Evidence-based guideline development in paediatric gastroenterology

Tabbers, M.M.

Citation for published version (APA):
Fermented milk containing *Bifidobacterium lactis* DN-173 010 in the treatment of childhood constipation: a randomised, double-blind, controlled trial

Abstract

Background
Constipation is a frustrating symptom affecting 3% of children worldwide. A fermented dairy product containing *Bifidobacterium lactis* strain DN-173 010 was effective in increasing stool frequency in constipated women. Our aim was to assess the effects of this product in constipated children.

Methods
In this randomised, double-blind, controlled trial, 159 constipated children (defecation frequency <3 times/week) were randomly allocated to receive either a fermented dairy product containing *Bifidobacterium lactis* DN-173 010 (n=79) or a control product (n=80) twice a day, for 3 weeks. Primary endpoint was the change in stool frequency from baseline to after 3 weeks of product consumption. Analyses were by intention to treat.

Results
11 children did not return to any follow-up visit – 5 in the probiotic group, 6 in the control group – and were therefore excluded from the final analysis. Thus, 74 children in each group were analysed. The change in stool frequency from baseline to after 3 weeks of product consumption increased in both groups, but the difference was not statistically significant (2.9±3.2 in probiotic group vs. 2.6±2.6 in control group, P=0.35). There were no serious adverse events.

Conclusions
In constipated children, the fermented dairy product containing *Bifidobacterium lactis* strain DN-173 010 did increase stool frequency, but this increase was comparable in the control group. There is currently not sufficient evidence to recommend fermented dairy products containing *Bifidobacterium lactis* strain DN-173 010 in this category of patients. Future studies should focus on whether higher doses or longer period of probiotic products are more effective.
Introduction
Chronic constipation is a common problem in childhood with an estimated prevalence of 3% in the Western world. Constipation is a debilitating condition characterised by infrequent painful defecation, faecal incontinence, and abdominal pain. The pathophysiology underlying functional constipation is undoubtedly multi-factorial, and not well understood. Withholding behaviour is probably the major cause for the development of constipation. A study in a tertiary hospital showed that despite intensive medical and behavioural therapy, 30% of patients who developed constipation before the age of 5 years continued to have severe complaints of constipation beyond puberty.

A recent systematic review evaluating the effects of using laxative treatment and dietary measures for the treatment of childhood constipation, showed that there is insufficient evidence to suggest that laxative treatment is better than placebo in children with constipation due to a lack of placebo-controlled trials.

Probiotics are defined as live micro-organisms which when administered in adequate amounts might improve the health of the host. A dysbiosis in the gut microbiota has been suggested as a mechanism behind constipation which might improve after the ingestion of probiotics. Furthermore, probiotics can lower the pH of the colon by producing lactic acid, acetic acid, and other acids. A lower pH enhances colonic peristalsis and, subsequently, decreases the colonic transit time. Two randomised, placebo-controlled trials with the fermented dairy product containing Bifidobacterium lactis DN-173 010 have been performed: one in adult patients with irritable bowel syndrome (IBS) and constipation, and one in constipated women with a defecation frequency <3 times/week. Both trials showed a significant increase in stool frequency in the probiotic group compared to the control group in subjects presenting less than 3 stools per week. No adverse events were reported. Therefore, we conducted a multi-centre, randomised, double-blind, controlled trial to assess the effects of this specific probiotic product in children with constipation.

Methods

Patients
The design and rationale of the study have been described in detail elsewhere. Children, 3-16 years of age were enrolled in 3 academic hospitals (In the Netherlands and Poland) and 12 Dutch non-academic hospitals. Patients were eligible for randomisation if they had been suffering from functional constipation according to Rome III criteria for the last 2 months. They had a defecation frequency of <3 times/week and one or more of the following criteria: faecal incontinence >1 episode/week, a large amount of stools that clog the toilet, painful defecation, withholding behaviour, or abdominal or rectal faecal impaction upon physical examination. Children had to be familiar with consumption of dairy products. Exclusion criteria
were: treatment for constipation less than 2 weeks before the start of the study, a diagnosis of either IBS or functional non-retentive faecal incontinence according to Rome III criteria; a diagnosis of mental retardation or metabolic disease (hypothyroidism), Hirschsprung’s disease, spinal anomalies, ano-rectal pathology, previous gastrointestinal surgery; lactose intolerance or known allergy to a product component; children who had taken antibiotics in the prior month or who were receiving medication influencing gastrointestinal motility (e.g., cisapride). Eligible patients were randomised. Random numbers were generated by a computer programme with an allocation ratio of 1:1 and with well-balanced blocks. Separate lists were generated for each study site. All investigators were unaware of product allocation. The randomisation lists were kept confidential by the person responsible for the preparation of the study products and their labelling. All children and/or their legal guardians gave written informed consent to participate in the study. This study was investigator-initiated and investigator-driven and performed in accordance with the principles of the Declaration of Helsinki and good clinical practice guidelines. The independent ethics committees of all participating hospitals approved the protocol.

**Study products**

The two study products were identical in weight, colour, smell, taste and packaging. All doctors, research staff, and patients with their caregivers involved remained unaware of the product administered to the patient. The probiotic product consisted of the fermented milk Activia® (125-g pot) manufactured with lactic cultures including *Bifidobacterium lactis* DN-173 010 (strain number I-2494 in French National Collection of Cultures of Micro-organisms (CNMC, Paris, France) [at least $4,25 \times 10^9$ cfu per pot]), yoghurt starter cultures (*Lactobacillus delbrueckii* ssp. *Bulgaricus* : CNCM strain numbers I-1632 and I-1519, and *Streptococcus thermophilus* :CNMC strain number I-1630) [at least $1.2 \times 10^9$ colony forming units (cfu) per pot]) and *Lactococcus cremoris* (CNMC strain number I-1631). The control product consisted of a milk-based, non-fermented dairy product (125-g pot) without probiotics and with a low content of lactose (< 4 g/pot). Both the probiotic and control preparations were checked according to national regulations for any contamination with known pathogens and macronutrient composition including lactose. Prior to the start and at the end of the study, the test product was analysed by counting *Bifidobacterium lactis* DN-173 010 (at least $4,25 \times 10^9$ cfu/pot) and *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (at least $1.2 \times 10^9$ cfu/pot).

Every patient had to take 2 pots per day: one at breakfast and one at the evening meal for 3 consecutive weeks. Products were kept in the refrigerator.

The study period was 5 weeks with 3 clinic appointments: inclusion visit (V1), randomisation visit (V2), and clinical evaluation visit (V3) ([Figure 1](#)). The first week was used to obtain baseline values, followed by a period of three days where enemas were given. Patients were then treated for three weeks with the study products. Products were delivered to homes by
study nurses using cool packages. The last visit was after 3 weeks of product consumption (Figure 1).

During the study, all children were instructed to try to defecate on the toilet for 5-10 minutes after each meal and to complete daily a standardised bowel diary. Intake of any other fermented dairy product or yoghurt was not allowed. During the product consumption period, patients were instructed to take bisacodyl 5 mg if they did not defecate for 3 consecutive days.

**Outcomes**

Frequency of defecation, frequency of faecal incontinence episodes, self-evaluation of digestive symptoms (abdominal pain and flatulence, [2-point scale, 1=yes, 2=no]), and self-evaluation of adverse effects (nausea, diarrhoea and bad taste, [2-point scale, 1=yes, 2=no]) were assessed daily using a subject diary. Stool consistency and pain during defecation (2-point scale, 1=yes, 2=no) were assessed for each passed stool using the same diary. Stool consistency was scored using the 7-point Bristol stool scale in which a score of 1 describes stools that are hard lumps, a score of 4 describes stools that are normal (smooth and soft), and a score of 7 describes stools that are watery stools (diarrhoea). The diary was also used to record the daily consumption of the study products, any unauthorised products, and bisacodyl as well as any other concomitant treatment.

The primary endpoint was the change in stool frequency from baseline (the week prior to randomisation) to after 3 weeks of product consumption. Secondary endpoints were the rate of success (defined as 3 or more bowel movements per week and less than 1 faecal incontinence episode in 2 weeks over the last 2 weeks of product consumption) and the rate of responders (with a responder defined as a subject who reports a stool frequency ≥
3 episodes during the last week of product consumption). Other secondary endpoints were calculated over the 3 week product consumption period: stool frequency, stool consistency, frequency of episodes of faecal incontinence, frequency of pain during defecation, frequency of digestive symptoms (abdominal pain and flatulence), frequency of adverse effects (nausea, diarrhoea and bad taste), and frequency of intake of bisacodyl.

Data collection was done by local physicians who completed case record forms. Independent Clinical Research Associates visited the recruiting sites in order to monitor all patients’ data. Adverse events were monitored by Independent Clinical Research Associates. After completing the study, but before any analysis or unblinding, 3 authors (MR, AC, MT) checked all primary and secondary endpoints with primary source data. Before any analysis and without knowledge of product allocation, the study group judged all exclusions, serious adverse events, and endpoints not fully specified in the protocol in individual patients. After agreement, analyses were done with blinding of the given products preserved. After revealing the results of the blinded analyses to the study group, the randomisation code was broken on Oct 26 2009.

Statistical analysis
Descriptive statistics were performed for baseline characteristics. Continuous variables were described by means and standard deviation or in case of skewed distributions by medians and 25th and 75th percentiles. Categorical variables were described by percentages. Almost all clinical outcomes were assessed three times during the intervention period, which led to the following statistical approach. For continuous outcomes, linear mixed models were made containing time (3 levels), product and the interaction between time and product, and the value of the outcome at baseline. Based on the linear mixed model we performed an overall test for difference in outcome between product groups across all time points and assessed the difference with 95% confidence interval at the third week of product consumption. In case of a binary outcome, a generalized estimating equations (GEE) logistic regression was made to take the correlated structure of the data into account. All analyses were done on the intention-to-treat population. All statistical tests were performed with a two-sided significance level of 5%. All analyses were done with SPSS (version 16.0).

The sample size was based on the percentage of success in both groups. In the intervention group (fermented milk containing *Bifidobacterium lactis* DN-173 010, toilet training, bowel diary) we expected this proportion to be around 35% and in the control group (acidified milk without ferments, toilet training, bowel diary) around 15%. The choice of 15% was justified by a previous study by van der Plas et al. showing that 15% of children with untreated chronic defecation problems were helped by an approach of toilet training and completing a daily bowel diary. To demonstrate such a difference, it required a total sample size of 146 using a two-sided significance (α) level of 0.05 and a power (β) of 80%. To allow for loss due to withdrawal, a total number of 160 subjects were randomised.
Results

Between February 2008 and November 2008, 186 children were assessed for eligibility of which 26 could not be included (Figure 2). A total of 160 children were randomised. However, there was one failure in the randomisation process in the sense that a non-existing child was randomised to the probiotic group. So, 159 children were correctly randomised to consume either the fermented dairy product (n=79) or the control (n=80) (Figure 2). Eleven children were lost to follow-up without having any outcome data during follow-up. These 11

Figure 2: Trial profile

186 children assessed for eligibility
- 26 children excluded
  - 9 not meeting inclusion criteria
  - 5 parents refused to participate
  - 12 other reasons

160 children randomised
- 1 failure of the randomisation system: the child did not exist

79 children assigned to probiotics
- 5 lost to follow-up, without any outcome data
- 6 discontinued intervention
  - 2 bad taste, 1 sore throat, 2 no second delivery of study products, 1 no specified reason
- During study product consumption
  - 2 used antibiotics for 7 days, 1 for 5 days, 1 for 3 days
  - 1 who discontinued intervention due to sore throat used antibiotics for 7 days
- 74 analyzed
- 5 excluded from analysis (lost to follow-up)

80 children assigned to placebo
- 6 lost to follow-up, without any outcome data
- 4 discontinued intervention
  - 2 bad taste, 1 sore throat and no second delivery of study products, 1 no specified reason
- During study product consumption
  - 1 used antibiotics for 5 days, 1 used microlax once
  - 1 who discontinued intervention due to sore throat and no second delivery of study products used antibiotics for 7 days
- 74 analyzed
- 6 excluded from analysis (lost to follow-up)
patients, 5 in the probiotic group and 6 in the control group, were excluded from the final analysis. Table 1 shows the baseline characteristics.

The mean stool frequency was 1.6 episodes per week at baseline in the probiotic group and 4.5 at week 3 compared to 1.3 episodes per week at baseline in the control group and 3.9 at week 3. The increase in stool frequency from baseline to end of study (primary endpoint) was therefore 2.9±3.2 in probiotic group vs. 2.6±2.6 in control group. This difference was not statistically significant (P=0.35) (figure 3). The test for a difference in stool frequency over 3 weeks was not statistically significant (P=0.51) (figure 3).

The rate of success was 38% (27/71) in the probiotic group versus 24% (17/72) in the control group, with a risk difference of 14% (95% CI -1 to 29%, p=0.06). The rate of responders was 72% (51/71) in the probiotic group versus 64% (46/72) in the control group, corresponding to a difference of 8% (95% CI: -7.3 to 23%; p=0.31).

Stool consistency was not statistically significantly different between the probiotic group and the control group (mean score 3.3 in the probiotic group versus 3.5 in the control group at week 3, p value over 3 weeks p=0.07). The test for difference in the proportion of patients with episodes of faecal incontinence showed a p-value of 0.19. The overall test for

Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Probiotics (n=79)</th>
<th>Control (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (SD)</td>
<td>7.0 (3.4)</td>
<td>6.5 (3.1)</td>
</tr>
<tr>
<td>Boys, n (%)</td>
<td>42 (53%)</td>
<td>41 (51%)</td>
</tr>
<tr>
<td>Mean duration of constipation in years (SD)</td>
<td>3.4 (2.7)</td>
<td>3.4 (2.6)</td>
</tr>
<tr>
<td>Mean stool frequency per week</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Stool frequency per week (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.6</td>
<td>8.8</td>
</tr>
<tr>
<td>0.5-1</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>1.5-2</td>
<td>58</td>
<td>55</td>
</tr>
<tr>
<td>Stool consistency score (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>hard</td>
<td>78</td>
<td>68</td>
</tr>
<tr>
<td>soft</td>
<td>2.6</td>
<td>12</td>
</tr>
<tr>
<td>watery</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>Pain during defecation score (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>54</td>
<td>58</td>
</tr>
<tr>
<td>sometimes</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>no</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Median number of faecal incontinence episodes per week (P25-P75)</td>
<td>0.1 (0-7)</td>
<td>2 (0-7)</td>
</tr>
</tbody>
</table>
difference in pain during defecation showed no statistically significant difference (p=0.14), nor for difference in abdominal pain (p=0.92). Flatulence was less frequently reported in the probiotic group compared to the control group with a difference of 13% at week 1, 24% at week 2 and 11% at week 3. The overall difference in flatulence over 3 weeks showed a significantly difference between groups in favour of the probiotic group (P=0.02). The overall test for difference of Bisacodyl intake showed a p-value of 0.12. Table 2 shows the overall test for differences during product consumption period and specific differences for all outcomes at week 3.

Table 2: Overall test for differences during product consumption period and specific differences at week 3 between the probiotic (P) and control group (C) for all outcome measures

<table>
<thead>
<tr>
<th>Outcome</th>
<th>P-value for overall differences</th>
<th>P (%)</th>
<th>C (%)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in stool frequency</td>
<td>0.35</td>
<td>2.9</td>
<td>2.6</td>
<td>0.3 (-1.45 ; 0.51)</td>
</tr>
<tr>
<td>Mean stool frequency</td>
<td>0.51</td>
<td>4.5</td>
<td>3.9</td>
<td>0.6 (-0.60 ; 1.20)</td>
</tr>
<tr>
<td>Mean stool consistency</td>
<td>0.07</td>
<td>3.3</td>
<td>3.5</td>
<td>-0.2 (-0.64 ; 0.03)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>P-value chi square test</th>
<th>P (%)</th>
<th>C (%)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of success</td>
<td>0.06</td>
<td>38</td>
<td>24</td>
<td>14 (-1 ; 29%)</td>
</tr>
<tr>
<td>Rate of responders</td>
<td>0.31</td>
<td>72</td>
<td>64</td>
<td>8 (-7.3 ; 23%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>P-value (GEE)</th>
<th>P (%)</th>
<th>C (%)</th>
<th>Estimated Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal incontinence</td>
<td>0.19</td>
<td>36.6</td>
<td>48.6</td>
<td>1.48 (0.83 ; 2.64)</td>
</tr>
<tr>
<td>Pain during defecation</td>
<td>0.14</td>
<td>48.6</td>
<td>41.4</td>
<td>0.67 (0.36 ; 1.15)</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>0.92</td>
<td>58.3</td>
<td>54.2</td>
<td>0.97 (0.56 ; 1.69)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>0.02</td>
<td>23.6</td>
<td>34.7</td>
<td>0.48 (0.26 ; 0.89)</td>
</tr>
<tr>
<td>Use of Bisacodyl</td>
<td>0.12</td>
<td>23.6</td>
<td>30.6</td>
<td>0.61 (0.32 ; 1.13)</td>
</tr>
</tbody>
</table>
Adverse events/safety

Two serious adverse events (SAE) occurred during this study, probably not related to consumption of the study products: one child broke his arm and one developed gynaecological pain for which she was admitted. Other adverse events which might related to consumption of the study products were gastroenteritis (intervention group n=1, control group n=3), nausea/vomiting (intervention group n=3, control group n=2), and Candida-infection of the anorectal region (control group n=1).

Discussion

This randomised, double-blind controlled trial in constipated children with a defecation frequency of <3 episodes per week revealed no significant difference in the increase in stool frequency from baseline to 3 weeks between the fermented dairy product containing *Bifidobacterium lactis* strain DN-173 010 group and the control group. Across all other clinical outcomes, differences in general were in favour of probiotics (table 2). These differences were small and not statistically significant with the exception of “flatulence”. No serious adverse events were reported.

This is the first, large, randomised, double-blind controlled trial conducted in constipated children to investigate the efficacy and safety of a specific probiotic product. A recent systematic review on the effects of laxative treatment and dietary measures in the management of childhood constipation found only 2 randomised controlled trials that evaluated the effects of probiotics. In the first small study, 45 children younger than 10 years with chronic constipation were randomly assigned to receive magnesium oxide (50 mg/kg/day (n=18), or 8 x 10^8 cfu/day of the probiotic *Lactobacillus casei rhamnosus* (n = 18), or placebo (n = 9) twice daily for 4 weeks. No statistically significant difference in the defecation frequency was found. However, patients receiving either the probiotic strain or the oral laxative had a significantly higher defecation frequency compared to the placebo group (defecation frequency [times/day 0.57±0.17 and 0.55±0.13, respectively, compared to 0.37±0.10, P=0.03). The second trial was conducted to determine if *Lactobacillus rhamnosus* GG (LGG) is an effective adjunct to lactulose for treating constipation in children. 48 children with constipation received 1 ml/kg/day of 70% lactulose plus 10^9 cfu of LGG or 1 ml/kg/day of 70% lactulose plus placebo, twice daily for 12 weeks. There were no significant differences in rates of product success (defined as ≥ 3 spontaneous stools per week with no faecal incontinence) at 12 and 24 weeks between the LGG group (rates: 72% and 64%, respectively) and the placebo group (rates: 68% and 65%, respectively). Both trials did not report any adverse events.

In contrast to our study, recent studies in adults have shown that the same fermented dairy product containing *Bifidobacterium lactis* DN-173 010 significantly reduced colonic
transit times in young and elderly healthy adults and in constipation-predominant IBS patients. Moreover, a randomised, double-blind, controlled trial performed in IBS patients with constipation showed a significant increase, as compared to controls, in stool frequency over the 6 weeks of product consumption in the subgroup of patients with a defecation frequency of <3 episodes/week and another clinical study performed in constipated women with a defecation frequency < 3 episodes/week showed the same result after 2 weeks of product consumption. The difference in efficacy of the fermented dairy product containing *Bifidobacterium lactis* DN-173 010 between adults and children underscores the hypothesis that constipation in children differs considerably from that in constipated adults with regard to its prevalence, onset, aetiology, symptoms, treatment, and prognosis.

We found in the control group a higher rate of success than expected, namely 24% instead of 15%. In an earlier study conducted in children with functional constipation, a response rate of 40% was found in the placebo group. Nurko et al. suggested a significant role of behaviour modification, including toilet training and parental positive reinforcement, in determining the high placebo response rate. In our study, toilet training in combination with keeping a bowel diary in addition with the consumption of a product could also have played an important role in achieving this high success rate in the control group. On the other hand, it could be due to a true placebo effect. This effect could be caused by the high level of expectancy of children and their parents participating in this study and the frequent contacts between the doctors and patients. In addition, it is unknown whether the control product, which contained an acidified milk with a low content of lactose, itself may also have had a laxative effect. However, this high placebo success rate must challenge clinical researchers to evaluate the effect of placebo compared to new compounds emerging for the treatment of childhood constipation.

The success rate, however is higher in the probiotic group, namely 38% versus 24% in the control group. The difference of 14% between both groups could be explained by coincidence. Although this difference is not statistically significant an increase in dosage of the probiotics or a longer consumption period might result in a significant difference in favour of probiotics.

In addition, quantitative analyses were runned as posthoc analyses. We found a significant difference in the mean number of episodes of faecal incontinence and the mean number of intake of *bisacodyl* per week and per group in favour of the probiotic product compared to the control group over 3 weeks (respectively 2.45 for the probiotic group versus 3.68 for control group for the episodes of faecal incontinence, p=0.0139; and 0.35 for the probiotic group versus 0.59 for the intake of *bisacodyl*, p=0.0069). The higher intake of laxatives in the control group compared to probiotic group could partially explain the absence of significant difference between the 2 groups on stool frequency.
Conclusion

The product containing *Bifidobacterium lactis* strain DN-173 010 did increase stool frequency in constipated children, but the increase was comparable in the control group. There is currently not sufficient evidence to support a general recommendation about the use of probiotics in the treatment of functional childhood constipation. Future studies should focus on whether higher doses or longer period of probiotic product consumption are more effective.

Investigators

*Clinical centres and investigators*

**Poland:** The Medical University of Warsaw, H. Szajewska

**The Netherlands:** Emma Children’s Hospital/Academic Medical Centre Amsterdam: M.A. Benninga, University Hospital Groningen, E.Rings, Amphia Hospital Breda, S.De Pont, Antonius Hospital Nieuwegein, A.Vlieger, Isala Hospital Zwolle, O.Norbruis, Zuwe Hofpoort Hospital Woerden, W.Verwijs, Academic Hospital Rotterdam, M.Y. Berger, Medical Centre Alkmaar, E.K.George, Gelderse Vallei Hospital Ede, GJ van der Burg, Spaarne Hospital Hoofddorp, J.Bokma, Catharina Hospital Eindhoven, NAJ de la Haye, R.Pelleboer, BG Werrij, Flevo Hospital Almere, M.Trijbels-Smulders, Rijnstate Hospital Arnhem, E.Leijn.

Acknowledgements

The authors wish to thank Danone Research, Palaiseau, France, for funding this study, especially for the supply of products. Danone Research is the sponsor of this study.
Reference List


