Lactobacillus reuteri DSM 17938 for the Management of Functional Abdominal Pain in Childhood: A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective To determine whether administration of *Lactobacillus reuteri* DSM 17938 is beneficial in functional abdominal pain (FAP) of childhood.

Study design A total of 101 children, aged 6-15 years, who fulfilled the Rome III criteria for FAP were enrolled in a randomized double-blind, placebo-controlled trial, and were randomly assigned to receive either *L reuteri* DSM 17938 or placebo for 4 weeks, with further follow-up of additional 4 weeks. Response to therapy was based on a self-reported daily questionnaire monitoring frequency and intensity of abdominal pain, using the faces scoring system by Hicks.

Results *L reuteri* (n = 47) was significantly superior to placebo (n = 46) in relieving frequency (1.9 ± 0.8 vs 3.6 ± 1.7 episodes/wk, $P < .02$) and intensity (4.3 ± 2.2 vs 7.2 ± 3.1 Hicks score/wk, $P < .01$) of abdominal pain following 4 weeks of supplementation. There was no difference in school absenteeism rate or other gastrointestinal symptoms, except for a lower incidence of perceived abdominal distention and bloating, favoring *L reuteri*.

Conclusions *L reuteri* DSM 17938, compared with placebo, significantly reduced the frequency and intensity of FAP in children. *(J Pediatr 2016;■:■-■)*.

Trial registration ClicalTrials.gov: NCT01180556.

Abdominal pain-related functional gastrointestinal disorders are very common in childhood and include 4 types: functional abdominal pain (FAP), irritable bowel syndrome (IBS), functional dyspepsia (FD), and abdominal migraine. This division has been based on the Rome III diagnostic criteria.[1] Although benign in nature, these disorders are commonly associated with significant anxiety, school absenteeism, frequent clinic visits, unnecessary testing, and a significant economic burden.[2] Children with IBS for instance represent up to 50% of all patients referred to pediatric gastroenterology clinics in the US.[3] Nevertheless, the therapeutic options for these common functional abdominal complaints are limited.[4]

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[5] Various probiotic agents have been proposed as optional therapy for functional gastrointestinal conditions. Several studies in adults have demonstrated some clinical benefit of particular probiotic agents, mostly in IBS.[6,7]

Pediatric literature data are scarce and controversial, as they present a wide variability of study design and type of microorganisms.[8-12] Most of the trials studied patients with IBS using largely *Lactobacillus GG*.[13] *Lactobacillus reuteri* has been shown to have significant benefit as a pain relieving probiotic strain in infantile colic.[14,15] In one study, administration of *L reuteri* DSM 17938[12] to pediatric patients with FAP reduced the intensity, but not the frequency, of abdominal pain.

Our aim was to examine in a well-designed prospective randomized, double-blind, placebo-controlled trial whether *L reuteri* DSM 17938 is effective in the management of childhood abdominal pain-related functional gastrointestinal disorders, according to the Rome III criteria.

Methods

This randomized, double-blind, placebo-controlled trial was carried out between March 2011 and October 2013 (ClinicalTrials.gov: NCT01180556). The study protocol was approved by the Institutional Review Board of the Faculty of Health Sciences, Ben-Gurion University.

A written informed consent was obtained from the children's parents. Children with recurrent abdominal pain, aged 6-15 years, were recruited at random from outpatient pediatric clinics at Soroka Medical Center and at 3 community childcare centers in the Beer-Sheva area. Children were excluded if they had any

FAP	Functional abdominal pain
FD	Functional dyspepsia
IBS	Irritable bowel syndrome

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Study products were manufactured, packed, and supplied free of charge by BioGaia AB, Stockholm, Sweden, which had no role in the conception, design, and conduct of the study, or in the analysis or interpretation of the data. Z.W. has served as a speaker for BioGaia AB. The other authors declare no conflict of interest.

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chronic illness, growth failure, previous abdominal surgery, or any alarming signs of organic conditions (such as vomiting, chronic diarrhea, bloody stools).[16] Subjects who were treated with antibiotics, probiotics, or prebiotics in the previous 8 weeks were excluded. All included children underwent a careful physical examination, including growth variables within normal limits. Baseline laboratory workup, including complete blood count, erythrocyte sedimentation rate, renal and liver function tests, amylase, lipase, celiac serology, and urinalysis, was within normal limits. Additional tests, including stools for occult blood, culture, ova, parasites, *Helicobacter pylori*, abdominal ultrasound, and a lactose breath test, also yielded negative results. Eligible children and their parents were fully informed about the trial and signed an informed consent. After a careful review of patient history, the patients were diagnosed by 1 physician as having an abdominal pain-related functional gastrointestinal disorder and were divided into 4 groups: FAP, IBS, FD, and abdominal migraine based on the Rome III diagnostic criteria.[1]

After informed consent, eligible patients entered a run-in phase of 2 weeks during which each participant and family completed a self-reported daily questionnaire. Only patients with at least 1 episode of abdominal pain per week were included in the study.

Subjects were randomly assigned to receive either *L reuteri* DSM 17938 (1×10^8 colony-forming units/d) or placebo, once a day, as identical chewable tablets for 4 weeks, with further follow-up phase of additional 4 weeks with no supplementation. In the preliminary study protocol, prior to recruitment, supplementation period was planned to last 6 weeks. Later on, this was changed to 4 weeks supplementation, with additional 4 weeks of follow-up, for better compliance. The amount and viability of the probiotic bacteria were monitored every 3 months. The placebo consisted of an identical formulation without the probiotic bacteria.

Randomization was performed by the random-digit method on the basis of computer-generated numbers. To avoid disproportionate numbers of subjects in each group, randomization was performed in blocks of 6, 3 for placebo and 3 for product. Allocation concealment was ensured by an independent person. Participants and the entire research team were blinded to code assignment. The code was revealed from vendor only when recruitment, data collection, and statistical analyses were completed.

Each participant and family completed a self-reported daily questionnaire throughout the 8 weeks of the study. This included daily monitoring of frequency and intensity of abdominal pain based on the validated face scoring system by Hicks.[17] Each of the 6 face scoring system ranked 0, 2, 4, 6, 8, or 10, where 0 = no pain (relaxed face) and 10 = very severe pain (miserable face). This scoring system was validated in children with a similar age range.[18] Any associated gastrointestinal symptoms (diarrhea, nausea, vomiting, dyspepsia, flatulence, bloating) and adverse events were recoded daily as well. Each participant was contacted by the study staff once a week to monitor progress, compliance, and filling out of daily diaries.

Each subject underwent a physical examination, including determination of growth variables, at baseline and at 4 and 8 weeks. Patients were also interviewed with nonleading questions regarding their symptoms and adverse events. They had to return unused tablets and containers to ensure compliance.

The primary outcome measures included frequency and intensity of abdominal pain. Secondary measures included school absenteeism because of abdominal pain, additional gastrointestinal symptoms, and adverse effects.

Statistical Analyses

For the assessment of abdominal pain frequency (dichotomous outcome), we calculated the sample size based on the assumption that relief of pain would be expected in 40% of the placebo group and 70% of the probiotic group. We estimated that, with a power of 80% and a significance level of 0.05, a sample of 38 children in each group will be required, to show a 30% difference between the groups.

For the assessment of differences in pain intensity (continuous outcome), we set the sample size at 34 per group to achieve a power of 80%, to show at least a difference of 2 (SD 2) in the intensity score between groups. In total, we planned to enroll 90 subjects to account for 20% follow-up losses.

The data from all patients were analyzed on an intention-to-treat basis. Categorical variables were tested using the χ^2 test or Fisher exact test as appropriate. Continuous variables were tested for normality, and if normality was confirmed, groups were compared using the Student *t* test. For nonnormally distributed variables, the Mann-Whitney U test was used. Differences were considered to be significant at the level of $P < .05$. All reported *P* values are 2-sided. The analysis was performed with SPSS 13.0 software (SPSS Inc, Chicago, Illinois).

Results

A total of 177 subjects were assessed for eligibility between March 2011 and October 2013; 54 were excluded because of exclusion criteria (**Figure 1**; available at www.jpeds.com). Following the initial diagnosis, 13 patients were diagnosed as having IBS and 5 patients as having FD. In view of the small size of these 2 groups, we have decided to include only patients with FAP. This intention-to-treat population consisted of 101 patients with FAP, which were randomly assigned to the probiotic group (n = 52) or to the placebo group (n = 49). All 8 failures were the result of poor compliance and violation of the protocol. None of them were therapy-related or because of adverse effects.

There were no significant differences between groups at randomization in terms of age at entry, sex, body weight, duration of symptoms, use of drug treatment for abdominal

pain, school absenteeism because of abdominal pain, self-reported frequency of pain, and self-reported intensity (Hicks score) of abdominal pain (Table).

The results of the primary outcome measures, frequency, and intensity of abdominal pain, are presented in Figures 2 and 3, respectively. At 4 weeks following supplementation, *L reuteri* was significantly superior to placebo in relieving frequency (1.9 ± 0.8 vs 3.6 ± 1.7 episodes per week, respectively, $P < .02$) and intensity (4.3 ± 2.7 vs 7.2 ± 3.1 Hicks scale, respectively, $P < .01$) of abdominal pain. At 8 weeks, following the 4-week follow-up phase, *L reuteri* was significantly better than placebo in relieving the intensity of abdominal pain (4.8 ± 3.3 vs 6.4 ± 4.1 Hicks scale, respectively, $P < .02$), but with no significant effect on pain frequency (3.4 ± 2.6 vs 4.4 ± 2.9 episodes per week, respectively, $P = .09$).

All secondary outcome measures pertaining to other gastrointestinal symptoms did not reveal any significant differences between groups, except for a lower incidence of perceived abdominal distention and bloating, favoring *L reuteri* (2.6 ± 1.3 vs 1.1 ± 1.7 episodes per week, in the placebo and the probiotic groups, respectively, $P = .013$). School absenteeism because of abdominal pain did not reveal any significant differences (2.7 ± 0.9 vs 1.9 ± 1.1 days per 4 weeks, in the placebo and the probiotic groups respectively, $P = .08$). Adverse effects were not noticed in any of the participants.

Discussion

Administration of *L reuteri* DSM 17938 reduced the frequency and intensity of abdominal pain in children with FAP. Most data on the potential efficacy of probiotics in abdominal pain-related functional disorders are derived from studies in adults with IBS.[19,20] Randomized controlled pediatric trials are uncommon and controversial, as they present a wide spectrum of study designs, probiotic strains, and outcomes.[8-12] Most of the 401 subjects included in these 5 pediatric trials[8-12] were diagnosed as having IBS (62%). Three of these studies used for probiotic supplementation *Lactobacillus* GG,[8-10] one used a multistrain product,[11] and one trial used *L reuteri* DSM 17938.[12] The present study is in accordance with the results of the last mentioned study, in which administration of the same microorganism to patients with FAP reduced the intensity, but not the frequency, of abdominal pain.[12] However, this trial presents methodologic limitations. The number of subjects is small and no adequate sample size calculation is provided. In addition, there is no allocation concealment and no intention-to-treat analysis as well.

A recent meta-analysis[21] evaluated the effect of *Lactobacillus* GG in abdominal pain-related functional gastrointestinal disorders in children, based on the above mentioned 3 trials.[8-10] The conclusion was that *Lactobacillus* GG moderately increases treatment success in children with these complaints, particularly among children with IBS.

The present study used randomization, double-blind allocation concealment, comparison of baseline characteristics, intention-to-treat analysis, blinding of outcome and monitoring of subjects lost to follow-up. Nevertheless, it has limitations. First, we used a subjective tool in the form of a daily self-reported face pain scale to assess the intensity of abdominal pain. However, this scoring system was validated in children of similar age[18] and has been used commonly in other well-designed studies.[22] Furthermore,

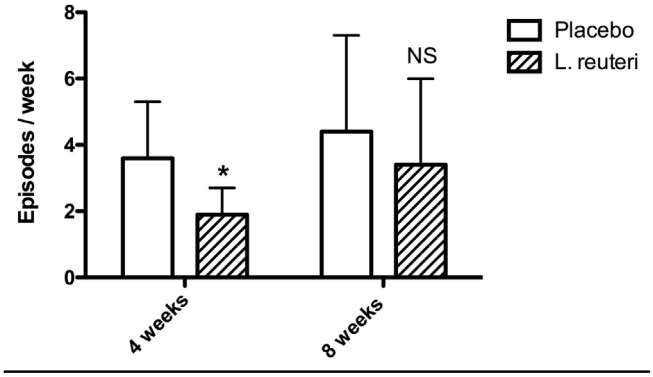

Figure 2. Frequency of abdominal pain at 4 and 8 weeks (episodes/w). Values are mean ± SD. *NS*, not significant. *$P < .02$.

Table. Baseline characteristics

	L reuteri (n = 47)	Placebo (n = 46)
Age (y)	12.2 ± 2.8	11.7 ± 3.2
Sex (male/female)	28/19	25/21
Body weight (kg)	44 ± 11	42 ± 14
Duration of symptoms (y)	1.8 ± 1.4	2.2 ± 1.9
Drug use for abdominal pain (n)	13	16
School absenteeism because of pain (n)	7	9
Self-reported frequency (episodes/wk)	4.2 ± 1.7	3.8 ± 2.1
Self-reported severity, Hicks score	6.8 ± 3.3	7.1 ± 2.8

Absolute numbers or mean ± SD. All *P* values were insignificant.

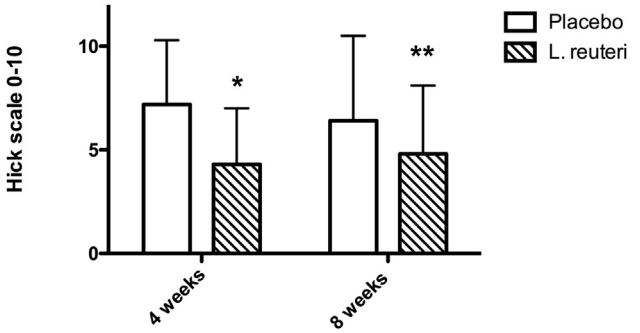

Figure 3. Intensity of abdominal pain at 4 and 8 weeks, Hicks score/wk. Values are mean ± SD. *$P < .01$; **$P < .02$.

the use of self-assessed outcome measures has been recommended for functional gastrointestinal disorders evaluation, as there are no other validated alternatives.[23] An additional limitation of the present trial is the lack of gut microbiota analysis. Studies suggest that patients with IBS have altered fecal flora compared with asymptomatic individuals, and probiotic agents that can modify microbial populations are proposed to treat this condition.[24] The use of advanced metagenomics allowed associating specific microbiome signatures with pediatric IBS.[25,26] These findings indicate the important association between gastrointestinal microbes and IBS in children, but trials of this type require large resources.

Several mechanisms of action have been suggested to explain the beneficial effect of probiotics in abdominal pain-related functional gastrointestinal disorders. Several in vitro and in vivo studies demonstrated the ability of various commensal bacteria to influence relevant gut functions, such as motility, gut barrier integrity, inflammatory activity, visceral sensation, and brain-gut interactions.[20] The probiotic strain used in the present study, *L reuteri* DSM 17938, has been shown to possess some of these mechanisms of action: stimulation of gastrointestinal motility[27,28] and reduction of pain perception.[29]

Data comparing probiotic species in a systematic and broad-based way have been scant and mostly derived from animal and laboratory studies.[30] Different types of probiotic bacteria exert different effect based on specific capabilities and enzymatic activities, even within one species.[31] Recent evidence suggests that probiotics are an effective treatment option for patients with IBS and that the effects of each IBS symptom are likely species-specific.[32]

When the variety of species and strain characteristics are considered, it becomes clear that a proven probiotic effect of 1 strain or species is not necessarily relevant to another. Therefore, more controlled clinical trials, comparing different types of bacteria for a specific indication, are required. Literature data concerning the management of abdominal pain-related functional gastrointestinal disorders in childhood are scant, and many of the published clinical trials suffer from inappropriate design. Thus, future high quality clinical research should focus on the ideal strategy in using probiotics for functional gut disorders, in terms of optimal strain, dose, formulation, and duration. ■

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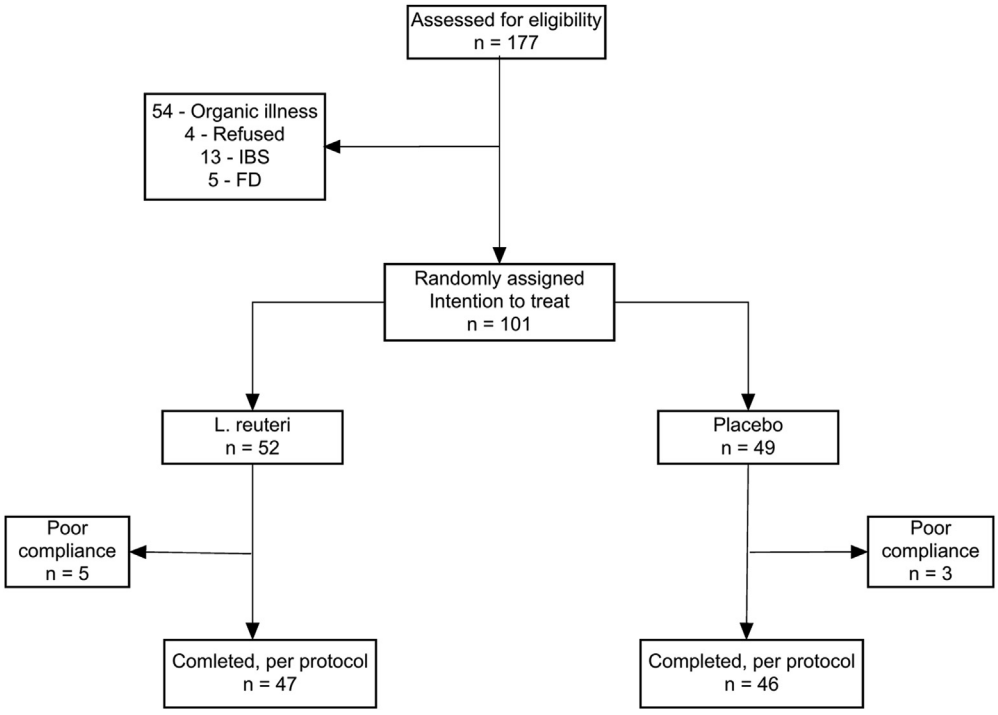

Figure 1. Flowchart showing the enrollment of patients.