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**Long-term safety and efficacy of perinatal probiotic intervention:
evidence from a follow-up study of four randomized, double-blind,
placebo-controlled trials**

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Long-Term Health Effects of Perinatal Probiotics

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Abstract page

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Long-term safety and efficacy of perinatal probiotic intervention: evidence from a follow-up study of four randomized, double-blind, placebo-controlled trials

Pediatric Allergy and Immunology

Abstract

BACKGROUND: Societies worldwide are faced with a progressive increase in immune-mediated health problems such as allergic, autoimmune and inflammatory diseases, as well as obesity. Perinatal administration of specific probiotic bacteria is an attractive approach in reducing the risk of these conditions, but long-term efficacy and safety data are lacking. The aim here was to evaluate the clinical benefit and long-term safety of specific probiotics administered during the perinatal period.

METHODS: The probiotic strains used were *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb-12, *Lactobacillus paracasei* ST11 and *Bifidobacterium longum* BL999. The children involved have subsequently undergone prospective long-term follow-up. In addition to physical examination, data were collected by structured questionnaires on non-

communicable diseases and continued probiotic use, and growth data from welfare clinics and school nurses.

RESULTS: Altogether 303 mother-infant pairs were included in the analysis. Seventy-six out of 163 (47%) children receiving perinatal probiotics had developed allergic disease compared with 79 out of 140 (56%) receiving placebo (OR 0.67, 95% CI 0.43-1.06, $p=0.09$). Fifty-nine out of 133 (44%) children receiving *Lactobacillus rhamnosus* GG perinatally had developed allergic disease, OR 0.62, 95% CI 0.38-0.99, $p=0.047$, as compared to placebo. We found no differences in growth or non-communicable disease prevalence between children receiving perinatally probiotics or placebo.

CONCLUSIONS: Perinatal probiotic administration is safe in long-term follow-up. Children receiving *Lactobacillus rhamnosus* GG perinatally tended to have decreased allergy prevalence.

Key words

Allergy, Asthma, *Bifidobacterium lactis* Bb-12, ST11, *Bifidobacterium longum* BL999, Child, Infant, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* GG, Probiotics/administration&dosage, Probiotics/therapeutic use

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Introduction

The most fully documented probiotic intervention with positive outcome is that comprising the treatment and prevention of acute infectious diarrhoea (1), while current research interest centres on reversing the increase in non-communicable diseases. Allergy, the most common chronic disease in children, is the first condition to manifest itself, and obesity, the most common nutritional problem worldwide, is reaching epidemic proportions with a high velocity of propagation in the paediatric population (2).

Aberrant compositional development of the gut microbiota, more specifically altered diversity, has been related to early-onset non-communicable diseases such as atopy (3,4), eczema (5), asthma and obesity (6). The underlying determinants characterized thus far are maternal atopy or excessive weight gain during pregnancy, delivery by caesarean section and antibiotic use (7). Clinical studies substantiate the potential of specific probiotics in the prevention of atopic eczema, provided the intervention is initiated during pregnancy (8). It has indeed been recognized that the risk of untoward consequences of dysbiosis culminates during the step-wise establishment of the gut microbiota, co-evolving with the immune and metabolic systems of the body (9).

On this basis, we here evaluated the long-term safety of specific probiotics administered during the perinatal period. One major issue relates to potential untoward effects on other chronic conditions. Based on the anticipated counter-regulatory properties between the immune phenotypes characteristic of allergic and many autoimmune diseases, it is conceivable that the

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attenuation of allergic immune responsiveness might increase the risk of other immune-mediated conditions. Nonetheless, there are data to suggest that allergic and autoimmune diseases share common environmental risk factors and immunological features which frequently coexist and that the immune pathology in these disorders may be ameliorated by tolerogenic immune signals (7). We, therefore, focused on the prevalence of non-communicable diseases in children exposed to perinatal probiotic intervention.

Methods

Settings

To establish whether perinatal probiotic administration exerts any long-term effects on non-communicable diseases, we combined data from four ongoing probiotic intervention studies with identical follow-up schedules and population base. The design and methods of these studies have been reported in detail elsewhere; study I (10), study II (11), study III (12), and study IV (13). In brief, all studies were double-blind, randomized, placebo-controlled trials conducted in a single tertiary center in Turku, Finland, between the years 1997 and 2012. The probiotic strains used were: *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG), alone in studies I (10) and IV (13), and in specific combinations (11,12) as indicated in the reports on the primary outcomes. The probiotic preparations were lactose free.

For the present study children who had been allocated to receive the respective study probiotics in the perinatal period were combined into the probiotic group

and those allocated to receive placebo, into the placebo group. Children were included in the LGG group if this strain had been administered perinatally alone or in combination.

This study was reviewed and approved by the Ethics Committee of the Hospital District of South-West Finland. Written informed consent was obtained from the children's parents.

Participants and Study Design

The target population comprised children at risk of disturbances in early host-microbe interaction resulting from preterm birth (13) or heightened allergy risk (10-12). The inclusion criteria for this study were attendance at a two-year follow-up visit and informed consent to participate. Exclusion criteria were incomplete data on non-communicable diseases, as recorded in the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (14) and the structured questionnaire (see below), and perinatal administration of prebiotics.

On regular study visits anthropometric measures were taken from child and mother and blood pressure was measured. Allergy symptoms were documented and skin prick tests performed. At each study visit a three-day dietary diary was collected for both mother and child, and continued probiotic consumption was checked for.

Recruitment for the present follow-up started in June 2013 and continued until January 2014. Out of the original 754 mother-infant pairs, 562 (75%) children were eligible. An invitation letter was sent to the caregiver incorporating the ISAAC questionnaire on allergy and asthma. In addition, families received a structured questionnaire assessing the presence of non-communicable diseases/ chronic inflammatory conditions such as allergic, gastrointestinal, neurological and autoimmune diseases, as well as operations or other hospital treatments, medications, vitamin D supplementation, sleeping habits, and form of day-care. In completing the questionnaire, parents gave information on whether or not their child was regularly receiving (more than twice a week) probiotics in capsules or drops, or any products such as juices or yoghurt enriched with probiotics. A total of 303 (54%) families returned the questionnaires.

In addition to completion of the structured questionnaires, children 4-5 years old (12,13) at the time of the present study were invited for clinical evaluation performed in the same fashion as in cohorts with 10-15 years' follow-up at that age. Additional growth data were obtained from welfare clinics and school nurses. Anthropologic measurements were available for 230 children (41%).

Outcome measures

All diagnoses of non-communicable diseases were based on the questionnaire information and made by one of the authors (KL) blinded to the study group the child was allocated to. Diagnoses of asthma, allergic rhinitis and atopic eczema were confirmed on International Study of Asthma and Allergies in Childhood (ISAAC) criteria (14). A child was considered to have allergic disease if asthma, allergic rhinitis, atopic eczema or food allergy or a combination of these was present.

Overweight and obesity were defined by international age- and sex-specific BMI cut-offs, corresponding to adult BMI cut-offs of 25 and 30 kg/m² at 18 years of age, ISO-BMI ≥ 25 , and ISO-BMI ≥ 30 (15).

Statistical analysis

We carried out an individual participant data meta-analysis using the one-step approach. Briefly, all data from individual participants were combined and the combined probiotic group and the subgroup receiving LGG were compared to the combined placebo group. All data from all studies were modeled simultaneously and taking account of the clustering of participants within individual studies. Baseline differences were tested between combined groups; t-test for independent samples and Mann-Whitney U test for continuous variables with normal and non-normal distributions, respectively. The Chi-squared test was used for categorical variables. The prevalence of asthma, allergic disease and overweight (BMI-ISO ≥ 25 kg/m²) after the follow-up were compared between children receiving probiotic intervention (or LGG alone or in combination) and children receiving placebo.

First, univariate logistic regression analysis was used to compare the combined probiotic and LGG groups to the combined placebo group. However, in the primary analysis, the particular study (studies I, II, III and IV) was included as a categorical factor, since it is not appropriate to analyze individual participant data as if they all derive from a single study. Also the effects of possible confounding factors or predictors (duration of total breastfeeding, duration of exclusive breastfeeding, mode of delivery, and continued regular use of

probiotics) were assessed using the Chi-squared test. The final models comparing probiotic to placebo are adjusted for relevant covariates. Results are given as odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was set at two-sided $p < 0.05$.

Analysis was performed using IBM SPSS Statistics for Windows (version 22.0, Armonk, NY, USA, IBM Corp.)

Results

The clinical characteristics of the study participants are presented in Table 1.

The most common chronic condition in the study population was allergic disease (Table 2) with an overall prevalence of 51.2%. It consisted in allergic rhinitis (prevalence 42.3%), atopic eczema (18.9%), asthma (12.7%) and food allergy (1.4%).

In children given specific probiotics or probiotic combinations perinatally there was a tendency to a decreased prevalence of allergic disease (atopic eczema, allergic rhinitis, food allergy or asthma), OR 0.67, 95% CI 0.43-1.06, $p = 0.09$. In those receiving LGG perinatally the decrease in the prevalence of allergic disease (59/133, 44.4%), as compared to placebo (79/140, 56.4%), was statistically significant, OR 0.62, 95% CI 0.38-0.99, $p = 0.047$.

The prevalence of asthma in the children receiving probiotics perinatally, 9.3%, was not statistically different from that in children given placebo, 15.8%; (OR 0.55, 95% CI 0.24-1.25, $p = 0.15$), nor was the prevalence of asthma in children receiving perinatally LGG alone or in combination

(10/96,10.4%), statistically different from that in children given placebo (18/114, 15.8%); (OR 0.62, 95% CI 0.27-1.42, p=0.26).

Two children in the placebo group and one child in the probiotic group had food allergy, and one in the placebo group presented with eosinophilic oesophagitis. A total of 62/161 (38.5%) children in the probiotic group and 65/139 (46.8%) in the placebo group had allergic rhinitis, while atopic eczema was present in 28/158 (17.7%) children in the probiotic group and in 38/138 (20.3%) children in the placebo group. There were no statistically significant differences in the occurrence of these two conditions.

In study populations with 10-15 years of follow-up (studies I, II and III), the baseline characteristics of the mother-infant pairs who participated in the present study were compared to non-participants, since not only the risk of allergic disease but also the original intervention and its outcome may have influenced the decision to participate. We found that of the participants 55.2% were originally assigned to the probiotic group and of non-participants 44.6% (p=0.007). In the same vein, the breastfeeding duration had been four months or longer in 89.4% of the participants vs. 76.3% of the non-participants (p<0.001). There were no differences in sex, delivery mode, parental smoking, skin-prick test positivity or atopic eczema prevalence at age 2 years between the participants and non-participants.

The prevalence of overweight (Table 3) in children receiving probiotic intervention perinatally, 20.4%, was not different from that in those given placebo, 18.6%; p=0.74. Five (4.6%) children in the probiotic group and four (3.5%) in the placebo group were obese (ISO-BMI \geq 30 kg/m²). No statistically

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significant difference was found between the risk of developing overweight in children receiving LGG perinatally (17/91, 18.7%) *versus* those on placebo (21/113, 18.6%) OR 1.01, 95% CI 0.50-2.04, $p=0.99$. Importantly, according to the multivariate logistic regression model (Table 3), a lower risk of overweight was associated with a breastfeeding duration of six months or more, compared to cases of shorter duration, OR 0.30, 95% CI 0.13-0.67, $p=0.003$. In children who regularly consumed probiotic products there was a tendency to a decreased overweight risk, OR 0.44, 95% CI 0.19-1.04, $p=0.06$.

Discussion

In this prospective follow-up study the perinatal probiotic intervention proved safe in long-term follow-up. We found no differences in growth patterns or non-communicable disease prevalence between children who had received perinatally probiotics or placebo. However, children given perinatally LGG alone or in combination with other defined probiotics had a lower risk of developing allergic disease (allergic rhinitis, eczema, food allergy or asthma) in long-term follow-up. Breastfeeding for six months or longer was associated with a decreased overweight risk. There was a tendency to a decreased risk of overweight in children who regularly consumed probiotic-containing products.

This series of investigations (studies I-IV and the present study) demonstrated that perinatal probiotic intervention yields benefits for allergy throughout childhood, beyond infancy (10,12,16,17,). In the primary prevention of atopic eczema, both pre- and postnatal administration of specific probiotics appears to be required. This conception is supported by meta-analyses, and the effect is

documented in both general and at-risk populations (8), and the mechanism has been substantiated in experimental studies. The therapeutic effect of probiotics may be reduced once colonization and the allergic phenotype are established, when compared with treatment at younger ages when there is greater potential for modulation (18).

The so-called T helper (Th)1/Th2 paradigm provided the initial immunological backbone for the hygiene hypothesis of allergic disease (19). The extended hygiene hypothesis (20,21) provides a sound rationale for the use of probiotics in supporting immunological maturation and the development of a tolerogenic immune environment and thereby reducing the risk of allergic and inflammatory disease. The results reported here on long-term follow-up in children receiving probiotic intervention perinatally support this rationale.

In the same vein, perinatal probiotic intervention as reported here for specific strains was not associated with excessive weight gain, as has been suggested for specific bacterial strains (22). In fact there was a tendency to a lower risk of overweight in children who during the follow-up regularly consumed probiotic-containing products. Previously, perinatal probiotic administration has been shown to modify the growth pattern by restraining excessive weight gain during the first years of life, most pronouncedly at the age of 4 years (23), which is the age considered critical for obesity development later in life (24). In our follow-up study, breastfeeding for six months or more was associated with a decreased risk of overweight, in accord with previous findings (25,26).

Our results should be interpreted in the context of the study's limitations. First, the relatively low response rate (54% of the eligible participants provided the questionnaires) raises concern about selection bias. In order to detect this bias, we compared the baseline characteristics of the mother-infant pairs who participated in this follow-up study to non-participants. The participants had received probiotics more often than the non-participants and they had been breast-fed longer. However, skin-prick test positivity or atopic eczema prevalence at age 2 years were not different between the study population and the drop-outs. Second, families were asked to participate in the follow-up study after unblinding, the impact of which, if any, remains unknown. Finally, this study may lack statistical power to address probiotic effects on less common conditions such as diabetes and coeliac disease. These limitations notwithstanding, the major strengths of this study include careful prospective follow-up of an at-risk population and prospective monitoring of continued probiotic consumption, as well as acquisition of objective documentation of health and growth from records and registers.

In clinical scenarios, in which probiotic treatment has been shown beneficial, it appears to be safe. The routine use of *Lactobacillus rhamnosus GG* in a neonatal intensive care setting has been safe and well-tolerated over a period of several years. (27).

In its guidelines for allergic disease prevention (28), the World Allergy Organization recommends the use of probiotics in pregnant women at high risk of having an allergic child; in women who breastfeed infants at high risk of developing allergy; and in infants at high risk of developing allergy, albeit

conditionally. This present follow-up study provides the evidence called for on the long-term safety of perinatal probiotic intervention. However, each probiotic strain has its specific genotypic and phenotypic properties and needs to be assessed separately for clinical evidence (29,30). We thus need to acknowledge that our safety results cannot be extended to other probiotic strains or combinations, nor can those of the primary studies (studies I-IV) on efficacy, many of the effects noted being strain-specific. Each probiotic strain is inherently different from others and similar health effects cannot therefore be expected even from closely related strains. It is important to characterize each probiotic to strain level and to select strains with documented properties.

A further interesting observation provided by the present study is that practice may apparently direct guidelines and not vice versa. The parents here had chosen to continue the consumption of probiotics, and the practice impacts on the estimation of the probiotic potential. Diet and the use of active nutrients and components should therefore be carefully monitored in clinical studies, both during and after the intervention period; many clinical studies thus far lack this monitoring. As any proof of causality requires well-powered clinical intervention studies in human populations, more such well-controlled research is necessary in at-risk populations. In view of the moving target of environmental exposures observed in the present study, population-based and epidemiological studies are also called for, as already reported for some of the probiotic strains.

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Table 1. Clinical characteristics

	Placebo* (N=140)	Probiotic* (N=163)	LGG ** (N=133)	Probiotic vs. Placebo P value***	LGG vs. Placebo P value***
Pregnancy					
Gestational age, median (range)	40.1 (34.9- 42.4)	40.0 (34.1- 43.0)	40.0 (34.1- 43.0)	0.18	0.16
Mode of delivery					
Caesarean section / vaginal delivery (%)	21/117 (15.2/84.8)	15/143 (9.5/90.5)	13/115 (10.2/89.8)	0.13	0.22
Child					
Female / male (%)	69/71 (49.3/50.7)	74/89 (45.4/54.6)	60/73 (45.1/54.9)	0.50	0.49
Birth weight (g), mean (range)	3577 (2090- 4800)	3537 (1930- 4860)	3521 (1930- 4860)	0.48	0.36
Duration of breastfeeding					
Exclusive breastfeeding (mo), median (range)	4.0 (0.0-6.5)	4.0 (0.0-8.0)	4.0 (0.0-8.0)	0.16	0.17
Total breastfeeding (mo), median (range)	10.0 (0.5- 36.0)	9.0 (0.0-24.0)	8.5 (0.5- 24.0)	0.25	0.25

*Combined data from four studies conducted in Turku, Finland, 1997-2012 (10,11,12,13)

**The LGG group is a subgroup of the Probiotic group including LGG as a single strain or LGG mixed with other probiotics.

***Groups were compared by independent samples test for birth weight and Mann-Whitney U test for other continuous variables. The χ^2 test was used for categorical variables

LGG, *Lactobacillus rhamnosus* GG

Table 2. Long-term follow-up prevalence of asthma and allergic disease in children participating in a perinatal probiotic intervention study

	Group	Prevalence	Unadjusted analysis*			Adjusted analysis**				
			OR	95% CI	P	Factors	OR	95% CI	P	
Asthma	Placebo	18/114 (15.8%)	1.00			Group	Placebo	1.00		
	Probiotic	10/107 (9.3%)	0.55	0.24 to 1.25	0.15		Probiotic	0.59	0.25 to 1.38	0.22
Allergic disease***	Placebo	79/140 (56.4%)	1.00			Study	Study I ****	1.00		
							Study II	0.91	0.38 to 2.17	0.83
	Probiotic	76/163 (46.6%)	0.67	0.43 to 1.06	0.09	Group	Study III ****	0.60	0.12 to 3.06	0.54
							Study IV	0.63	0.07 to 5.53	0.68
							Placebo	1.00		
							Probiotic	0.71	0.44 to 1.14	0.16
Study	Study I	1.00								
	Study II	0.81	0.44 to 1.47	0.49						
	Study III	0.69	0.38 to 1.26	0.23						
					Study IV	0.15	0.03 to 0.72	0.02		

* Univariate logistic regression analysis

** Multivariate logistic regression analysis. The original study was included as a categorical covariate.

***A child with allergic rhinitis, eczema, food allergy or asthma was considered to have allergic disease.

****Early effect on atopic eczema at 24 months of age: Study I, reduced risk OR 0.51, 95% CI 0.32-0.84; study III reduced risk OR 0.17 95% CI 0.08-0.35

Table 3. Long-term follow-up prevalence of overweight (ISO-BMI ≥ 25 kg/m²) in children participating in a perinatal probiotic intervention study.

		Unadjusted analysis*			Adjusted analysis**			
Group	Prevalence	OR	95% CI	P	Factors	OR	95% CI	P
Placebo	21/113 (18.6%)	1.00			Group	Placebo	1.00	
Probiotic	22/108 (20.4%)	1.12	0.58 to 2.18	0.74		Probiotic	1.02	0.46 to 2.25 0.97
					Study	Study I	1.00	
						Study II	0.67	0.27 to 1.64 0.38
						Study III	1.13	0.32 to 3.93 0.85
						Study IV	1.19	0.23 to 6.10 0.84
						Breastfeeding ≥ 6 months	0.30	0.13 to 0.67 0.003
						Continued use of probiotic	0.44	0.19 to 1.04 0.06

* Univariate logistic regression analysis (N=221)

** Multivariate logistic regression analysis (N=187), where the original study, breast-feeding and continued use of probiotic were included as categorical covariates.