than 24 months of age should at least open their bowels once daily. Therefore we suggest to adjust the Rome III criterion, defecation frequency, to <1 per day rather than <3 per week for constipation in infants 24 months and younger.

Interestingly, 38 infants from our cohort were treated with laxatives for other reasons than low defecation frequency such as hard stools, small amounts of stools, straining but also for abdominal distension. Since the Rome III criteria lack clear definitions for constipation in the younger age group, clinical experience is often the reason to start laxative treatment. The results of this study in combination with the use of the Amsterdam infant stool form scale hopefully will lead to a more scientific based treatment of infants with defecation problems. Besides defecation
frequency, hard stool is a good candidate to characterize constipation in infancy. This study showed that hard stool was one of the main reasons to start laxative treatment. Previously, Loening-Baucke also showed that hard consistency of stools and hard bowel movements were found in the majority of constipated patients younger than 2 years. Moreover, the latter study also showed that only 13% of the constipated children had defecation frequency <2 per week. Therefore, large prospective or cross-sectional studies including infants from different age groups are necessary to further describe specific stool characteristics using a standardized stool scale. Additionally, quantification of exact frequency and abdomen circumference is needed for complaints concerning low defecation frequency and abdominal distension respectively.

This study had also shortcomings such as the lost-to follow-up rate of 33% over the two years follow-up which was higher compared to other studies, 19-21%, with follow-up period between 3-4 years. A possible explanation for this difference might be that our population was partly healthy and not followed by any physician making the follow-up data more difficult to obtain. Even those followed in the outpatient clinic, had no close relation to the gastro-intestinal outpatient clinic conducting the study.

In conclusion this prospective study demonstrated that breast feeding was positively correlated with defecation frequency in only the first two weeks after birth. Furthermore, term born infants and preterm born infants had comparable defecation frequency, of at least 1 bowel movement per day, from the second week of life up to the age of 24 months. Therefore, we recommend changing the item defecation frequency in the Rome III criteria for constipation in children younger than 4 years. The defecation frequency in those infants younger than 24 months should be < once per day rather than a defecation frequency < 3/week. Additional symptoms such as hard stools or straining, together with defecation frequency<1/week would justify starting laxative treatment for infants.
REFERENCE LIST


PART II

Pathophysiology
The role of colorectal chloride secretion in childhood constipation

Noor Bekkali
Hugo de Jonge
René van den Wijngaard
Liedeke van der Steeg
Jan Taminiau
Jehan-François Desjeux
Marc Benninga
BACKGROUND

Disturbance in fluid secretion, driven by chloride channels, might play a role in constipation. However, disturbed chloride secretion in those patients has yet to be evaluated.

Aim

Compare chloride secretion in rectal biopsies of children with functional constipation (FC) and children without constipation.

Methods

To measure changes in short circuit current (Isc in µA/cm²) reflecting chloride secretion, intestinal biopsies from children with constipation, to either exclude or diagnose Hirschsprung’s disease, and from children without constipation (controls) undergoing colonoscopy for screening of familial adenomatous polyposis, juvenile polyps or inflammatory bowel disease (IBD), were compared and studied in Ussing chambers. Following electrogenic sodium absorption blockade by amiloride, chloride secretory responses to calcium-linked (histamine, carbachol) and cAMP-linked (IBMX/forskolin) secretagogues were assessed.

Results

Ninety six patients (46 FC) participated; 9 FC patients (n=1 congenital syndrome and n=8 technical problems) and 13 controls (n=6 IBD; n=7 technical problems) were excluded. No significant difference was found in mean (±SE) basal chloride currents between children with FC and controls (8.7±6.4 vs. 9.0±6.3; p=0.78, respectively). Responses to amiloride and calcium-linked chloride secretagogues were higher in controls but only reached statistical significance following chloride secretion activation with histamine (33.0±3.0 vs. 24.5±2.3, respectively; p=0.03).

Conclusion

Rectal chloride secretion is disturbed in children with FC following stimulation with histamine. Whether this defect occurs at the level of histamine receptors, components of receptor-linked signal transduction pathways or basolateral Ca²⁺-sensitive K⁺ channels enhancing the electrical driving force for apical chloride secretion, remains to be explored.
INTRODUCTION

The intestinal epithelium has important ion transport capacity with chloride as the predominant electrolyte driving fluid secretion in the colon. The intestine and the organs draining into it, secrete approximately 8 litres of fluid per day of which only 200 ml leaves the body within the stool. It is evident that pathological consequences will follow when chloride secretion is increased or reduced as occurs in secretory diarrhoea or cystic fibrosis, respectively. Two major proteins required for active chloride secretion are the Na/K/2Cl cotransporter (NKCC1) for chloride import and the cystic fibrosis transmembrane conductance regulator (CFTR) for chloride export, both located mainly in the crypts. In cystic fibrosis, many newborns present with meconium ileus, where thickened, dehydrated intestinal secretions cause intestinal obstruction shortly after birth. Therefore, disturbance in fluid secretion might also play a role in constipation. Chloride secretion stimulators act as selective chloride channel activators in the apical membrane of the gastrointestinal epithelium increasing intestinal chloride secretion and thereby intraluminal fluid collection in the gut. Just recently, our group showed that lubiprostone stimulates chloride secretion by activation of the CFTR channel rather than by the earlier described chloride-2 channel. Interestingly, in adults with constipation, those stimulators were found to be efficient in increasing defecation frequency and softening stools. However, a disturbance in gut chloride secretion or CFTR expression in patients with constipation remains to be evaluated, e.g. by monitoring transepithelial chloride secretion in Ussing chambers.

Although Ussing chambers are widely used for the characterization of gastrointestinal ion transport in animal models, studies in human tissue have been fairly limited due to the scarce availability of adequately sized samples. In recent years, miniaturised container inserts which enclose smaller biopsy specimens fitting into existing Ussing chambers, have been developed to facilitate studies in human tissue. In this study, using such a miniaturised Ussing chamber system (figure 1), we evaluated changes in chloride currents (as a measure for chloride secretion) ex vivo, in the rectal biopsies of children with functional constipation or controls following stimulation with secretagogues, as well as expression of CFTR in those biopsies.
METHODS

Patients & biopsies
Between April 2006 and June 2009 children with functional constipation (FC) according to the Rome III criteria, who were resistant to laxative therapy and were undergoing rectal suction biopsies to diagnose or to exclude Hirschsprung’s disease (HD) participated into the study. Two biopsies of suspected HD patients were consecutively taken upon endoscopy using a biopsy forceps with diameter 3.7 mm (Olympus). Two biopsies of children without constipation, undergoing colonoscopy, to screen for familial adenomatous polyposis, to remove juvenile polyps or to screen for inflammatory bowel disease (IBD), were control biopsies. Biopsies of children with proven IBD or congenital disorders were excluded from analysis.

Ussing chambers protocol
The colonic tissue obtained was transferred to ice-cold isotonic medium composed of 136.9 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl\(_2\)2H\(_2\)O, 0.5 mM Na\(_2\)HPO\(_4\)12H\(_2\)O and 1.5 mM KH\(_2\)PO\(_4\), pH 7.4. Then, the tissue was mounted in modified Ussing chambers (window area 0.025 cm\(^2\)). The tissue was short-circuited using a dual voltage clamp (WPI, Berlin, Germany) and transepithelial current changes in response to secretagogues added to the bath were recorded ex vivo using Powerlab (AD Instruments, Spechbach, Germany).

Two biopsy specimens per patient were used for Ussing chambers experiments. The bath solution was a modified Meyler buffer composed of 105 mM NaCl, 4.7
mM KCL, 1.3 mM CaCl$_2$$\cdot$2H$_2$O, 1.0 mM MgCl$_2$$\cdot$6H$_2$O, 20.0 mM NaHCO$_3$, 0.4 mM NaH$_2$PO$_4$$\cdot$H$_2$O, 0.3 mM Na$_2$HPO$_4$$\cdot$2H$_2$O, 10.0 mM HEPES, pH 7.4. The tissue was kept at 37°C using hot water jackets. Carbogen (95%O$_2$/5% CO$_2$) was constantly perfused through the chambers to oxygenate the tissue. After mounting the tissue, indomethacin was added to the bath in order to reduce the endogenous production of prostaglandins. Thereafter, biopsy specimens were allowed to equilibrate for 20 minutes to achieve stable measurements, after which experimental treatments were applied.

As depicted in figure 2, first, amiloride (10 μM) was added to the mucosal side of the tissue to inhibit the contribution of epithelial Na$^+$ channels (ENaC) to the current. The amiloride-sensitive current (DIsc) was defined as the difference between baseline Isc before and after addition of amiloride. After 10 minutes, the calcium-linked secretagogue histamine (50 μM) was added to the serosal side (S). Then, after reaching stable currents (~10 min), another calcium-linked secretagogue, carbachol (200 μM) was added to S. Finally, after a new steady-state was reached (~10 min), a cocktail of cAMP-linked agonists, forskolin (10 μM) and IBMX (0.1mM) was added to S.

![Figure 2](image)

**Figure 2.** Time line study protocol

**Mechanisms of action of the secretagogues in human colon**

Histamine and carbachol stimulate chloride secretion mainly through activation of calcium dependent K$^+$ channels (presumably of the KCa3.1/SK4 type) in the basolateral membrane.$^{19, 20}$ The resulting membrane hyperpolarisation increases the electrochemical driving force for Cl$^-$ exit through partially open CFTR channels in the apical membrane. Forskolin stimulates chloride secretion through adenylyl cyclase activation and cAMP formation. Cyclic AMP, through activation of cAMP-dependent protein kinase, phosphorylates and activates apical CFTR Cl$^-$ channels and basolateral K$^+$ channels (presumably KV7.1/KCNQ1).$^{19, 20}$ IBMX is a phosphodiesterase (PDE) inhibitor which indirectly augments cAMP levels. All secretagogues result in stimulation of chloride secretion with subsequent increase in Isc. The DIsc was measured as the difference between baseline Isc (before addition of the secretagogues) and the peak level of the Isc after addition of the secretagogues.
**Immunocytochemistry and confocal microscopy**

Cryo-sections (8 µm) of human rectal biopsies were stored at -80°C and analysed according to the previously described protocol by Broere et al. where sections were fixated for 10 min with 4% paraformaldehyde and with 20 minutes in methanol, followed by washing in 0.5% protifar (low-fat babymilk) and 0.15% glycine in PBS, (=PBS+). Then, sections were incubated for 90 min in PBS+ containing anti-CFTR monoclonal antibody (528, 1:50) kindly donated by Prof. J.R. Riordan, University of North Carolina at Chapel Hill. Subsequently, sections were washed with PBS+, incubated for 60 min with Cy3-labeled anti-mouse IgG (1:100, Jackson Immunoresearch Laboratory, West Grove, PA) and mounted with Vectashield (Vector Laboratories Inc., Burlingame, CA). Immunofluorescence micrographs were captured using a Zeiss LSM510 confocal microscope equipped with a 25 milliwatt argon laser. Fluorescence emission after excitation (488 nm for CFTR) was detected using 10x/0.3 or 40x/1.3 numerical aperture oil immersion lenses, a dichroic beam splitter reflecting 488-nm excitation light, and a 505-530 bandpass emission filter. The images were scanned using a 75-µm pinhole. For (semi)quantification of signal from colonic crypts, images from longitudinal sections at Z-steps of 0.5 µm were analysed by determining total background-subtracted fluorescent signal using KS400 Zeiss software. The same threshold was utilized for all images from one slide. Three individual crypts of each biopsy were analysed in a blinded fashion.

**Medical Ethical committee**

All parents and children aged > 12 years gave written consent. This study was approved by the medical ethical committee of the Academic Medical Centre of Amsterdam.

**Statistical analysis**

A sample size of 37 samples from each group was required to achieve 80% power at a significance level of 0.05 to detect a difference in means of twice the current change between the two groups. Such a difference was anticipated on the basis of earlier data by Bijlsma et al. The latter study showed preliminary evidence for a dramatically reduced response to the secretagogues histamine and carbachol (by >85%) in the epithelium of constipated patients. Baseline characteristics and difference in change of current between constipated and control tissue, were described and analysed using the student’s t-test (SPSS version 14.0 statistical software). A p-value below the 0.05 was considered statistically significant.
RESULTS

Baseline characteristics of participating patients

A total of 55 children with functional constipation (25 male) with mean constipation duration of 3.6±3.1 years and 50 (23 male) controls participated (table 1). Eighteen biopsies were excluded in the FC group, 1 biopsy was provided from a patient with a congenital disease (trisomy 12) and 8 other experiments were excluded due to technical problems with the equipment (see also figure 3). A total of 13 control biopsies were excluded, 6 patients had IBD and in 7 patients the experiments could not be conducted due to technical problems.

Table 1. Baseline characteristics of the controls and patients with constipation

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=37)</th>
<th>Constipation (n=37)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Mean ±SE age (yrs)</td>
<td>10.7±0.6</td>
<td>6.3±0.6</td>
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<tr>
<td>Duration constipation symptoms (yrs)</td>
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<td>3.6±0.5</td>
<td>-</td>
</tr>
<tr>
<td>Mean ±SE defecation frequency/ week</td>
<td>10.0±7.2</td>
<td>0.7±1.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean ±SE faecal incontinence frequency/ week</td>
<td>0.3±0.1</td>
<td>8.2±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number with hard stool consistency</td>
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<td>25</td>
<td>&lt;0.001</td>
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Figure 3. Flow chart participating patients.


**Chapter 4**

**Basal properties of human rectal mucosa**

Under basal conditions, after 20 minutes stabilisation in the Ussing chamber, basal Isc of the control tissue biopsies was $9.2 \pm 0.8 \mu$A/cm$^2$ compared to $9.6 \pm 1.1$ in the FC patients; $p=0.75$.

**Effect of amiloride and correlation with defecation frequency**

Amiloride caused a change in current of $-11.8 \pm 7.0 \mu$A/cm$^2$ in FC biopsies compared to $-14.7 \pm 2.2 \mu$A/cm$^2$ in control biopsies ($p=0.25$). In constipated and control biopsies, no correlation was found between amiloride-induced current changes and defecation frequency per week, ($r=-0.01$; $p=0.95$ and $r=0.01$, respectively; $p=0.92$).

**Effect of secretagogues and correlation with defecation frequency**

As depicted in table 2, carbachol induced a higher current change in controls but this difference did not reach statistical significance. Histamine caused a current change of $24.5 \pm 2.3 \mu$A/cm$^2$ in FC compared to $33.0 \pm 3.0 \mu$A/cm$^2$ in controls ($p=0.028$). In controls, a correlation was found between the currents, following stimulation with histamine ($r=0.4$; $p=0.001$) and carbachol ($r=0.5$; $p<0.001$), and

**Table 2.** Mean (±SE) change in current ($\Delta$Isc; µA/cm2) after addition of amiloride, histamine, carbachol and forskolin/IBMX to the mucosal (M) or serosal (S) bath in the Ussing chamber.

<table>
<thead>
<tr>
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<th>Controls (n=37)</th>
<th>Constipation (n=37)</th>
<th>p-value</th>
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</thead>
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<tr>
<td>Amiloride (M, 10 µM)</td>
<td>-14.7±2.2</td>
<td>-11.8±7.0</td>
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<tr>
<td>Histamine (S, 50 µM)</td>
<td>33.0±3.0</td>
<td>24.5±2.3</td>
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<tr>
<td>Carbachol (S, 200 µM)</td>
<td>33.6±3.4</td>
<td>26.4±2.7</td>
<td>0.10</td>
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<tr>
<td>Forskolin (S, 10 µM)+ IBMX (S, 200 µM)</td>
<td>15.1±2.2</td>
<td>15.4±2.4</td>
<td>0.94</td>
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</tbody>
</table>

**Figure 4.** CFTR immunostaining of control biopsies (a) and constipated children (b)
defecation frequency. No correlation was found between defecation frequency and currents following IBMX-forskolin ($r=-0.2; \ p=0.24$) in controls. In constipated biopsies no such correlations were found with any secretagogue.

**Correlation of the current responses with age**

No correlation was found between age and amiloride-induced current changes ($r=-0.07; \ p=0.43$). Also for the secretagogues histamine ($r=-0.02; \ p=0.80$), carbachol ($r=-0.07; \ p=0.44$) and IBMX-forskolin ($r=-0.01; \ p=0.9$), no correlation was found with age.

**Immunocytochemistry**

As shown in figure 4, CFTR immunostaining was confined almost exclusively to the apical membrane of colonic crypt cells, in agreement with a previous immunolabeling study reporting the absence of apical staining by the same monoclonal anti-CFTR antibody in rectal biopsies from CF patients.\(^{14}\) Quantification of the immunofluorescence staining intensity of the CFTR channel protein did not reveal a significant difference between control and FC biopsies (results not shown), indicating that the expression and distribution of CFTR protein was not affected significantly by the FC condition.

**DISCUSSION**

In this study we showed that calcium-linked chloride secretion in the rectum is disturbed in constipated children when compared to controls. Also, a positive correlation was found between calcium-linked chloride secretion, as induced by histamine and carbachol, and defecation frequency in control biopsies but not in biopsies from constipated patients (FC). However neither the amount of CFTR chloride channel protein nor its apical localization in the colonic crypts was affected by the FC condition.

To our knowledge this is the first study evaluating chloride secretion in tissue of constipated children. Constipated children had overall disturbed calcium-linked chloride secretion but statistical significance compared to non-constipated children was only reached following stimulation with histamine. Because the measurements are performed *ex vivo* and the secretagogues are added exogenously, the reduction in transepithelial chloride secretion in response to histamine in the FC group can not be explained by possible differences in local histamine levels. Histamine was only included in our protocol for its potent calcium-linked secretagogue effect.\(^{23}\) However, just recently a correlation between mast cells and nerve fibres in rectal mucosa was found in children with chronic constipation.\(^{24}\) In the latter study
constipated children showed an increase in mast cell area. Considering the potential histamine release by mast cells, exogenous histamine might not have reached its optimal effect due to desensitization of the histamine 1 receptor in constipated children from our study.\textsuperscript{25, 26} Given the latter, further research to elucidate the possible role of mast cells and their mediators in constipated children is justified. Considering the diminished chloride secretory response to calcium-linked secretagogues but not to cAMP-linked secretagogues (forskolin-IBMX) in constipated children, and the positive correlation of defecation frequency with calcium dependent secretagogues in controls, the disturbance in chloride secretion in the FC group is most plausibly due to a defect in intracellular calcium signalling. Whether this defect occurs at the level of the histamine receptor, components of the receptor-linked signal transduction pathway (e.g. phospholipase C, inositol 1,4,5-trisphosphate-triggered calcium release), or basolateral Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels (presumably SK4) that enhance the electrical driving force for apical chloride secretion through the CFTR Cl\textsuperscript{−} channel, remains to be explored. The transient character of the current response to calcium linked secretagogues, closely following the transient increase in intracellular Ca\textsuperscript{2+}, might suggest that in constipated children an enhanced storage or buffering capacity of intracellular Ca\textsuperscript{2+} rather than a reduced mobilization could be responsible for the difference in current changes. This is however unlikely as we measured the maximal change in current after secretagogues addition which occurred within one minute, i.e. before the reuptake of calcium into cellular reservoirs begins. Therefore, the CFTR channel activator lubiprostone, which does not to elicit a rise in cellular Ca\textsuperscript{2+} levels, would still be an obvious candidate to treat children with constipation.\textsuperscript{7} The latter is even more important as we found a positive correlation of chloride secretion with defecation frequency in controls.

In contrast to the findings with Ca\textsuperscript{2+}-linked secretagogues, the chloride current response to cAMP-linked agonists (IBMX-forskolin), was not different between controls and constipated children. Moreover, the expression level and apical distribution of the CFTR protein in the colonic crypts was also not affected by functional constipation. Both findings strongly indicate that the components of the cAMP signalling pathway, i.e. cAMP-dependent protein kinase, apical CFTR chloride channels, basolateral K\textsuperscript{+} channels (KCNQ1) and the NaKCl\textsubscript{2} cotransporter (NKCC1) were functioning normally in the colon of the constipated children, and stress the specificity of the defect in components of the calcium signalling pathway that are not shared with the cAMP pathway.

In adults, drugs stimulating chloride secretion are already successfully applied in treating functional constipation and IBS-C.\textsuperscript{10, 27} For children, no data are published
concerning treatment with those agents. Childhood functional constipation treatment encloses a combination of behavioural and medical therapy. Polyethylene glycol (PEG) is the first line drug used in these children and aims to soften the stools, thereby contributing to a break-through of the vicious circle of defecation avoidance caused by pain during defecation. 28 As only 60% of constipated children accomplish successful treatment with PEG, we recommend conducting randomized controlled trials to assess the effect of PEG versus chloride secretion stimulators such as lubiprostone or linaclotide.29

In conclusion, disturbed chloride secretion is found in constipated children following stimulation with histamine. Furthermore, calcium dependent chloride secretion was positively correlated with defecation frequency in control biopsies. Given these results, it is recommendable to conduct trials to investigate the role of drugs stimulation chloride secretion in constipated children.

Acknowledgment
We wish to thank Alice Bot and Flore Dossou for their advice and training in using the Ussing chamber technique, and Huub Jorna for conducting the immunocytochemical experiments.


CHAPTER 5

Muscularis mucosae of the rectum in children with functional constipation and Hirschsprung’s disease

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Submitted
ABSTRACT

Background
Difference in rectal muscularis mucosae (RMM) morphology in children with functional constipation (FC), Hirschsprung's disease (HD) and controls is not described.

Aim
To evaluate RMM characteristics in FC, HD and controls and assess correlation between RMM thickness and duration of constipation symptoms.

Methods Rectal suction biopsies from children with intractable FC were evaluated for HD. Endoscopically obtained tissue without evidence for pathology was control tissue. An experienced pathologist reviewed biopsies assessing RMM hypertrophy (thickness >80μm) and/or disorganisation (RMM extension in between the crypts). Two observers, measured exact RMM thickness.

Results
Fifty-six biopsy specimens were eligible, of which 50 analysed. RMM hypertrophy was found in 13/28 FC and 4/22 HD (p=0.024) patients and disorganisation in 12/28 and 2/22 (p=0.01), respectively. Mean RMM thickness was 117±61μm and 70±44μm in FC and HD, respectively (p=0.001). Controls (n=10) had smaller RMM thickness (51±17μm) compared to FC (117±61μm; p=0.003) but similar to HD (p=0.20). RMM thickness and symptom duration were correlated in FC (r=0.50; p=0.02).

Conclusion
Children with FC have hypertrophied and disorganised RMM compared to HD and controls. In FC RMM thickness was correlated between RMM thickness and duration of constipation symptoms. These findings suggest that RMM changes are secondary consequences of constipation.
INTRODUCTION

Constipation is common during childhood with a median prevalence of 8.9% in the western world and is characterised by infrequent painful defecation in combination with involuntary faecal incontinence.\(^1\) Organic causes of constipation are only found in 5-10% of cases.\(^2\) Withholding behaviour is suggested to be the major cause of functional constipation in children.\(^3\)

Defecation is a complex interplay between the autonomic and somatic nervous system and the group of muscles controlling the anal sphincters and the pelvic floor muscles. Consequent faecal stasis might result in higher intraluminal pressure and may cause histological changes in rectal colonic musculature. Moreover, it is suggested that changes in muscularis mucosae such as hypertrophy can be expected in gastrointestinal disease.\(^4\) For example, the rectal muscularis mucosae (RMM) in solitary rectal ulcer syndrome in adults, is characterized by hypertrophy and disorganisation.\(^5, 6\) In this condition, ischemia, atherosclerosis as well as colonic stasis are suspected factors in the pathogenesis.\(^7\)

While paediatric patients with intractable constipation are still considered to suffer from a disease of functional nature, recent studies showed structural abnormalities of the colonic enteric nervous system (ENS) in those patients. These include, morphological changes or reduced numbers of ganglia cells and/or glial cells \(^8-11\), abnormal nerve fibre density in the circular muscle layer \(^12\) and a reduced or abnormal number of interstitial cells of Cajal \(^8, 9, 13-15\) have been observed. In addition, colonic smooth muscle defects of patients with slow transit constipation have been reported.\(^16, 17\) These structural changes may play a crucial role in the pathophysiology of colorectal motility disorders such as constipation. But consequential histological changes as a result of constipation symptoms have yet to be evaluated in children. Therefore we hypothesized that paediatric patients with constipation have different rectal mucosal musculature and that these changes are associated with duration of constipation symptoms.

METHODS

Specimens

(Rectal suction biopsy specimens)

Sixty-five rectal suction biopsy specimens, taken between November 2000 and June 2008, were retrospectively evaluated from patients (aged 0-18 years) presenting to the department of paediatric gastroenterology or paediatric surgery with intractable constipation to diagnose or to exclude Hirschsprung’s disease (HD). For HD diagnostics, rectal suction biopsies between 2 and 4 cm
from the dentate line were taken and directly snap frozen. Slides from the frozen biopsies were stained by hematoxylin-eosin and assayed for acetylcholinesterase activity. The latter was determined as previously described by Karnovsky and Roots. 18 Nonspecific acetylcholinesterase was inhibited by 2.5x 10^6 mol/L tetra-isopropylpyrophosphoramide (ISO-OMPA). 19 Rectal suction biopsies were considered positive for HD when acetylcholinesterase activity was elevated in combination with absence of ganglion cells. If ganglion cells were present, HD was excluded. Patients excluded for HD were diagnosed with functional constipation (FC).

Resection specimens & endoscopically obtained control tissue

In HD patients with poor quality rectal suction biopsies, resection specimens obtained by the paediatric surgeon were used for RMM thickness evaluation. In patients suspected for inflammatory bowel disease colonoscopy was performed. When no macro- and/or microscopical evidence were found for colonic pathology, obtained rectal tissue was analysed as control samples (n=10).

The resection and endoscopic specimens were fixed in 4% formaldehyde buffered with phosphate-buffered saline (PBS), dehydrated, and embedded in paraffin. Tissue sections were made at 5 µm, stretched and dried at 57°C overnight. The sections were rehydrated in an alcohol series, endogenous peroxidase activity was blocked by 1% H₂O₂ in methanol for 1 hour.

Protocol

Semi-quantitative evaluation

An experienced pathologist, blinded for the patient’s diagnosis, first screened the biopsy specimens for muscle thickness measurement suitability. Rectal suction biopsies were considered suitable for measurement when the entire thickness of the RMM was visible. Biopsies were discarded when the limits of the muscularis mucosae were not clear due to absence of the border of the submucosa. Furthermore, semi-quantitative evaluation of the RMM was performed by the pathologist. We defined RMM hypertrophy when its thickness exceeded 80 µm. The pathologist determined RMM thickness with a light microscope (Olympus U-SD03) equipped with a x20 objective. Muscle architecture was also evaluated and disorganisation of the RMM was defined by the pathologist as extension of the RMM in between the crypts.

Quantitative evaluation

After the first screening of the biopsies, two medical doctors (NB and MMT), blinded for the patient’s diagnosis, measured independently of each other the exact thickness (in µm) of the RMM of all suitable specimens (20x magnification; Leica DM 5000B). These observers measured the maximal and minimal muscle thickness
of each specimen while taking photographs (Leica Microsystems). The mean of the maximal and minimal measurements of both observers was used for analysis.

**Statistical analysis**

Statistical analysis of the data was conducted using the statistical software package SPSS (version 14.0; Inc, Chicago, IL). Baseline characteristics of the group were analysed in a descriptive way. Differences in evaluation of muscle hypertrophy and organisation, as described by the pathologist (yes/no), between FC, HD and control patients were analyzed by Fisher’s exact $\chi^2$ statistics. Agreement between the two observers was determined using the Pearson correlation test. Difference in exact muscle thickness between FC, HD and control patients was calculated using the student t-test. Correlation between muscle thickness ($\mu$m) and duration of symptoms (months) or age (months) was calculated using the Pearson correlation test. A p-value <0.05 was considered significant.

**RESULTS**

**Baseline**

Fifty six rectal suction biopsy specimens were included (fig. 1). Six specimens were excluded by the pathologist because of poor quality, these included five rectal suction biopsies from the FC group and one from the HD group. Children with FC (n=28) and HD (n=22) had a median age (range) of 8.0 (0.2-288) months and 5.4 (0.2-22.0) months (p=0.003), respectively. Control patients (n= 10) had a median age of 12.4 (7.2-18.2) years. Mean duration of constipation symptoms was 33.1±54.0 months and 6.5±9.2 months in respectively FC and HD patients, respectively.

**Hypertrophy and disorganisation of the RMM**

Rectal muscularis mucosae was significantly thicker in FC patients compared to HD patients and controls as shown in table 1 and illustrated in figure 2. When resection specimens were excluded from analysis, difference in hypertrophy of the RMM remained statistically significant between HD and FC patients (p=0.004). Both hypertrophy as well as disorganisation of the RMM was found in 9 and 2 patients of FC and HD patients, respectively. In three FC patients RMM disorganisation was found without hypertrophy.

A total of 10 patients from the HD group compared to 18 FC patients had no hypertrophy and nor RMM disorganisation in the same biopsy specimen (p=0.002).
The muscle thickness measurements, as shown in table 2, were well correlated ($r=0.90; p<0.001$) between the two observers. In FC patients significant correlation between muscle thickness and duration of constipation symptoms was found ($r=0.5; p=0.01$). In HD patients, this correlation was not found ($r=-0.1; p=0.66$).
Figure 2. Photographs of rectal muscularis mucosae of (a.) a child with functional constipation, (b.) a child with Hirschsprung’s disease, and (c.) a control patient.
Mean muscle thickness and correlation with age

No correlation between age and RMM thickness was found in FC patients ($r=0.3; p=0.18$), HD patients ($r=0.06; p=0.80$), or controls ($r=0.27; p=0.45$).

DISCUSSION

This study demonstrated that rectal muscularis mucosae (RMM) is hypertrophied in and disorganised in 43–46% of children with functional constipation. Furthermore, a clear correlation was found between muscle thickness and duration of functional constipation symptoms.

In the gut propulsive forces are generated mainly by the muscularis propria but also by the muscularis mucosae. As a result of faecal impaction, present in 30–75% of children with functional constipation, the RMM may undergo constant elevated intraluminal pressure. This chronic rectal faecal stasis requires more contraction of the colonic wall muscles. Consequently, the muscles remain presumably contracted with consequential hypertrophy of the RMM. Another explanation is that due to expulsion of large amounts of stools, often reported in constipated children, higher muscle force is necessary to facilitate defecation (Laplace’s law).

The effect of chronic constipation on RMM morphology was further emphasized as we demonstrated that longer symptom duration was correlated with thicker RMM. Other factors might also contribute to RMM hypertrophy such as chronic inflammation as described in Crohn’s disease. Interestingly, inflammation of the mucosa including infiltration with eosinophils is described in constipated children with cow’s milk allergy or (food) intolerance. The latter results suggest that cow’s milk allergy or intolerance should be considered as a cause of chronic constipation.

In patients with HD no correlation between RMM thickness and symptom duration was found despite constipation symptoms. This might be explained by differences in pathophysiology. Due to absence of ganglion cells in the distal colon...
rectal muscularis mucosae in constipation

Chapter 5

Rectal muscularis mucosae in constipation (the rectum), faeces do not reach the level at which biopsies are taken. Faeces remain impacted above the biopsied level and therefore no changes are found in histological characteristics of the RMM. The latter further emphasizes that the histological changes found in FC children such as hypertrophy and disorganisation, might well be the consequence of faecal impaction.

Hirschsprung’s disease is a developmental disorder of the enteric nervous system characterized by the absence of ganglion cells along a variable portion of the distal intestine. This results in a functional obstruction caused by dysmotility of the diseased segment. Delayed passage of meconium is the cardinal symptom in neonates with HD which is in contrast with healthy term born neonates. Approximately 80-90% of all HD cases present with symptoms of constipation, abdominal distension and vomiting during the neonatal period. In accordance with earlier studies, in 80% of the children in this study, HD was diagnosed before the age of 6 months. In contrast, children with functional constipation usually present with symptoms at the time of toilet training. Consequently, children with HD in this study were younger compared to the children with functional constipation. Therefore, we evaluated correlation between age and RMM thickness. No correlation however was found between age and RMM thickness in any group, indicating that the correlation between constipation symptom duration and RMM thickness was not due to age.

Interestingly, the histochemical characteristics might be helpful additional tools to distinguish functional constipation from HD in inconclusive rectal suction biopsies. The latter problem is found when rectal suction biopsies contain too superficial specimen and do not contain muscularis mucosae. Rectal suction biopsies are frequently repeated due to inconclusive findings which may increase the risk of complications. Some clinicians even choose for full thickness biopsies, if rectal suction biopsies are inconclusive. Therefore, more histological characteristics such as disorganisation and hypertrophy of the rectal muscularis mucosae might help prevent repeated testing.

In solitary rectal ulcer syndrome in children, RMM can be hypertrophied and disorganised as well. Disorganisation of the RMM in the latter syndrome includes distortion of the crypt architecture as one of the features which we did not find in our group of patients. This could be explained by the more severe presentation of patients with solitary ulcus syndrome compared to our constipated patients. Although the syndrome is believed to be associated with a disorder of evacuation, the pathophysiology remains uncertain. This study had also shortcomings. Resection specimens of patients with HD are processed differently as they are embedded in paraffin which may shrink
the tissue. Consequently the RMM measured from paraffin embedded tissue may appear thinner. In contrast, the rectal suction specimens are snap frozen at -80°C, a process which may swell the tissue. This implies that frozen tissue may appear more distended compared to the tissue embedded in paraffin. This could be a confounder in our study. However, when we analysed the rectal suction biopsies only, by excluding resection specimens, difference in muscle thickness remained between the constipated and HD patients. Another shortcoming was the retrospective character of our study which made evaluation between symptoms of constipation, such as faecal impaction and RMM thickness difficult to assess.

In conclusion, in this study we demonstrated that rectal muscularis mucosae is hypertrophied and disorganised in patients with functional constipation. In addition, we found a correlation between muscle thickness and symptom duration of functional constipation. Further prospective studies are needed to confirm our findings and the effect of constipation on the rectal muscularis mucosae. Interestingly, these findings contribute to more insight into the pathophysiology of functional constipation and might also be useful additional diagnostic criteria for pathologists distinguishing constipation from Hirschsprung’s disease in inconclusive rectal suction biopsies.
REFERENCE LIST


CHAPTER 6

MRI of the lumbosacral spine in intractable constipation or non-retentive faecal incontinence: a prospective study

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In press
ABSTRACT

Background
Magnetic resonance imaging (MRI) revealed lumbo-sacral spine (LSS) abnormalities in 9% of children with intractable constipation.

Aim
To determine the prevalence of LSS abnormalities in children with defecation disorders, intractable constipation or non-retentive faecal incontinence (NRFI), and to evaluate whether LSS abnormalities on MRI can clinically be detected by neurological examination.

Methods
MRI of the LSS and complete neurological examination, by a paediatric neurologist who was blinded to the MRI, were performed in patients with intractable defecation disorders.

Results
One hundred and thirty patients with intractable constipation (76 male) median (range) age 11 (6-18) years and 28 NRFI (18 male) with median age 10 (7-15) years participated. One occult spina bifida (OSB) and three terminal filum lipomas were found, with normal neurological examination. One patient had a terminal filum lipoma and neurological complaints. Gluteal cleft deviation was found in 3/4 constipated patients with LSS abnormalities. Neurosurgical treatment was not needed in any patient during the 12 weeks follow-up.

Conclusion
MRI showed LSS abnormalities in 3% of patients with defecation disorders and normal neurological examination who all had symptom relief at 12 weeks follow-up, without neurosurgical intervention. It remains therefore unclear whether LSS abnormalities play a role in defecation disorders.
BACKGROUND

Constipation and faecal incontinence are common defecation disorders in childhood. The symptoms vary from relatively mild to severe chronic constipation with faecal impaction and faecal incontinence. In more than 90% of children with defecation problems no organic abnormalities are found and thus considered as functional disorders. Only recently however, a retrospective study showed lumbo-sacral spine abnormalities (LSS) in 9% of children with intractable constipation. None of these patients presented with major neurological symptoms. The authors described constipation symptom relief in 8 of 9 of these patients post-neurosurgery.

In adults, LSS abnormalities are associated with faecal incontinence. No such studies have been performed in children with faecal incontinence without clinical or physical signs of constipation. These children are hence diagnosed with functional non-retentive faecal incontinence, a diagnosis found in approximately 20% of children presenting with functional defecation disorders in our practice.

Based on the results of the MRI study mentioned above, the North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition (NASPAGHN) adapted her recommendations to include MRI in the standard work-up of paediatric intractable defecation disorders. However, the true prevalence of LSS abnormalities in these children remains to be established in a prospective study. Therefore the aim of this study was to determine the prevalence of LSS abnormalities in children with defecation disorders (constipation or non-retentive faecal incontinence), to prospectively follow-up all patients for 12 weeks during treatment and to assess the relation between LSS abnormalities and neurological symptoms found upon neurological examination.

METHODS

Study design & patients

Between February 2006 and January 2009, paediatric patients aged 6-18 years referred to our tertiary paediatric motility centre with symptoms of intractable functional defecation disorders, constipation or non-retentive faecal incontinence, were included in this 12 weeks prospective study. Children with known organic cause for constipation (such as Hirschsprung’s disease) were excluded. Children with known spinal cord abnormalities, mental retardation and claustrophobia were also excluded.
Neurological examination

All patients were subjected to a history focusing on neurological complaints such as (back) pain, pareses and numbness, walking difficulties and sphincter disturbances. A paediatric neurologist performed neurological examination with special attention to pareses, reflex or sensibility disturbances of the lower extremities and lumbosacral region. Additionally, dermatological signs of the lower back and orthopaedic signs like scoliosis or feet deformities were evaluated. The neurologist was blinded to the MRI results.

Imaging

*Magnetic Resonance Imaging (MRI)*

For this study the lumbosacral spine was, based on clinical availability, either imaged with Siemens Magnetom Avanto 1.5 T (Siemens AG, healthcare sector, Erlangen, Germany) or Philips Panorama 1.0 T (Philips Medical Systems, Best, the Netherlands) MR system based on availability of the magnets. Imaging protocol consisted of the following sequences: sagittal $T_1$ SE, sagittal $T_2$ TSE, axial $T_2$ TSE, at the level of the intervertebral foramina $T_{12-S_5}$. Special attention was made to detect an occult spina bifida, a terminal filum lipoma or conus lipoma, thickened terminal filum (> 2 mm diameter at the $L_5-S_1$ region) or tethered cord. The cord was considered tethered when the conus medullaris extended below $L_2$ vertebral level with a terminal filum thicker than 2 mm. No sedation or anesthesia for the MRI was required, as only children aged > 6 years were included.

*Conventional radiography*

As part of the clinical work-up of patients with defecation disorders, some patients underwent a marker study assessing colonic transit time (CTT) by performing an abdominal radiograph. The abdominal radiographs were also used to evaluate skeletal anomalies of the LSS. As the ingested markers are non-ferromagnetic they had no negative impact on the quality of the MRI exams.

Standardized treatment

At intake a standardized bowel questionnaire was completed by all patients as well as a complete physical examination. All children received behavioral recommendations during the study period. Patients were recommended and instructed to sit on the toilet for 5 minutes three times per day after each meal. Furthermore, they were instructed to immediately go toileting when feeling sensation of urge. Children diagnosed for constipation received additional disimpaction treatment for the first 3 days consisting of daily rectal enemas (dioctylsulfosuccinate sodium (Klyx®)). Subsequently, they received maintenance therapy containing of oral poly-ethylene glycol laxatives.
Children diagnosed with non-retentive faecal incontinence did not receive additional laxatives but underwent a strict toilet training program. At study entry all patients were given a standardized bowel diary to record their bowel habits during the full study period (12 weeks).

RESULTS

Overall group
A total of 158 (96 male) patients were eligible with a median (range) age of 10 (6-18) years (table 1). For unknown reasons MRI was not planned by the physician in three constipated patients. Three other patients did not undergo MRI: one refused MRI after neurological examination revealed no abnormalities and two patients did not show up for MRI appointment without any specific reason (figure 1). Abnormalities of the LSS using MRI were found in five patients (3%). Neurological examination revealed no neurological abnormalities in all these five patients while one patient complained of neurological problems. A total of 60 (46%) and 20 (71%) plain abdominal radiographs were available from the constipated and the NRFI patients, respectively, showing no abnormalities.

Figure 1. Flow chart of all participating patients.
Patients with constipation

One hundred and thirty (78 male) constipated patients with median (range) age of 11 (6-18) years and mean (±SD) symptom duration of 58±41 months were included, of whom 125 underwent MRI (table 2). Faecal incontinence was found in 105 (80%) patients, whereas urinary incontinence was found in 22 (17%) patients; of these patients, 19 (15%) had both faecal and urinary incontinence. MRI of the LSS was abnormal in 4 (3%) patients: one patient with occult spina bifida and three patients with a terminal filum lipoma. Five patients (4%) did not return for neurological examination. Neurological examination revealed neurological abnormalities in four patients with normal MRI findings. Gluteal cleft deviation was found in 3 out of 4 patients with abnormal MRI.

Table 1. Characteristics of participating patients. NRFI= non-retentive fecal incontinence

<table>
<thead>
<tr>
<th></th>
<th>Constipation</th>
<th>NRFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number (male)</td>
<td>130 (78)</td>
<td>28 (18)</td>
</tr>
<tr>
<td>Median age (min-max)</td>
<td>11 (6-18)</td>
<td>10 (7-15)</td>
</tr>
<tr>
<td>Mean duration symptoms in months (±SD)</td>
<td>58±41</td>
<td>50±34</td>
</tr>
<tr>
<td>Abnormal MRI</td>
<td>4 (3%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Abnormal neurological examination</td>
<td>4 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Presence fecal incontinence</td>
<td>105 (80%)</td>
<td>28 (100%)</td>
</tr>
<tr>
<td>Presence urinary incontinence</td>
<td>22 (17%)</td>
<td>6 (21%)</td>
</tr>
<tr>
<td>Mean fecal incontinence/ wk</td>
<td>Intake</td>
<td>8.9 ±9.2</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>1.6 ±3.0</td>
</tr>
<tr>
<td>Mean defecation frequency/ wk</td>
<td>Intake</td>
<td>2.4 ±2.9</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>6.7 ±3.6</td>
</tr>
</tbody>
</table>

Abnormal MRIs and normal neurological examination

Terminal filum lipoma

MRI revealed a small terminal filum lipoma (of 1 mm width and undeterminable length) in 2 patients, I and II as depicted in table 2. In patient I, pes planus and gluteal cleft deviation was found without neurological complaints. In both patients no skeletal anomalies of the LSS were found on plain abdominal radiographs. After 12 weeks of laxative treatment, symptoms of constipation improved in both patients.

Occult spina bifida

In one child, patient IV, MRI showed an occult spina bifida and conus medullaris just below L2 as well as an anterior sacral cyst connected to the dural sac. Furthermore, a lipoma was found (of maximal diameter 3.8 mm) in the terminal filum as well as in the sacral meningocele. At intake no neurological abnormalities were found by the paediatric neurologist or reported by the patient. However, a subtle gluteal cleft deviation was found (figure 2; online). Skeletal anomalies of the LSS were not
found on plain abdominal radiographs. At 12 weeks follow-up, faecal incontinence episodes resolved and defecation frequency normalized without neurosurgical intervention. After 18 months follow-up however, the patient presented with neurological complaints consisting of head ache, leg and back pain suggestive for tethered cord. The patient had a normal defecation frequency without faecal incontinence. Repeated neurological examination revealed no dermatological or orthopaedic abnormalities. A second MRI also performed at follow-up, confirmed previous findings. This patient is now under consideration for neurosurgical treatment.

Abnormal MRI with neurological complaints
One patient, III in table 2, had a terminal filum lipoma of a maximum width of 2.8 mm reaching from L₁ up to S₁ with normal position of the medullar conus. The neurologist found subtle gluteal cleft deviation in this patient but no further objective neurological abnormalities. However, this patient complained of sensibility disturbances in the right leg increasing in severity during the day. No abdominal radiograph was performed in this patient. At intake faecal incontinence as well as urinary incontinence was present; both resolved after 12 weeks of follow-
up (table 2) without neurosurgical treatment. Given the neurological clinical complaints, this patient was referred to the neurosurgeon for further assessment.

**Normal MRI and abnormal neurological examination**
In four (3%) patients abnormal neurological examination was found but without abnormalities upon MRI. In three patients the anal reflex (anal wink) could not be elicited and one patient had symptoms fitting the earlier diagnosed (mild) hemiparesis, resulting from neonatal asphyxia.

**Patients with non-retentive faecal incontinence**
Twenty seven of 28 (18 male) patients with non-retentive faecal incontinence aged 10 (7-15) years with mean symptom (±SD) duration of 50±34 months underwent MRI (table 1). Urinary incontinence was found in 6 (21%) of these patients. MRI was abnormal in one patient with normal neurological findings upon neurological examination. One patient (4%) did not return for appointment at the neurologist (figure 1). Abnormal neurological findings were not found in this group of patients.

**Abnormal MRI and normal neurological examination**
**Terminal filum lipoma**
MRI revealed a terminal filum lipoma (of 1 mm width and undeterminable length) in one child, patient V in table 2, with normal neurological findings. No skeletal anomalies of the LSS were found on plain abdominal radiograph. After 12 weeks of intensive toilet retraining, episodes of faecal incontinence resolved (table 2). No neurosurgical treatment was needed.

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**Table 2. Clinical characteristics of patients with lumbo-sacral spine abnormalities on MRI. Patients I-IV: constipation; patient V: non-retentive fecal incontinence.**

<table>
<thead>
<tr>
<th>MRI</th>
<th>Intake</th>
<th>12 weeks FU</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GCD</td>
<td>UI</td>
<td>FL/wk</td>
</tr>
<tr>
<td>Patient I</td>
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<td>No</td>
</tr>
<tr>
<td>Patient II</td>
<td>Lipoma L3</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patient III</td>
<td>Lipoma S2</td>
<td>Yes</td>
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</tr>
<tr>
<td>Patient IV</td>
<td>OSB S2</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Patient V</td>
<td>Lipoma S2</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Lipoma= terminal filum lipoma; OSB= occult spina bifida; GCD: Gluteal cleft deviation; UI= urinary incontinence; FI/ wk= fecal incontinence/ week; DF/wk= defecation frequency/ week. FU= follow-up; BR= behavioral recommendations; LT= laxative treatment.
DISCUSSION

To our knowledge this is the first prospective study evaluating the prevalence of lumbosacral spine (LSS) abnormalities in children with defecation disorders. Using MRI we found LSS abnormalities in a low percentage of children with defecation disorders of whom only one patient presented with neurological complaints. During the twelve week follow-up period all patients with LSS abnormalities had symptom relief of their defecation problems with simple toilet training advice and/or oral laxative treatment without any neurosurgical intervention.

The prevalence of lipoma is reported in 1.5-19% of asymptomatic adults, whereas the prevalence of LSS abnormalities is comparable between children with OSB (8%) and children presenting with functional urinary or bowel problems (10%). We found LSS abnormalities in 3% of children presenting with defecation disorders in accordance with the prevalence documented in asymptomatic adults. The latter further emphasizes the poor clinical relevance of MRI findings in defecation disorders. In addition, no LSS abnormalities were found upon MRI in our patients with abnormal neurological examination and urinary incontinence and/or faecal incontinence.

In contrast to Rosen et al. who found 9% LSS abnormalities in a comparable study population with constipation we only found 3% LSS abnormalities in children with defecation disorders. Given the difference in symptom duration, 28 months and 58 months in Rosen’s and our own study respectively, one would expect more abnormalities in our population. A possible explanation for the discrepancy between the two studies might be the difference in defining intractable constipation. In the study by Rosen et al. intractable constipation was defined as defecation frequency <3 times per week for more than three months while not responding to laxative treatment. In our study population, intractable constipation was defined as constipation not responding to laxative treatment prescribed by primary and secondary centres but not necessarily presenting with defecation frequencies <3 times per week. More intractable cases may present with lower defecation frequencies. Indeed all constipated patients with abnormal findings on MRI in our study had a defecation frequency less than three per week at intake.

The child with occult spina bifida (OSB) from our current study had gluteal cleft deviation without neurological or dermatological abnormalities upon physical examination at intake. Therefore no neurosurgical treatment was initially offered. However, in OSB syndrome an increased tension on the cord is expected to occur. Chronic tension may lead to deformation of the medullar conus, impaired local spinal blood flow and, finally, local spinal cord ischemia causing neurological dysfunction such as urinary incontinence. Those complaints can occur at any age from childhood up to adulthood. Indeed, after 18 months of follow-up, our
OSB patient presented at the age of 11 years for the first time with neurological symptoms (head ache, back pain and walking disturbances). Consequently this patient was referred to the neurosurgeon.

Four patients in our study had a terminal filum lipoma upon MRI; of whom one with neurological complaints which could not be confirmed upon neurological examination. Spinal cord lipomas have been suggested as a common cause of cord tethering which subsequently leads to progressive neurological defects and hence complaints. Retrospective studies show good outcome of preventive neurosurgical treatment in asymptomatic patients presenting with spinal lipoma. Interestingly, they showed that asymptomatic patients remained symptom free at follow-up in 74-93% of cases. However, prospective randomized controlled trials evaluating the effect of preventive neurosurgical treatment of spinal lipomas are lacking making conclusions with respect to natural history difficult. Since all patients with terminal filum lipoma in our study had symptom relief after 12 weeks of conservative treatment, the role of neurosurgery in our neurological asymptomatic patients with defecation disorders is rather questionable.

In constipation, loss of spinal inhibition produces alterations in the hindgut function leading to constipation. Furthermore, damage to the cord above the sacral nerves results in abnormal left-sided colonic transit. Conversely, the pathophysiology of non-retentive faecal incontinence is unknown, while faecal incontinence in constipated children is believed to be the consequence of overflow in an already impacted rectum. In one recent study, an association between disturbed pudendo anal-reflex, suggesting a defect in the external anal sphincter innervation (by pudendal nerve, deriving at S2-4), and non-retentive faecal incontinence or constipation was only found in children with OSB. In addition, other studies in adults and children with OSB could not show an association between spinal cord abnormalities and defecation disorders. Therefore the role of spinal cord abnormalities in non-retentive faecal incontinence has yet to be elucidated.

Urinary incontinence is more common in OSB children suffering from constipation compared to those diagnosed with non-retentive faecal incontinence. In one paediatric study 22% of occult spina bifida subjects presented with both urinary and faecal incontinence. Moreover, in spinal cord lesions urinary incontinence may be the only manifestation of underlying spinal cord pathology. However, only two patients with abnormal MRI from our study presented with urinary incontinence which resolved during follow-up.

The neurological abnormalities found in this study consisted of absence the anal reflex (wink) most likely due to an increased tension of the gluteal muscle of the child undergoing physical examination. More interestingly, in 3 out of 4 patients with constipation and abnormal MRI findings, presence of gluteal cleft deviation was found. We therefore suggest to especially perform a lumbar MRI in patients
with intractable defecation disorders presenting with the latter asymmetries. Furthermore MRI of the LSS should be performed in patients presenting with pilonidal dimple and/or a tuft of hair, midline pigmentary abnormalities, sacral agenesis and orthopaedic signs to rule out organic disorders.\textsuperscript{32}

In conclusion, using MRI, LSS abnormalities are found in only 3\% of patients with defecation disorders. This prevalence is low and comparable to LSS abnormalities found in healthy neurologically asymptomatic patients. As all patients with an abnormal MRI achieved defecation symptom relief without neurosurgical intervention, we suggest that performing MRI to assess LSS abnormalities is not required in the standard work-up of children with intractable constipation or non-retentive faecal incontinence. Based on our results we recommend performing MRI in those children presenting with neurological complaints and/or physical symptoms such as gluteal cleft deviation, suggestive for spinal cord abnormalities.
REFERENCE LIST


PART III

Treatment
CHAPTER

Rectal faecal impaction treatment in childhood constipation: enemas versus high doses oral PEG

Noor Bekkali
Maartje van den Berg
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Michiel van Wijk
Olivia Liem
Marc Benninga

ABSTRACT

Background
Rectal faecal impaction (RFI) is a common finding in childhood constipation. No data are available comparing the effect and tolerability of disimpaction with enemas versus oral laxatives polyethylene glycol (PEG).

Aim
To evaluate whether enemas and PEG are equally effective and/or equally tolerated and to assess colonic transit time (CCT) during disimpaction.

Methods
Children (4-16 yrs) with functional constipation and RFI participated. One week prior to disimpaction, rectal examination was performed, symptoms of constipation recorded and CCT$_1$ measurement started. If RFI was determined, patients were randomly assigned to receive enemas once daily or PEG (1.5 g/kg/day) for 6 consecutive days. During this period CCT$_2$ measurement started and a child’s behaviour questionnaire assessed. Primary outcome: success (rectal disimpaction); secondary outcomes: defecation and faecal incontinence frequency, abdominal pain, watery stools, CCTs (before and after disimpaction) and behaviour scores.

Results
Ninety-five patients were eligible; of whom 90 patients (60 male) participated with mean age of 7.5±2.8 years. Forty-six received enemas and 44 PEG, with 5 drop-outs in both groups. Successful disimpaction was achieved with enemas (80%) and PEG (68%); p=0.28. Faecal incontinence and watery stools were more frequently reported with PEG (p<0.01) but defecation frequency (p=0.64), abdominal pain (p=0.33) and behaviour scores were comparable between groups. CCT normalized equally (p=0.85) in both groups.

Conclusion
Enemas and PEG are equally effective in treating RFI in constipated children. Compared to enemas, PEG caused more faecal incontinence with comparable behaviour scores. Both treatments should therefore be equally considered as first line therapy for RFI.
INTRODUCTION

Functional constipation is a common condition in childhood with a worldwide prevalence of 7-30%.\(^1\) Approximately 30-75% of children with longstanding functional constipation have abdominal and/or rectal faecal impaction upon physical examination, which results in severe faecal incontinence in 90% of the patients.\(^2-4\) Faecal impaction has been defined as a large faecal mass, noted either by abdominal palpation or rectal examination, which is unlikely to be passed on demand.\(^5\) It is important to assess the presence of rectal faecal impaction (RFI) in children with constipation, as disimpaction should be achieved before initiation of maintenance therapy.\(^6,7\) If initial disimpaction is omitted, treatment with oral laxatives may paradoxically result in an increase of faecal incontinence due to overflow diarrhoea.

Despite the lack of scientific data, enemas have long been advocated the best first line treatment for severe RFI. It is often assumed however that children strongly dislike enema administration.\(^3,8\) Manual evacuation of faeces under general anaesthesia may diminish the stress for the child. One study however described the risk of structural injury to the anal sphincter following manual disimpaction in constipated adults. Manual disimpaction not only contributes to sphincter weakness in some patients but is also an expensive procedure.\(^9\) Two recent studies showed that oral administration of a high dose of polyethylene glycol (PEG), for 3-6 consecutive days, is effective in as much as 95% of patients to clear rectal faecal impaction.\(^3,10\) However, while Youssef et al. performed an uncontrolled trial where possible adverse events, e.g. faecal incontinence, were not documented, Candy et al. applied an unclear definition for faecal impaction.

We hypothesized that 1) enemas and oral laxatives are equally effective in removing a faecal mass from the rectum but 2) an enema is less tolerated and 3) colonic transit time (CCT) improves during disimpaction. Therefore the aim of our study was to evaluate the efficacy and tolerability of enemas versus high doses of oral PEG in disimpaction of children with functional constipation and RFI. Furthermore, we aimed to evaluate the effect of disimpaction on bowel habits and colonic transit time.\(^11-13\)

METHODS

Study setting & design

Between February 2005 and July 2008 a randomized controlled trial was carried out at a tertiary hospital, Emma Children's Hospital in Amsterdam. The hospital’s
medical ethics committee approved the research protocol. All parents and children aged > 12 years gave written consent.

**Subjects**

Patients were eligible if they were aged between 4 and 16 years and had evidence of RFI upon rectal examination. Furthermore, they had to fulfil at least one of the other Rome III criteria for functional constipation present for at least 8 weeks, being: 1) defecation frequency <3 per week, 2) ≥ 1 faecal incontinence episode per week, 3) history of retentive posturing or excessive volitional stool retention, 4) history of painful or hard defecation, 5) history of large diameter stools which may obstruct the toilet.\(^{14}\) Patients with a history of colorectal surgery or organic cause for constipation were excluded.

**Protocol**

The protocol design is depicted in the figure below:

**Definition of rectal faecal impaction (RFI) & successful disimpaction**

Before study entry, presence of RFI was evaluated by the physician performing a rectal digital exam. Rectal impaction was defined as a large amount of hard stool in the rectum (faecaloma). Successful disimpaction was defined as absence of faecaloma upon rectal examination. If patients were too scared to undergo a second rectal examination an abdominal X-ray was performed to assess RFI.

**Standardized questionnaire & bowel diary**

The standardized questionnaire at intake included questions regarding medical history, age at onset of defecation problems, current bowel habits and laxative use. The standardized bowel diary recorded defecation and faecal incontinence frequency, consistency of stools and abdominal pain.

**Colonic transit time (CTT)**

Whole CTT and segmental CTT were determined using the Bouchoucha et al method.\(^{11}\) Radiograph localization of markers was based on the identification...
of bony landmarks and gaseous outlines as described by Arhan and colleagues.\textsuperscript{11} Patients ingested 1 capsule with 10 radio-opaque markers (sitzmarks\textsuperscript{®}, Bipharma, Weesp) for 6 consecutive days. Subsequently an abdominal X-ray was taken on day 7 in order to count the markers present in the colon and rectosigmoid. The number of markers multiplied by 2.4 determines the total CTT in hours. A total CTT of more than 62 hours, an ascending colon transit time > 18 hrs, a descending transit time > 20 hrs and a recto-sigmoid transit time (RSTT) of > 34 hours was considered delayed.\textsuperscript{11}

**Disimpaction & maintenance treatment**

One group received rectal enemas (dioctylsulfosuccinate sodium (Klyx\textsuperscript{®})) once daily for 6 consecutive days, children < 6 years 60 ml and children \(\geq 6\) years 120 ml. The other group received oral PEG 3350 with electrolytes (Movicol\textsuperscript{®}, 1.5 g/kg/day) for six consecutive days. Maintenance treatment was started after 6 days disimpaction treatment and consisted of oral PEG 3350 with electrolytes (Movicol\textsuperscript{®}, 0.5 g/kg/day) for at least 2 weeks (follow-up).

**Behaviour score: parents’ assessment of how stressful the disimpaction week was**

A child’s behaviour questionnaire containing 7 questions (table 4) evaluating the association between behaviour and laxative treatment was completed by all parents at the end of the disimpaction week.

**Outcome measurements**

The primary outcome was successful disimpaction. Secondary outcome measures defecation and faecal incontinence frequency per week, abdominal pain, watery stools, CTT values and the child’s behaviour, were calculated for children who completed the study protocol.

**Adequacy of sample**

A total sample size of 90 was required to achieve 80% power at a significance level of 0.05 to detect a 20% difference in proportions successful disimpaction between both treatment groups by a two-sided \(\chi^2\)-test, assuming that 75% of children receiving oral laxatives would be treated successfully.

**Data analysis and interpretation**

Patients’ characteristics were documented descriptively. All patients, including those not completing the two study periods according to the protocol, were analyzed according to the intention-to-treat analysis to describe the primary
outcome variable. Comparison of the proportions successful disimpaction between the two groups was performed using the $\chi^2$-test. Difference in defecation and faecal incontinence frequency was analyzed using the Student’s t-test. For CTT analysis, differences in CTT values within groups, between pre-disimpaction and after six days of disimpaction, were assessed by a paired samples t-test; differences between the groups after six days of disimpaction were assessed by analysis of covariance in order to adjust for scores at baseline. Segmental transit times (delayed/ not delayed) were evaluated using $\chi^2$ statistics. Difference in presence (yes/no) of abdominal pain or watery stools was tested using (Yates’ continuity corrected) $\chi^2$ statistics or Fisher’s exact test, depending on cell frequencies. Statistical significance was defined as $p< 0.05$. All analyses were performed using the statistical software package SPSS (version 14.0; Inc, Chicago, IL).

RESULTS

Baseline
Between February 2005 and July 2008, 627 patients with constipation visited our outpatient clinic (figure 2); of whom 90 participated. Forty six and 44 patients were randomized for enemas and PEG, respectively. As depicted in table 1 baseline characteristics were balanced between the two treatment groups. Prior to study enrolment 39% (n=18) and 36% (n=16) from the enema and the PEG group respectively, had a history of enema use ($p=0.83$). A total of 10 patients were drop-outs (figure 2). In the enema group, drop outs were due to receiving 5 enemas instead of 6 (n=1), hospitalization during the study (n=1), non-compliance in recording bowel diaries (n=1) or patients not showing up at the outpatient clinic (n=2). The patient hospitalized during the study, required clinical oral lavage with Klean-prep (1.5 L/day= 88.5 g PEG) for 7 consecutive days and was therefore excluded from analysis. In the PEG group, drop-outs were due to administering low PEG dose (0.5 g/kg/day instead of 1.5 g/kg/day) (n=3), non-compliance in recording bowel diaries (n=1) and one patient did not return for follow-up.

Enemas versus oral PEG
Successful disimpaction was achieved in 37 (80%) and 30 (68%) patients from the enema group and PEG group ($p=0.28$), respectively (figure 2). Three patients from the enema group with unsuccessful initial disimpaction, achieved successful disimpaction after extension of the rectal treatment with one enema for one day in combination with PEG maintenance treatment. Patients who initially failed oral disimpaction (n=9), achieved successful disimpaction with addition of one enema
daily for a total of 3 days in 4 of them. Patients who failed a second intensive oral or rectal disimpaction regime were admitted to the clinic for colonic lavage (figure 2).

**Bowel habits & symptoms**

As shown in table 1 and 2, a significant increase in defecation frequency was achieved in both groups after the disimpaction week. The frequency of faecal incontinence per week was significantly lower in the enema group (p<0.001) during

**Table 1.** Baseline characteristics, including (left columns) and excluding (right columns) the drop-outs. PEG: poly-ethylene glycol. FU: follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Total included patients</th>
<th>Patients with successful FU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enema</td>
<td>PEG</td>
</tr>
<tr>
<td>Number</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Mean ±SD age in years</td>
<td>7.9±2.9</td>
<td>7.2±2.6</td>
</tr>
<tr>
<td>Mean ±SD defecation/wk</td>
<td>1.9±2.4</td>
<td>1.5±1.8</td>
</tr>
<tr>
<td>Mean symptom duration</td>
<td>5.2±3.3</td>
<td>4.7±2.8</td>
</tr>
<tr>
<td>Presence abdominal faecal mass</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Mean ±SD daytime faecal incontinence/wk</td>
<td>15.7±13.1</td>
<td>16.6±12.4</td>
</tr>
<tr>
<td>Mean ±SD night time faecal incontinence/wk</td>
<td>1.2±2.4</td>
<td>1.0±2.4</td>
</tr>
<tr>
<td>No. abdominal pain</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>No. watery stools</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
disimpaction but not at follow-up (p=0.58). Furthermore, watery stools were more frequently reported in the PEG group during disimpaction (10 vs. 28; p<0.001) and at follow-up (4 vs. 13; p=0.03)

**Colonic transit time**

Two patients in the enema group and six patients in the PEG group were not able to ingest the radio-opaque markers. Before disimpaction, delayed CTT was found in 42 (95%) and 37 (97%) patients from respectively the enema and the PEG group; delayed RSTT was found in respectively 33 (75%) and 33 (87%) of the patients (table 3). As shown in table 3, a significant decrease in colonic transit time was found

### Table 2. Bowel habits and gastro-intestinal symptoms after six days of disimpaction & follow-up (2 weeks after disimpaction). PEG: poly-ethylene glycol

<table>
<thead>
<tr>
<th></th>
<th>Disimpaction</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enema (n=46)</td>
<td>PEG (n=44)</td>
</tr>
<tr>
<td>Defecation frequency/ week ±SD</td>
<td>5.8±3.6</td>
<td>8.8±8.5</td>
</tr>
<tr>
<td>Faecal incontinence/ week ±SD</td>
<td>3.4±4.3</td>
<td>13.6±12.6</td>
</tr>
<tr>
<td>Patients with abdominal pain</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Patients with watery stools</td>
<td>10</td>
<td>28</td>
</tr>
</tbody>
</table>

### Table 3. Total and segmental colonic transit times

<table>
<thead>
<tr>
<th>Transit time (hours)</th>
<th>Enema Intake (n=44)</th>
<th>Enema Disimpaction (n=41)</th>
<th>PEG Intake (n=38)</th>
<th>PEG Disimpaction (n=39)</th>
<th>*p-value</th>
<th>**p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Median</td>
<td>14.4</td>
<td>7.2</td>
<td>21.6</td>
<td>12.0</td>
<td>0.24</td>
<td>0.47</td>
</tr>
<tr>
<td>· Percentiles 25-75</td>
<td>7.2-43.2</td>
<td>2.4-21.6</td>
<td>9.0-50.4</td>
<td>7.2-24.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Delayed &gt; 18 hrs</td>
<td>46%</td>
<td>33%</td>
<td>59%</td>
<td>44%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Descending colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Median</td>
<td>21.6</td>
<td>9.6</td>
<td>24.0</td>
<td>7.2</td>
<td>0.69</td>
<td>0.48</td>
</tr>
<tr>
<td>· Percentiles 25-75</td>
<td>9.6-50.4</td>
<td>2.4-19.2</td>
<td>12.0-39.0</td>
<td>4.2-21.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Delayed &gt;20 hrs</td>
<td>51%</td>
<td>23%</td>
<td>56%</td>
<td>32%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recto-sigmoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Median</td>
<td>57.6</td>
<td>24.0</td>
<td>61.2</td>
<td>20.4</td>
<td>0.57</td>
<td>0.07</td>
</tr>
<tr>
<td>· Percentiles 25-75</td>
<td>38.4-79.2</td>
<td>8.4-42.0</td>
<td>43.2-79.8</td>
<td>11.4-24.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Delayed &gt; 34 hrs</td>
<td>75%</td>
<td>29%</td>
<td>87%</td>
<td>13%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Median</td>
<td>117.6</td>
<td>37.2</td>
<td>120.0</td>
<td>43.2</td>
<td>0.89</td>
<td>0.78</td>
</tr>
<tr>
<td>· Percentiles 25-75</td>
<td>86.4-136.4</td>
<td>24.6-67.8</td>
<td>98.4-141.6</td>
<td>27.6-67.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Delayed &gt; 62 hrs</td>
<td>95%</td>
<td>72%</td>
<td>97%</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-values for difference between the groups at * intake and during ** disimpaction Segmental and total CTT significantly decreased after disimpaction in both groups; p<0.001
between intake and disimpaction in all colonic segments (p<0.001). No significant differences were found in CTT between the two groups at all time points.

**Behaviour score: parents’ assessment of how stressful the disimpaction week was**

A total of 38 patients of the enema group (93%) and 31 (79%) patients of the PEG group completed the questionnaires (table 4). Struggle to administer medication as well as actions necessary to enable treatment and levels of anxiety were equally

| Table 4. Behavior score: parents’ assessment of how stressful the disimpaction week was, completed at the end of disimpaction week (t=2). PEG= poly-ethylene glycol |
|---------------------------------------------------------------|-----------------|-----------------|-----------------|
| Struggle to administer oral or rectal treatment               | Enema n=38      | PEG n=31        | p-value         |
| - Yes                                                         | 24              | 17              | 0.18            |
| - No                                                          | 14              | 14              |                 |
| Actions necessary to enable treatment (e.g. distraction)      |                 |                 |                 |
| - Yes                                                         | 21              | 18              | 0.25            |
| - No                                                          | 17              | 13              |                 |
| More anxious during disimpaction                              |                 |                 |                 |
| - Yes                                                         | 36              | 25              | 0.13            |
| - No                                                          | 2               | 6               |                 |
| Abdominal pain soon after treatment                           |                 |                 |                 |
| - Yes                                                         | 31              | 16              | 0.008           |
| - No                                                          | 7               | 15              |                 |
| If abdominal pain, how long did the pain last?                |                 |                 |                 |
| - <5 min                                                      | 6               | 5               |                 |
| - 5-15 min                                                    | 10              | 3               |                 |
| - 15-30 min                                                   | 7               | 2               |                 |
| - 30-60 min                                                   | 2               | 1               |                 |
| - > 1 hour                                                    | 5               | 2               |                 |
| - Not applicable or not recorded                              | 8               | 18              |                 |
| Who administered the enema to the child?                      |                 |                 |                 |
| - Father                                                      | 5               |                 |                 |
| - Mother                                                      | 22              |                 |                 |
| - Both                                                        | 9               |                 |                 |
| - Someone else                                                | 2               |                 |                 |
| - Not applicable                                              | 0               | 31              |                 |
| After how much time did defecation occur?                     |                 |                 |                 |
| - <5 min                                                      | 5               |                 |                 |
| - 5-15 min                                                    | 25              |                 |                 |
| - 15-30 min                                                   | 6               |                 |                 |
| - 30-60 min                                                   | 1               |                 |                 |
| - Not applicable                                              | 1               | 31              |                 |
reported in both groups. Abdominal pain directly after administration of the laxative was more frequently reported in the enema group (n=31) compared to the PEG group (n=16; p=0.008). Abdominal pain occurring directly after enema use resolved within 30 minutes in 23 out of 30 patients (77%).

**DISCUSSION**

This is the first prospective randomized controlled study demonstrating that enemas and high doses PEG (1.5 g/kg) are equally effective in treating rectal faecal impaction in children with constipation. However, children receiving enemas reported less faecal incontinence episodes and watery stools but more abdominal pain directly after enema application. Defecation frequency increased in both groups and the occurrence of abdominal pain during the day as reported in the bowel diaries was not different between the groups. Surprisingly, extra effort to administer medication as well as tricks necessary to enable treatment was equally reported in both groups.

The dosage (PEG 1.5g/kg/day) and duration (6 days) of oral and rectal disimpaction was based on previous studies showing a mean disimpaction time of 3-7 days. Using this regimen successful disimpaction was achieved with enemas and PEG in respectively 80% and 68% of the children in our study. These results are in accordance with other studies in which success with high doses of oral PEG was reached in 92-97% of cases. In a retrospective chart review extracting clinical outcomes across 5 hospitals in England and Wales, it was found that enemas were successful in 73% of children with faecal impaction compared to 97% in those receiving PEG. It is however not possible to compare our results with the latter study since a definition for faecal impaction is lacking. Furthermore, it is unclear how the investigators confirmed disimpaction in their study. The strength of this study was that only children were included and re-evaluated after therapy by either rectal examination or abdominal X-ray.

As expected a high dosage of PEG resulted in an increase of the faecal incontinence frequency during the disimpaction period. Poly-ethylene glycol is a soluble inert polymer which acts by hydrogen bonding water molecules, to expand the volume in the large intestine resulting in softer and more watery stools. Until the faecaloma has been cleared, soft stool leaks along the faecal mass in the rectum. This increase in faecal incontinence episodes was also found in a randomized controlled trial evaluating the efficacy of PEG 3350. In contrast, rectal enemas (dioctylsulfosuccinate) are hypertonic and stimulate direct contraction of the colon. Direct contraction stimulates the rectum to empty the faecal mass which explains why faecal incontinence episodes appeared less with enemas. However, as
expected, due to its contractile effect, abdominal pain directly after treatment was more frequently reported in the enema group. The increase in peristalsis might be experienced as cramping and hence abdominal pain. Nevertheless, the majority (77%) had abdominal pain relief within 30 minutes and overall abdominal pain, as reported in the bowel diaries, was not different between the treatment groups. Probably, parents and children qualified the abdominal pain directly after enemas differently.

Faecal incontinence is associated with lower quality of life regarding both physical and psychosocial functioning as reported by parents and constipated children. Therefore it is important to inform child and parents that disimpaction with oral PEG is likely to cause more episodes of faecal incontinence compared to disimpaction with enemas. In accordance with an earlier study, we observed a significant decrease in faecal incontinence episodes after the intensive disimpaction period in the current study.

This is the first study comparing change in behaviour in constipated children, by using a questionnaire, between treatment with enemas and treatment with oral laxative therapy. In accordance with the general assumed opinion towards enema use in children, we indeed found that 95% of children receiving enemas exhibited fearful behaviour. But we also found fearful behaviour in 81% of children receiving oral laxative treatment. Given this comparable behaviour in both groups, disimpaction with enemas should not necessarily be withheld to prevent anxiety. We did not find more fearful behaviour in the enema group which might be explained by the administration of enemas by parents at home instead of a nurse in an unfamiliar environment (hospital) which is more commonly done in practice. In adults retrograde colonic irrigation, which is applied by the patients themselves, improved both quality of life and bowel habits.

Rectal examination, to confirm the diagnosis constipation is controversial. Many paediatricians advocate avoidance of rectal examination and invasive treatments, such as rectal enemas to prevent uncomfortable, painful and/or embarrassing situations. However, the recently published NASPGHAN guidelines for constipation in infants and children recommend however at least one digital examination of the anorectum to evaluate the amount and consistency of stool and its location within the rectum and to identify organic disorders. In our centre, rectal examination is performed routinely in children presenting with constipation. If faecal impaction is present, rectal disimpaction is performed using enemas. This treatment regimen was based on a small study suggesting that rectal disimpaction shortly after the onset of symptoms is more effective than less aggressive means of therapy. Since this study shows that enemas are not superior to oral laxatives we question the need of rectal examination as prerequisite for the choice of either oral or rectal treatment. We suggest only performing rectal examination in children in whom the diagnosis of constipation is uncertain when they only meet one symptom of
the Rome-III criteria for constipation. Furthermore a rectal examination should be performed when symptoms of constipation persist after initial oral or rectal disimpaction. Even though anatomic problems are rare, a rectal examination may be necessary in these children.

In this study colonic transit time measurements were used as a non-invasive tool to localize delay of colonic transit and to verify the effect of disimpaction. In contrast to previous observations in children with constipation, both total and RSCTT were more delayed in our current study. In our study however, only children with a large palpable rectal mass were included. These children have significantly longer colonic transit times than children with symptoms of constipation without rectal faecal impaction. The latter phenomenon, outlet obstruction, delay of transit at the level of the rectum, is found in both constipated children and constipated adults. Indeed in our study we found in 75-87% of the patients a delay in RSTT. We also demonstrated that both CTT and RSTT improved while defecation frequency increased during both oral and rectal disimpaction. This is in concordance with the suggestion that a distended rectum, with faeces, slows down the motor activity of the colon and the existence of an inhibitory recto-colonic feedback mechanism. It was remarkable however that even after disimpaction 72-75% still had delayed CTT. This percentage is higher compared to earlier studies in a comparable group constipated children with rectal faecal impaction in 30-36%. It is likely that in our current study we included children with more severe motility disorders given the impacted rectum in all these children and the presence of palpable abdominal faecal mass in 37-66% of them.

This study had also its limitations. As we included children with a history of enema use as well as those without, the findings concerning fearful behaviour might be confounded. However, it is unclear whether children with positive history of enema would be more or less anxious towards enemas. The latter could not be extracted from the behaviour questionnaires we used in our study. A second limitation is the assessment of behaviour scores only after start of disimpaction. Nevertheless, the questions were addressed in a way to detect changes in behaviour rather than general behaviour at a single point in time.

In conclusion, we demonstrated that enemas and oral laxatives were equally effective in treating rectal faecal impaction in functional childhood constipation. Therefore, rectal enemas and oral laxatives should be equally considered as the first line therapy.
**REFERENCE LIST**


The role of a probiotics mixture in the treatment of childhood constipation: a pilot study

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Maartje van den Berg
Olivia Liem
Marc Benninga

ABSTRACT

Background
Inconsistent data exist about the efficacy of probiotics in the treatment of constipation. Several studies in adults with constipation showed positive effects of probiotics on constipation. Inconsistent data exist regarding the effect of a single probiotic strain in constipated children. The aim of this pilot study was to determine the effect of a mixture of probiotics containing bifidobacteria and lactobacilli in the treatment of childhood constipation.

Methods
Children aged 4-16 years with constipation as defined by the Rome III criteria were eligible for the study. During a 4 week period, children received a daily mix of 4x10⁹ colony forming units of a probiotic mixture (Ecologic® Relief) containing Bifidobacteria (B.) bifidum, B. infantis, B. longum, Lactobacilli (L.) casei, L. plantarum and L. rhamnosus. Primary outcome measures were frequency of bowel movements (BMs) per week and stool consistency. Secondary outcome measures were number of faecal incontinence episodes per week, abdominal pain and side effects.

Results
Twenty children, 50% male, median age 8 (range 4-16) were included. The frequency of BMs per week increased from 2.0 (1.0-5.0) to 4.2 (0.0-16.0) in week 2 (p=0.10) and 3.8 (2.1-7.0) in week 4 (p=0.13). In 12 children presenting with <3 BMs/ week, BMs per week increased significantly from 1.0 (0.0-2.0) to 3.0 (0.0-7.0) in week 2 (p=0.01) and 3.0 (0.0-10.0) in week 4 (p=0.01). The stool consistency was reported as hard in 7 children at baseline, in 4 children at week 2 (p=0.23) and in 6 children after 4 weeks of treatment (p=1.00). A significant decrease in number of faecal incontinence episodes per week was found in the entire group: 4.0 (0.0-35.0) to 1.5 (0.0-14.0) in week 2 (p=0.01) and 0.3 (0.0-7.0) in week 4 (p=0.001). The presence of abdominal pain decreased significantly from 45% to 25% in week 2 (p=0.04) and 20% at week 4 (p=0.006). No side effects were reported.

Conclusion
This pilot study shows that a mixture of probiotics has positive effects on symptoms of constipation. To confirm these findings, a large randomised placebo controlled trial is required.
BACKGROUND

Functional constipation is a common and frustrating phenomenon in children. The prevalence of childhood constipation in the western world is 1-30% 1. No organic cause is found in 90% to 95% of those constipated children 2. This functional defecation disorder is characterized by infrequent defecation less than three times per week, more than two episodes of faecal incontinence per week, the passage of large and painful stools which clog the toilet and retentive posturing. Upon physical examination a palpable faecal mass is often found in the abdomen and the rectum 3, 4.

Childhood constipation is usually treated with a combination of toilet training, a bowel diary and oral laxatives such as lactulose or polyethylene glycol (PEG). Laxatives aim to soften the stools, thereby contributing to a break-through of the vicious circle of defecation avoidance caused by pain during defecation. Only 60% of constipated children accomplish successful treatment with laxatives 5. It is clear that development of other treatment options is required.

There is growing interest in the use of probiotics in organic and functional gastrointestinal disorders. Probiotics are live microbial food ingredients which are reported to be effective in the treatment of IBD, travellers diarrhoea and constipation 6-9. Colonic microflora influences the peristalsis of the colon 10. Therefore, imbalance in the colonic microflora has been suggested to play a role in gastro-intestinal diseases such as constipation. Probiotics, such as Bifidobacteria (B.) and lactobacilli (L.), both produce lactic, acetic and other acids resulting in a lowering of pH in the colon. A lower pH enhances peristalsis of the colon and subsequently decreases colonic transit time which is beneficial in the treatment of constipation 11, 12. The latter hypothesis was confirmed showing a decrease in colonic transit time in healthy adults consuming B. animalis as supplement 13. To date, several studies have been performed, mainly in adults, in order to determine the effects of probiotics on symptoms of constipation 12, 14-18. It has been shown that probiotic strains, such as L. shirota and the B. infantis, increase defecation frequency and soften stools in adults with constipation and IBS 19, 20. A recent study in children with constipation showed an increase in defecation frequency and a decrease in abdominal pain using the strain L. rhamnosus 21. In contrast to the latter study however, the probiotic strain Lactobacillus GG did not have an additional positive effect on constipation symptoms, when used as an adjunctive therapy with lactulose 22.

In constipated elderly, two different strains the Lactobacillus rhamnosus and propionibacterium freudenreichii resulted in a small but significant increase in defecation frequency, whereas the use of a single strain did not affect defecation frequency 23.
The interpretation of these clinical trials is difficult to compare due to the differences in endpoints, variations in probiotics used, dose and strains. Nonetheless, a recent review suggested that overall, sufficient evidence is available to warrant further evaluation

Therefore we hypothesized that a combination of several strains of bifidobacteria and lactobacilli might be effective in the treatment of childhood constipation. In a pilot study, we aimed to determine the therapeutic effect of a combination of probiotics strains, containing the bifidobacteria *B. bifidus*, *B. infantis* and *B. longum* and the lactobacilli *L. casei*, *L. plantarum* and *L. rhamnosus*, on childhood constipation.

**METHODS**

**Subjects**

Children between 4 to 16 years of age referred to the outpatient clinic of the Emma Children’s Hospital in Amsterdam, the Netherlands, with constipation were eligible for study entry. Childhood constipation was defined by the Rome III criteria as having at least 2 out of 6 of the following symptoms: bowel movements <3 times/week; faecal incontinence >2 times/week; large amounts of stools obstructing the toilet once in 10 days; painful defecation; withholding behaviour; palpable abdominal or rectal mass on physical examination. Exclusion criteria were the use of any oral laxative < 4 weeks before intake, mental retardation, metabolic disease, functional non-retentive incontinence, and a history of gastro-intestinal surgery. All children older than 12 years and/or parents gave informed consent. This pilot was approved by the medical ethical committee of the Academic Medical Centre of Amsterdam.

**Study design**

Seven days prior to baseline assessment and during the treatment period all children recorded frequency of bowel movements, the number of faecal incontinence episodes, stool consistency, abdominal pain, flatulence and pain during defecation as well as adverse effects such as vomiting and diarrhoea in a standardized bowel diary.

At baseline assessment, a medical history and information on the current defection pattern was collected. Additionally, a physical examination including a rectal digital exam was performed to assess whether an abdominal or rectal faecal mass was present.

Before start of the probiotics treatment, all children received once daily for 3 days a rectal enema (Klyx: sodium-dioctylsulfosuccinate and sorbitol) in order
to accomplish rectal disimpaction. After rectal disimpaction, children were administrated a daily probiotics mixture of $4 \times 10^9$ colony forming units (CFU), containing *Bifidobacteria* (*B.*) *bifidum*, *B. infantis*, *B. longum*, *Lactobacilli* (*L.*) *casei*, *L. plantarum* and *L. rhamnosus* (*Ecologic® Relief, Winclove Bio Industries BV, The Netherlands*) for 4 weeks. During the treatment period children were instructed to start toilet training. Toilet training consisted of sitting on the toilet 3 times per day for 5 minutes after each meal with the intention of trying to defecate. The use of laxatives was not allowed during the short treatment period.

Evaluation was conducted during visits to the outpatient clinic at 2 and 4 weeks after start of treatment. During each visit the physician assessed the patient’s daily bowel diary and examined the child.

**Outcome measures**

Primary outcome measures were frequency of bowel movements per week and stool consistency. Stool consistency was rated by the patients as hard, normal or watery. Secondary outcome measures were number of faecal incontinence episodes per week, presence of abdominal pain and incidence of adverse effects such as vomiting and diarrhoea.

**Analysis**

Descriptive statistical measures were calculated for baseline characteristics using SPSS version 12.0.1 statistical software (SPSS Inc, Chicago, Ill). Change of frequency of bowel movements and faecal incontinence was assessed using the non-parametric paired Wilcoxon test. For the analysis of change of stool consistency, the Mc Nemar test was used. For the comparison of abdominal pain between baseline and the evaluation time points, the Wilcoxon rank test was used. All continuous values were expressed as median (range). A p-value < 0.05 was considered significant.

**RESULTS**

Between February 2006 and July 2006, 20 children were enrolled into this pilot study and all patients completed the study. Baseline characteristics are summarized in table 1. In 85% of the children, onset of constipation symptoms was between 0 to 4 years of age.

The frequency of bowel movements (BMs) per week increased from 2.0 (1.0-5.0) to 4.2 (0.0-16.0) in week 2 (p=0.10) and 3.8 (2.1-7.0) in week 4 (p=0.13) (figure 1). In 12 children presenting with <3 BMs per week, BMs per week increased significantly...
Table 1. Baseline characteristics.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>20</td>
</tr>
<tr>
<td>Age in years</td>
<td>8 (4-16)</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>10</td>
</tr>
<tr>
<td>Time of constipation before intake (years)</td>
<td>3.5 (0.3-6.5)</td>
</tr>
<tr>
<td>Treatment time before intake (months)</td>
<td>12 (0-48)</td>
</tr>
<tr>
<td>Bowel habits, n (%)</td>
<td></td>
</tr>
<tr>
<td>• Bowel movements &lt; 3 / week</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>• Faecal incontinence ≤ 2/ week</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>• Faecal incontinence &gt; 2/ week</td>
<td>15 (80%)</td>
</tr>
<tr>
<td>• Large amounts of stools</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Stool consistency, n (%)</td>
<td></td>
</tr>
<tr>
<td>• Hard stools</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>• Normal stools</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>• Watery stools</td>
<td>0</td>
</tr>
<tr>
<td>Painful defecation, n (%)</td>
<td></td>
</tr>
<tr>
<td>• No pain</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>• Sometimes painful</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>• Always painful</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Abdominal pain, n (%)</td>
<td></td>
</tr>
<tr>
<td>• No abdominal pain</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>• Sometimes abdominal pain</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>• Often abdominal pain</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Physical examination, n (%)</td>
<td></td>
</tr>
<tr>
<td>• Abdominal scybala</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>• Rectal scybala</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>• Anal Fissures</td>
<td>0</td>
</tr>
</tbody>
</table>

from 1.0 (0.0-2.0) to 3.0 (0.0-7.0) in week 2 (p=0.01) and 3.0 (0.0-10.0) in week 4 (p=0.009) (figure 2).

The stool consistency was reported as hard in 7 children at baseline, in 4 children at week 2 (p=0.23) and in 6 children at week 4 (p=1.00). At week 4, hard stools appeared in 5 children who also had hard stools at baseline. One child, with normal stools at baseline, reported hard stools only at the end of the study. Two of the 7 children, who presented with hard stools, reported normal stools at the end of the study. The number of faecal incontinence episodes per week decreased significantly from 4.0 (0.0-35.0) to 1.5 (0.0-14.0) in week 2 (p=0.007) and 0.3 (0.0-7.0) in week 4 (p=0.001) (figure 3).

The presence of abdominal pain decreased significantly from 45% (n=9) to 25% (n=5) in week 2 (p=0.04) and 20% (n=4) at week 4 (p=0.006). There were no side effects such as vomiting, bloating and increased flatulence during the study period.
DISCUSSION

This pilot study showed that a probiotics mixture containing different strains of bifidobacteria and lactobacilli, increases the frequency of bowel movements in constipated children presenting with a defecation frequency of less than 3 times per week. This probiotic mixture was also effective in decreasing the number of faecal incontinence episodes and in reducing the presence of abdominal pain. No significant changes in stool consistency were found.

Given their safety profile, probiotics could be an attractive compound to manipulate gastrointestinal motility in constipated children. However, exact mechanisms underlying enhancement of gastrointestinal transit are not yet unraveled. Based on the results of our pilot study we hypothesise that a mixture of bifidobacteria and lactobacilli producing lactic, acetic and other acids resulting in a lowering of pH in the colon are effective in enhancing motility of the colon, subsequently leading to a decrease in colonic transit time. A large randomised placebo controlled trial is necessary to confirm these findings.

In this study, we found that administration of a mixture of probiotics had a positive effect on frequency of bowel movements and consequently leading to a decrease in faecal incontinence episodes. In contrast to our findings, Banaszkiewicz showed no additional effect of lactobacillus GG (LGG) to placebo in children with constipation who were all treated with lactulose. The authors suggested that the probiotic strain LGG may not provide clinical benefits in the treatment of constipation. Furthermore they assumed that the failure of LGG to provide synergistic effects (with lactulose) occurred despite the tempting notion that the concurrent use of lactulose with proven probiotic properties (ie, it promotes growth of lactobacilli in the colon) should enhance the therapeutic effects of LGG. A second study in which a group receives LGG alone, is needed to more directly examine this issue.

It has been assumed that probiotics soften the stools by stimulating water and electrolyte secretion. However, we were not able to show a significant softening of stools after 4 weeks of treatment. As only a minority of children (35%) had hard stools at baseline, it is necessary to investigate whether this probiotic mixture has a positive effect on hard stools in a larger randomised controlled trial.

A significant decrease in abdominal pain was found after 4 weeks of treatment with the probiotics mixture. This is in accordance with one paediatric study and several adult studies performed in irritable bowel syndrome (IBS) patients with abdominal pain/discomfort, distension/ bloating and difficult defecation. A recent randomised placebo controlled trial conducted in 360 women with IBS showed that the strain B. infantis was associated with significant improvement of both abdominal pain and the subjects’ global assessment of symptoms. This positive effect on abdominal pain occurred irrespective of any effect on stool frequency which indicates that the observed effect was not attributable to either a laxative
or anti-diarrhoeal effect. It has been suggested previously that abdominal pain
and bloating may decrease as a consequence act of probiotics diminishing visceral
hypersensitivity by its anti-inflammatory effect on the enteric mucosa. No
side effects of the probiotics were found in our study. This is in accordance with
literature about the safety of probiotics. The safe use of especially bifidobacteria
is supported by the long historical consumption of fermented milk and growing
knowledge about bifidobacteria taxonomy and physiology. Furthermore, studies
performed with lactobacilli and bifidobacteria showed to be well tolerated in adults
and children.

The interpretation of clinical trials of probiotic strains in functional gastrointestinal
disorders is complicated by several factors. Results between studies are difficult to
compare due to differences in endpoints, variations in probiotics dose and strains.
Whereas one group uses mixtures of probiotics, others use single isolates, making
it difficult to determine what were the active moieties. Nonetheless, a recent
review suggested that overall, sufficient evidence is available to warrant further
evaluation.

In conclusion, this non randomised non placebo controlled pilot study evaluating
the effect of a mixture of probiotics, showed beneficial effects on symptoms of
constipation and a decrease of abdominal pain. Therefore a randomised placebo
controlled trial is now required to confirm these data.
REFERENCES


Summary & future perspectives
SUMMARY

More than 99% of term born infants pass their first stool, meconium, within 48 hours after birth. Preterm born infants, however have a delayed first passage of their stools. Information concerning the total duration of meconium passage is however lacking. In the first part of this thesis duration of passage of meconium as a measure for colonic motility was prospectively compared between preterm and term born infants. Furthermore, characteristics of infant stools such as amount, consistency and colour, were evaluated and served as basis for the development of the ‘Amsterdam’ infant stool form scale.

Constipation, is found in 10.4% of children in both Western and Asian countries, when defined as defecation frequencies below three times per week without evidence for organic disease. In 95% of patients presenting with constipation no organic cause is found. Therefore the second part of this thesis focussed on two specific pathophysiological mechanisms, the lumbar spinal cord and chloride secretion in the rectum, which might be involved in the development of childhood constipation.

In the last part of the thesis, new treatment regimens as well as existing therapies for constipation were compared in clinical studies.

PART I Stool characteristics and bowel habits

In part I we focused on describing factors responsible for differences in bowel habit aspects, such as stool frequency and consistency, between preterm and term infants. In Chapter 1 meconium passage was compared between preterm and term born infants. This study showed, similar to previous studies, that first passage of meconium was delayed in the preterm born infant. In addition, our new data showed that total duration of meconium stools was also prolonged in preterm infants. We found a mean duration of meconium passage of 8 days in preterm infants compared to 3 days in term born infants. Hence, meconium stools are not only delayed but also prolonged in the premature born infant. A possible explanation might be delayed motility of the gastrointestinal tract in premature born infants. In Chapter 2 we described the development of an infant stool form scale, the “Amsterdam Infant Stool Form Scale”, for infants <24 months of age. This scale can be used as a tool for parents and physicians to describe stool characteristics facilitating diagnosis such as constipation or diarrhoea. The scale describes stool amount, consistency and colour. The use of this scale showed good inter-observer and intra-observer agreement scores. However, future studies are necessary to validate the applicability and validity of this scale for practical and research purposes. In Chapter 3 a cohort of term and preterm born infants were
followed from the first day of life up to the age of 24 months. In the first and second week, breastfed infants had 2.41 more episodes of defecation per week compared to the formula-fed infants. A higher median defecation frequency was only found in week 1 in the term compared to the preterm group. Overall median defecation frequency was 16 (7-30) per week from birth up to the age of 24 months over the total group of participating infants. Based on these findings we conclude that term and preterm born infants have comparable defecation frequency from the second week of life up to the age of 24 months. Additional stool characteristics such as stool colour and consistency were also comparable between both groups during the follow-up period.

PART II Pathophysiology

In part II we focused on elucidating possible underlying pathophysiological mechanisms for functional constipation in children. In Chapter 4 we evaluated chloride secretion in the rectum of children with intractable functional constipation compared to controls. Chloride secretion is a measure for fluid secretion in the colon and plays an important role in water and electrolytes homeostasis. In our study we showed rectal chloride secretion disturbance in children with functional constipation following stimulation with histamine. Whether this defect occurs at the level of the histamine receptor, components of the receptor-linked signal transduction pathway or basolateral Ca²⁺-sensitive K⁺ channels enhancing the electrical driving force for apical chloride secretion, remains to be explored. In Chapter 5 we critically evaluated rectal suction biopsies of children with functional constipation, children with Hirschsprung’s disease and controls. It was demonstrated that the rectal muscularis mucosae was thicker in functional constipation compared to Hirschsprung’s disease and controls. No differences were found between characteristics of the muscularis mucosae of controls and children with Hirschsprung’s disease. Interestingly, we found a correlation between duration of functional constipation symptoms and thickness of the muscularis mucosae. The latter finding suggests that the rectal muscularis mucosae hypertrophy might be secondary to constipation symptoms such as rectal faecal impaction. In Chapter 6 we evaluated the role of spinal cord lesions in childhood constipation. Using magnetic resonance imaging (MRI) we found lumbar sacral spine abnormalities in 3% of children presenting with intractable constipation. Without neurosurgical interventions, all children were successfully treated with laxative and behavioural treatment. Therefore, the relevance of the found lumbo-sacral abnormalities, such as lipomas, remains questionable as all children reached success with conservative treatment only.
PART III Therapy

In part III we evaluated new and compared existing therapeutic regimens for treating childhood functional constipation.

In Chapter 7 two treatment regimens for rectal faecal disimpaction were compared in a randomised controlled manner. Children with constipation and rectal faecal impaction were either randomised for enema therapy or for high doses oral laxatives during 6 consecutive days. We found no statistically different success percentage between both treatment options indicating that rectal enemas (80% success) are not superior to oral laxatives (68% success) for treating rectal faecal impaction. Furthermore fearful behaviour was not more reported in those receiving enemas compared to the group receiving oral laxatives. As expected, oral laxatives were associated with more frequent episodes of faecal incontinence and an increase of watery stools compared to those receiving enema therapy. Given these results, both treatments should be equally considered as first line therapy for treating rectal faecal impaction. Nonetheless, one should take into account the preference, of both the child and/or parents, for prescribing either an oral or rectal intervention. After disimpaction, maintenance therapy with oral laxatives is necessary. In many countries polyethylene glycol (PEG) is the first line maintenance therapy for children with constipation. In Chapter 8, a mixture of probiotics was evaluated in the treatment of childhood constipation. In this uncontrolled study we found that especially children presenting with a defecation frequency <3 times per week had a significant increase in defecation frequency. In addition, all participating children had a decrease in faecal incontinence frequency and episodes of abdominal pain but no difference was found in stool consistency. Since this uncontrolled study showed beneficial effects on symptoms of constipation a randomised placebo controlled trial is justified to evaluate the role of this mixture probiotics in treating functional constipation in children.
DISCUSSION AND FUTURE PERSPECTIVES

To date, little is known about normal bowel habits in the first years of life. The necessity for more prospectively obtained data concerning infants and young children is mandatory, not only to define normal bowel habits but also to recognise abnormal bowel habits such as constipation. Since the Rome III criteria lack clear definitions for constipation in infants, clinical experience is often the reason to start laxative treatment. The results of previous studies evaluating bowel habits in infants, in combination with the use of the Amsterdam infant stool form scale hopefully will lead to a more scientific based treatment of infants with defecation problems. Previously, hard consistency of stools and painful bowel movements were found in the majority of constipated patients younger than 2 years. Accordingly, we showed in chapter 3 of this thesis that hard stools were one of the main reasons to start laxative treatment. Therefore, in addition to defecation frequency, hard stools would also be a good candidate to characterise constipation in infancy. In 2006 the Rome-III committee decided that a defecation frequency fewer than 3 per week is one of the criteria for functional constipation in neonates and toddlers. However we and others clearly showed that the normal defecation frequency in infants < 2 years is 1-2 per day. Therefore we suggest to change the Rome III criterion, defecation frequency, to <1 per day rather than <3 per week for infants 24 months and younger with constipation.

The aetiology of childhood constipation has yet to be further clarified. We therefore evaluated different aetiological aspects of constipation in this thesis. In chapter 4, we found disturbed rectal chloride secretion in children with functional constipation following stimulation with histamine. Whether this defect occurs at the level of the histamine receptor, components of the receptor-linked signal transduction pathway or basolateral Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels that enhance the electrical driving force for apical chloride secretion, remains to be explored. Yet we only assessed chloride secretion in the rectum rather than in the total colon. In future experiments, assessment of chloride secretion in the different parts of the colon would provide more insight into the importance of disturbed chloride secretion. Lubiprostone is a fatty acid derivative of a metabolite of prostaglandin E1 and acts as a chloride channel activator to increase intestinal fluid secretion and transit. Indeed, several clinical trials showed that chloride secretion stimulation with this chloride channel activator is effective in the treatment of chronic constipation in adults. In children however, such clinical studies have yet to be conducted. Withholding behaviour is suggested to be the major cause of constipation in children. This conscious contraction of the pelvic floor muscles and consequent faecal stasis in the rectum may cause histological changes in the rectal muscularis mucosae (RMM). Indeed, we found that the RMM was hypertrophied and
disorganised in children with constipation compared to controls and children with Hirschsprung’s disease. More interestingly, we found a correlation between muscle thickness and duration of symptoms. These findings suggest that muscularis mucosae hypertrophy might be secondary to constipation symptoms such as rectal faecal impaction. To investigate the latter, a study assessing muscle thickness in constipated children before and after therapy (rectal disimpaction and maintenance laxative therapy) is recommended.

Constipation is a common symptom in children with spinal cord lesions. The mechanism by which spinal cord lesions produce constipation is unknown. A retrospective study showed 9% lumbo-sacral abnormalities in children presenting with intractable constipation. More importantly, 88% had symptom relief following neurosurgical treatment. In this thesis we prospectively described a lower prevalence of 3% lumbo-sacral abnormalities in Dutch children with intractable constipation. In healthy adults a comparable prevalence of 1.5-5% spinal lipomas is found. Given the latter and the finding that children from our study reached success with conservative treatment consisting of laxatives, the relevance of the found lumbar sacral abnormalities remains questionable. Therefore we concluded that performing MRI to assess lumbo-sacral spine abnormalities is not required in the standard work-up of children with intractable constipation. The question whether preventive neurosurgery is recommended in lumbo-sacral spine abnormalities, such as lipomas, should be answered by performing large prospective controlled studies in this group of children.

The treatment of children with constipation is mainly based on clinical experience and usually consists of a behavioural strategy with oral and sometimes rectal laxatives. The treatment of chronic constipation in childhood is based on 4 important phases: 1) education, 2) disimpaction, 3) prevention of re-accumulation of faeces and 4) follow up. Up to now we start with the administration of rectal enemas in the majority of children with severe rectal faecal impaction. Our new findings demonstrate however, that rectal enemas and high doses of oral laxatives are both equally effective in removing a rectal faecal mass. As expected, the high dosage of PEG results in a softening of stools and consequently increasing the faecal incontinence frequency. Until the faecaloma has been cleared, soft stool leaks along the rectal faecal mass. In contrast, a rectal enema contains a hypertonic solution which stimulates contraction of the rectosigmoid. These huge contractions result in immediately clearing of the rectal faecal mass, which explains the almost immediate disappearance of faecal incontinence. It is therefore important to inform child and parents that disimpaction with oral laxatives is likely to cause more episodes of faecal incontinence compared to disimpaction with enemas.
For maintenance therapy, despite adequate treatment with laxatives, after one year of laxatives intake, success is achieved in only 62% of children with chronic constipation. Therefore novel therapies, such as probiotics, are studied. Probiotics, defined as live microbial microorganisms, that when administered in adequate amounts confer a health benefit on the host, are now entering mainstream medicine. While an increasing number of potential health benefits are being attributed to probiotic therapies, only a few have been confirmed in well-designed and conducted randomized controlled trials. More importantly, there is a major need for evaluating high-dose probiotic use in healthy infants and children with respect to their safety and tolerance for extended periods. Only one randomised controlled trial is available in the literature evaluating the effect of a probiotic strain in children with constipation. This trial concluded that Lactobacillus GG, as used in this study, was not an effective adjunct to lactulose in treating constipation in children. Just recently a study in healthy infants was conducted using a mixture of probiotics rather than one strain, showing that an infant formula supplemented with a mixture of probiotics, Lactobacillus paracasei ssp. paracasei and Bifidobacterium animalis ssp. Lactis, resulted in more frequent and softer stools compared to infants receiving a formula without the addition of this probiotic mixture. A trial using a probiotics mixture in constipated children was for the first time conducted and described in this thesis. In this uncontrolled trial, we found that a mixture of probiotics, containing lactobacilli and bifidobacteria, decreased abdominal pain as well as faecal incontinence episodes in constipated children. However larger randomised controlled trials are needed to confirm these preliminary data.

Constipation and abdominal pain are the most common gastrointestinal diseases in children. Painful infrequent defecation and involuntary faecal incontinence have great social impact on the child and the family. Nevertheless, well-designed studies are still scarce with respect to pathophysiology, diagnostic work-up, therapy and long term follow-up of children with chronic constipation. To improve the latter, national and international co-operation is needed in order to create consortia aiming to include large numbers of patients.
Samenvatting & toekomst perspectieven
SAMENVATTING

Bij 99% van de gezonde zuigelingen vindt de eerste ontlasting, meconium (zwarte/donkergroene ontlasting), binnen 48 uur plaats. Echter, slechts 66% van de te vroeg geboren kinderen (prematuur) en de zuigelingen met een te laag geboortegewicht in verhouding tot de zwangerschapsduur (dysmatuur) produceren meconium binnen deze termijn en is de meconiumlozing dus vaak vertraagd. Het is echter onbekend of deze groep zuigelingen ook een verlengde duur van de meconiumlozing heeft ten opzichte van de voldragen zuigelingen. Om deze reden werd de duur van meconiumlozing als maat voor de darmmotiliteit (darmwerking) in dit proefschrift onderzocht. Tevens werd informatie verzameld betreffende karakteristieken van de ontlasting zoals ontlastingsconsistentie, hoeveelheid en kleur om een ontlastingsschaal voor kinderen onder de twee jaar te ontwikkelen, de zogenoemde ‘Amsterdam infant stool form scale’.

Obstipatie (verstopping), komt zowel bij zuigelingen als bij oudere kinderen veel voor. De prevalentie van obstipatie op de kinderleeftijd in Westerse en Aziatische landen is 9%. In 95% van de gevallen wordt geen lichamelijke oorzaak voor obstipatie gevonden en spreekt men van functionele obstipatie. Het is dus van groot belang om meer inzicht te verkrijgen in de pathofysiologie van dit symptomencomplex. In dit proefschrift wordt de rol van de waterhuishouding van de darm welke onder andere bepaald wordt door chloorsecretie, met betrekking tot kinderen met obstipatie, onderzocht.

Obstipatie is een moeilijk te behandelen ziektebeeld en vergt veel geduld van de patiënt, de ouders en de hulpverleners. Na 1 jaar intensieve behandeling heeft 50% van de kinderen nog altijd klachten en is afbouwen van de medicatie vaak onmogelijk. In het laatste deel van dit proefschrift wordt de effectiviteit van nieuwe behandelmethodes onderzocht.

DEEL I Het onlastingspatroon in de eerst levensjaren

Deel I van het proefschrift beschrijft het onlastingspatroon en de bijbehorende onlastingskarakteristieken bij voldragen en te vroeg (prematuur) geboren zuigelingen. Zowel direct na de geboorte als gedurende de eerste levensjaren werden deze kinderen vervolgd.

In hoofdstuk 1 onderzochten we het verschil in duur van het lozen van meconium tussen voldragen en te vroeg geboren zuigelingen. In overeenstemming met eerder onderzoek toonden wij aan dat de eerste meconiumlozing vertraagd was bij prematures. Daarnaast liet deze studie zien dat de totale duur van meconiumlozing bij prematures gemiddeld 8 dagen is ten opzichte van 3 dagen bij voldragen zuigelingen. Deze bevindingen zijn mogelijk een gevolg van vertraagde
motilititeit van het maagdarmstelsel bij deze groep zuigelingen. In hoofdstuk 2 wordt een nieuw ontwikkelde onttlastingsschaal (Amsterdam Infant Stool Form Scale) voor kinderen onder de 24 maanden gepresenteerd. Met deze gegevens kunnen zowel ouders als hulpverleners de onttlasting karakteristieken van het kind objectiveren. De schaal beschrijft naast de consistentie ook de hoeveelheid en kleur van de onttlasting. Aan de hand van deze schaal kan een arts met behulp van de beschrijving van ouders sneller en efficiënter de diagnose obstipatie of diaree stellen. Ondanks de gevonden goede inter- en intra-observer scores voor deze schaal, dienen in de toekomst de validiteit en praktische toepassing van deze schaal verder geëvalueerd te worden. In hoofdstuk 3 is een cohort van prematuren en voldragen zuigelingen beschreven die van geboorte tot de leeftijd van 2 jaar vervolgd werden. Deze studie toonde aan dat 1) alleen in de eerste twee weken na de geboorte, borstgevoede zuigelingen 2.41 keer meer onttlasting produceerden dan flesgevoede zuigelingen en 2) dat voldragen zuigelingen alleen in de eerste week meer onttlasting produceerden dan de prematuur geboren zuigelingen. Bij follow-up bleken de andere onttlasting karakteristieken vergelijkbaar tussen beide groepen zuigelingen.

DEEL II Pathofysiologie

Deel II van het proefschrift beschrijft mogelijk nieuwe onderliggende oorzaken van obstipatie op de kinderleeftijd.

De chloorsecretie speelt een belangrijke rol bij de samenstelling van de onttlasting. Zo is chloor het belangrijkste mineraal dat de waterhuishouding in de dikke darm reguleert. Kinderen met obstipatie hebben vaak harde onttlasting en om deze reden zou de chloorsecretie een rol kunnen spelen in het ontstaan van obstipatieklachten. In hoofdstuk 4 wordt onderzocht of de chloorsecretie bij kinderen met obstipatie gestoord is ten opzichte van kinderen zonder obstipatie. Tot onze verrassing is inderdaad de chloorsecretie van de dikke darm gereduceerd in de groep kinderen met obstipatie na stimulatie van het darmepitheel met histamine. Histamine is een biogen amine dat een belangrijke rol speelt in signaal transmissie in de darm. Bij toediening van histamine worden via toename van het calcium, kaliumkanalen geactiveerd. Deze activatie leidt vervolgens tot hyperpolarisatie van de cel dat vervolgens leidt tot opening van de CFTR chloorkanalen waardoor chloor en water de darm inkomen. De vraag of dit defect zich afspeelt op het niveau van de histaminereceptor, componenten van de ‘receptor-linked’ signaal transductie ‘pathway’ of basolaterale calciumgevoelige kaliumkanalen die de elektrische grote kracht zijn voor apicale chloorsecretie, dient verder onderzocht te worden.
Ophoudgedrag wordt beschouwd als de belangrijkste oorzakelijke factor voor het ontstaan van obstipatie op de kinderleeftijd. Langdurig ophoudgedrag resulterend in ophoping van ontlasting in de endeldarm kan mogelijk gevolgen hebben voor het darmslijmvlies. De muscularis mucosae is een onderdeel van het darmslijmvlies en bestaat uit een dun laagje glad spierweefsel, dat op de grens ligt tussen mucosa (slijmvlies) en submucosa (bindweefsellaag onder het slijmvlies). In hoofdstuk 5 zijn de karakteristieken van de muscularis mucosae van de endeldarm van kinderen met obstipatie, kinderen met de ziekte van Hirschsprung en gezonde controles, beschreven. Uit deze studie bleek dat de muscularis mucosae bij kinderen met obstipatie verdikt en meer opgetrokken is ten opzichte van patiënten met de ziekte van Hirschsprung of controle patiënten. Daarnaast werd een correlatie gevonden tussen de duur van obstipatieklachten en de dikte van deze spier. Deze correlatie suggereert dat de verdikking van de muscularis mucosae het gevolg is van obstipatie. De peristaltiek van de darm wordt verzorgd door een samenspel van spieren en zenuwen. Wanneer de anatomische aanleg van bijvoorbeeld het zenuwweefsel gestoord is, bijvoorbeeld bij spina bifida (open rug), kan obstipatie het gevolg zijn. In hoofdstuk 6 wordt een onderzoek beschreven dat een eventuele aanlegstoornis in het lumbo-sacrale wervelkanaal via ‘magnetic resonance imaging’ (MRI) in kaart brengt. Spinale wervelkolom afwijkingen zoals lipomen, werden bij 3% van de kinderen met obstipatie gevonden. Dit percentage is vergelijkbaar met percentages die gevonden worden bij gezonde volwassenen zonder tekenen van obstipatie. In tegenstelling tot onderzoek uit de Verenigde Staten, waar kinderen met obstipatie en lumbo-sacrale afwijkingen bijna allen (8 van de 9) succesvol geopereerd werden, werden alle kinderen met obstipatie en MRI afwijkingen uit onze studie, succesvol behandeld met alleen laxeermiddelen of gedragsadviezen. Op basis van de resultaten van ons onderzoek dienen kinderen met obstipatie en kleine lumbo-sacrale afwijkingen eerst conservatief met laxeermiddelen behandeld te worden alvorens neurochirurgie wordt overwogen.

DEEL III Therapie

In deel III van het proefschrift wordt de effectiviteit en het bijwerkingenprofiel van nieuwe en bestaande laxeermiddelen voor obstipatie met elkaar vergeleken. Fecale impactie is een frequent voorkomend symptoom (30-75%) bij kinderen met obstipatie. Van fecale impactie wordt gesproken indien sprake is van ophoping van een harde en grote hoeveelheid ontlasting in de endeldarm. In hoofdstuk 7 worden twee behandelingsmogelijkheden voor fecale impactie met elkaar vergeleken. Kinderen met obstipatie en fecale impactie in de endeldarm (rectum), werden gerandomiseerd voor behandeling met een klysma of voor een behandeling met een hoge dosering oraal laxeermiddel. Tot onze verrassing was er geen significant
verschil in effectiviteit (disimpactie van het rectum) tussen de rectale (80%) en orale (68%) behandeling. Wel was behandeling met het orale laxans geassocieerd met meer fecale incontinentie episodes en meer waterige ontlasting. Daarentegen, verdween de fecale incontinentie vrijwel direct na het starten met de klysma’s. Ook was het opvallend dat de gerapporteerde angst vergelijkbaar was tussen de kinderen die gedurende 6 dagen klysma’s kregen en de kinderen die oraal gelaxeerd werden. Deze resultaten illustreren dat beide behandelingen even effectief zijn in het ledigen van het rectum. Echter, rekening dient gehouden te worden met de voorkeur van patiënt en/ of ouders voor de orale of rectale interventie.

Probiotica, ‘levende micro-organismen, die de gezondheid van de gastheer bevorderen indien toegevoegd in adequate hoeveelheden, zijn in. Ze worden wereldwijd steeds meer gebruikt, zowel op eigen initiatief van de consument als op voorschrift van medici en paramedici. Kleine en vaak niet goed uitgevoerde studies bij volwassenen met obstipatie hebben positieve resultaten laten zien nadat zij behandeld werden met probiotica. In hoofdstuk 8 wordt een studie beschreven met een mix van verschillende probiotica als therapie voor kinderen met obstipatie. Uit deze studie bleek dat voornamelijk de groep kinderen die zich presenteerde met een ontlastingsfrequentie lager dan drie maal per week een toename had van de ontlastingsfrequentie na vier weken behandeling. Daarnaast hadden kinderen een afname in buikpijnklachten en een afname van de fecale incontinentie episodes. Er werd geen verbetering in de consistentie van de ontlasting gevonden. De positieve resultaten van dit onderzoek geven aanleiding om een grote placebogecontroleerde studie bij kinderen met obstipatie uit te voeren om de resultaten van dit onderzoek te bevestigen dan wel te ontkrachten.
DISCUSSIE EN TOEKOMSTPERSPECTIEVEN

Studies betreffende het normale ontlastingspatroon bij kinderen gedurende de eerste levensjaren zijn schaars. De behoefte aan meer prospectief verkregen data rondom de ontlasting van zuigelingen en jonge kinderen is groot, niet alleen om het normale ontlastingspatroon te definiëren maar vooral ook om een afwijkend defecatiepatroon, zoals bij obstipatie beter te herkennen. De huidige Rome III gelden voor kinderen vanaf 4 jaar en op dit moment ontbreekt nog een definitie voor obstipatie bij zuigelingen en jonge kinderen. Het is belangrijk om ook in deze groep kinderen een consensus te bereiken over de definitie van obstipatie. Niet alleen voor artsen om te bepalen wanneer zij een behandeling moeten beginnen maar ook voor wetenschappers die onderzoek doen naar kinderen in deze leeftijdscategorie.

Eén van huidige Rome III criteria voor obstipatie is een ontlastingsfrequentie minder dan 3 maal per week. Voor kinderen jonger dan 2 jaar hebben wij en anderen echter een normale ontlastingsfrequentie van minstens eenmaal per dag beschreven. Daarom zouden wij als één van de criteria voor obstipatie bij kinderen jonger dan 2 jaar, een ontlastingsfrequentie van minder dan 1 maal per dag voorstellen. Verder komt uit eerder onderzoek naar voren dat harde ontlasting één van de voornaamste redenen voor artsen is om te starten met orale laxantia bij jonge kinderen. Daarom zou naast de ontlastingsfrequentie, harde ontlasting het tweede criterium moeten zijn voor het beschrijven van obstipatie voor kinderen jonger dan 2 jaar. Ten slotte is het belangrijk de consistentie en hoeveelheid ontlasting te omschrijven. Hierbij kan de nieuw ontwikkelde ‘Amsterdam infant Stool Form Scale’ hulp bieden. Het opstellen van een goede definitie van obstipatie voor kinderen jonger dan 2 jaar zal leiden tot betere herkenning en hopelijk tot een betere behandeling van deze jonge kinderen met ontlastingsproblematiek.

Anno 2009 is er nog altijd weinig bekend over het ontstaan van obstipatie bij zowel kinderen als volwassenen. Het huidige wetenschappelijke onderzoek richt zich op een aantal verschillende factoren die waarschijnlijk van invloed zijn op de defecatie. Op dit moment wordt het bewust of onbewust ophouden van de ontlasting als belangrijkste oorzakelijke factor voor het ontstaan van obstipatie op de kinderleeftijd gezien. Echter, naast het gedrag spelen voeding, aanleg van zenuwen en spieren, functie van de dikke darm (colon), de endeldarm (rectum) en de bekkenbodemspieren waarschijnlijk ook een belangrijke rol in het ontstaan van obstipatie.

Dit proefschrift toont voor het eerst aan dat de chloorsecretie in het rectum gestoord is bij kinderen met obstipatie. Dit leidt waarschijnlijk tot harde en dus pijnlijke ontlasting wat het ontstaan van obstipatie op de kinderleeftijd mogelijk verklaart. Helaas werden er alleen uit het rectum biopten verkregen waardoor nog onbekend is of de chloorsecretie ook gestoord is in de rest van het colon. Onderzoek naar de chloorsecretie in de andere delen van het colon, door meerdere
biopten te nemen bij coloscopie, kan ons meer inzicht geven of deze stoornis beperkt blijft tot het rectum. Lubiprostone is een nieuw geneesmiddel dat in staat is om de chloorsecretie te stimuleren en dus de waterhuishouding in de darm te optimaliseren. Verschillende placebo gecontroleerde studies beschrijven inderdaad dat lubiprostone de onlastingsfrequentie verhoogt en de onlasting zachter maakt bij volwassenen met obstipatie. Bij kinderen dienen dergelijke studies nog uitgevoerd te worden.

Zoals boven beschreven wordt ophoudgedrag beschouwd als de belangrijkste factor bij het ontstaan van obstipatie op de kinderleeftijd en leidt dit tot fecale ophoping in het rectum. Mogelijk leidt fecale stase (ophoping) tot histologische veranderingen van het darmepitheel. In hoofdstuk 5 beschreven we inderdaad een dikkere muscularis mucosae bij kinderen met obstipatie. Deze retrospectief verkregen resultaten suggereren dat een verdikte muscularis mucosae waarschijnlijk het gevolg is van fecale stase. Vervolg onderzoek moet de vraag beantwoorden of kinderen met een verdikte muscularis mucosae een slechtere prognose hebben. Een prospectieve studie waarbij de muscularis mucosae van kinderen met obstipatie bij fecale ophoping en na succesvolle behandeling wordt geëvalueerd zou hier antwoord op kunnen geven.

Afwijkingen in het spinale wervelkanaal kunnen ook aanleiding geven tot defecatie problematiek. Obstipatie en fecale incontinentie komen bij ongeveer 80% van de kinderen met spina bifida (open rug) voor. Het exacte mechanisme voor het ontstaan van obstipatie bij deze anatomische afwijkingen is merendeels onbekend.

In tegenstelling tot Amerikaanse data, waarbij 9% van de kinderen met hardnekkige obstipatie spinale wervelkolom afwijkingen vertoonde, vonden wij slechts bij 3% van de ernstig geobstipeerde kinderen spinale wervelkolomafwijkingen. Dit percentage komt overeen met de 1.5-5% spinale wervelkolom lipomen die als toevalsbevinding worden gezien bij gezonde volwassenen. Gezien dit feit en het gegeven dat alle kinderen uit onze huidige studie klachtenvrij werden na standaard behandeling (laxeermiddelen en gedragsadviezen), zonder neurochirurgisch ingrijpen, blijft de relevantie van de gevonden spinale wervelkolom afwijkingen discutabel. Op basis van ons onderzoek is het verrichten van een MRI om spinale wervelkolom afwijkingen bij kinderen met chronische obstipatie uit te sluiten, niet geïndiceerd.

Het blijft echter de vraag of deze kinderen preventief geopereerd dienen te worden om neurologische klachten, zoals urine-incontinentie, in de toekomst te voorkomen. Om deze vraag te beantwoorden dient bij deze groep kinderen een prospectieve gerandomiseerde gecontroleerde studie (preventief versus conservatief) verricht te worden bestaande uit een grote patiëntenpopulatie.

Behandeling van kinderen met obstipatie is vooral gebaseerd op klinische ervaring en behelst vaak de combinatie van gedragsadviezen en orale laxeermiddelen. Deze behandeling bestaat uit 4 fases: 1) educatie, 2) desimpactie (opruimen
fecale ophoping) 3) preventie van ophoping van ontlasting en 4) follow-up. Tot op heden bestaat desimpactie bij de meerderheid van kinderen die zich presenteert met ernstige fecale ophoping uit het toedienen van rectale klysma’s. Zoals eerder beschreven, laten onze data zien dat hoge dosering orale laxantia (PEG) even effectief zijn als rectale klysma’s. Maar zoals verwacht werd er vaker fecale incontinentie gezien bij kinderen die orale laxeermiddelen gebruikten. Het is om deze reden belangrijk om ouders en kind hierover te informeren, vooral omdat bekend is dat fecale incontinentie gepaard gaat met een lagere kwaliteit van leven, zowel psychisch als lichamelijk. De vraag of een kortere desimpactieduur van bijvoorbeeld 3 dagen even effectief is als de huidige desimpactieduur van 6 dagen dient om deze reden in de toekomst geëvalueerd te worden.

Na desimpactie volgt onderhoudstherapie met orale laxantia. Als deze kinderen voor langere tijd vervolgd worden zien we dat ondanks onderhoudstherapie met adequate doseringen laxantia na één jaar, maar 62% succes bereikt. Om deze reden worden nieuwe alternatieve behandelmethode onderzocht zoals het gebruik van probiotica. Probiotica zijn levende micro-organismen, die de gezondheid van de gastheer kunnen bevorderen indien toegevoegd in adequate hoeveelheden. Terwijl een groot scala aan potentiële gezondheidsvoordelen wordt toegeschreven aan probiotica-gebruik, zijn goed opgezette studies hiernaar schaars. Verder is de vraag naar het evalueren van probioticagebruik, zowel betreffende effectiviteit als veiligheid, op de kinderleeftijd groot. Toch is slechts één gerandomiseerder gecontroleerde studie verricht bij kinderen met obstipatie. In deze studie bleek de stam lactobacillus GG, gebruikt als adjunct bij het laxeermiddel lactulose, niet effectief. Een studie waarbij een cocktail van probiotica wordt gebruikt in plaats van één stam bij kinderen met obstipatie werd voor het eerst in dit proefschrift beschreven. In deze studie nam zowel de buikpijn als de fecale incontinentie af en nam de ontlastingsfrequentie toe. Echter, gezien het kleine patiëntenaantal van twintig en het ongecontroleerde karakter van onze studie, dienen de resultaten eerst in grotere gerandomiseerde studies bevestigd te worden alvorens harde conclusies te trekken.

Obstipatie is naast buikpijn de meest voorkomende gastro-intestinale aandoening op de kinderleeftijd. Infrequente pijnlijke ontlasting in combinatie met het onvrijwillig verliezen van ontlasting heeft grote impact op het kind en het gezin. Het is om deze reden belangrijk de oorzaak te ontrafelen die aan obstipatie ten grondslag ligt. Uiteindelijk kan dit leiden tot betere behandelstrategieën voor deze kinderen. Intensieve samenwerking tussen nationale en internationale consortia is noodzakelijk om in de komende jaren antwoorden te geven op het terrein van pathofysiologie (oorzaak), diagnostiek, therapie en follow-up van obstipatie op de kinderleeftijd.
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This thesis


Other


Acknowledgments / Dankwoord
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**De poeppoli dudes**, uit het beste poeplab ter wereld!

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Kinder-MDL:

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