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A randomized controlled trial

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Probiotics in late infancy reduce the incidence of eczema: A randomized controlled trial

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Abstract page

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ABSTRACT

Background

Allergic diseases are common and represent a considerable health and economic burden worldwide. We aimed to examine the effect of a combination of two probiotic strains administered in late infancy and early childhood on the development of allergic diseases and sensitization.

Methods

In this double-blind, placebo-controlled intervention trial, participants were randomized to receive a daily mixture of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subsp. *lactis* or placebo – starting prior to attending daycare. The intervention period was 6 months, and the parents answered web-based questionnaires on allergic symptoms and doctor's diagnosed allergic disease monthly. IgE was measured at baseline and follow-up.

Results

A total of 290 participants were randomized; 144 in the probiotic group and 146 in the placebo group. Mean age at intervention start was 10.1 months. At follow-up (mean age 16.1 months), the incidence of eczema was 4.2% in the probiotic group and 11.5% in the placebo group (p = 0.036). The incidence of asthma and conjunctivitis did not differ between groups, and no children presented with rhinitis. Sensitization was equal in the two groups at intervention start (7.5% and 9.5% respectively), and two children in each group were sensitized during the intervention.

Conclusions

We observed a significantly lower incidence of eczema in the probiotic group compared to the placebo group. The probiotics were administered in late infancy – prior to attending day care – suggesting a broader window of opportunity using probiotics in the prevention of eczema. The incidence of asthma, rhinitis, conjunctivitis and sensitization did not differ.

Clinical Trial Registration

Effect of Probiotics in Reducing Infections and Allergies in Young Children Starting Daycare (ProbiComp), NCT02180581. https://clinicaltrials.gov/ct2/show/NCT02180581

Keywords

Allergy, allergic diseases, atopy, *Bifidobacterium animalis* subsp. *lactis, Lactobacillus rhamnosus,* RCT, sensitization

INTRODUCTION

Allergic diseases in childhood consist of eczema, asthma, rhino-conjunctivitis and food allergies. In 2014 it was estimated, that 11.6% of children under the age of 18 years suffer from eczema, 8.4% from rhinitis, 10% from respiratory allergies, and 5.4% from food allergies in the United States (1). In a 2015 estimate, one third of children in Denmark and Sweden was affected by at least one allergic disease at 5 years of age (2). Allergic diseases present a considerable health and economic burden; and might diminish the quality of life (3, 4), making the prevention of the development of these diseases an important task.

In the last decades, the interest in the preventive effects of probiotics (defined by the World Health Organization (WHO) as "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*" (5)) has increased.

In 2012, Pelucchi *et al*. published a meta-analysis on the use of probiotics in prevention of atopic dermatitis (6). They concluded that probiotics play a moderate role in preventing atopic dermatitis if administered in pregnancy/early life to mother, child or both. A similar conclusion was drawn by Cuallo-Garcia *et al.* in a systematic review and meta-analysis from 2015, whereas no preventive effect on other allergic diseases was observed (7).

Due to the beneficial effects on the development of eczema, The World Allergy Organization guideline panel suggested in 2015 to use probiotics in pregnant women at high risk of having an allergic child; women, who breastfeed infants at high risk of developing allergy; and infants at high risk of developing allergy (8). In a systematic review from 2014 on food allergy, The European Academy of Allergy and Clinical Immunology (EAACI) did not find evidence to support the use of probiotics in the prevention of food allergy (9).

Overall, studies on probiotics have shown conflicting results, and the heterogeneity of studies on probiotics and the development of allergic diseases is a pitfall in the interpretation of the results (10).

Most studies investigating the prevention of the development of allergic diseases by probiotics administer probiotics either to the mother during pregnancy, to the infant during early infancy, or both, whereas administration in late infancy has not previously been examined.

As part of the ProbiComp Study (11), we aimed to investigate the effect of *Lactobacillus rhamnosus* (LGG) in combination with *Bifidobacterium animalis* subsp. *lactis* (BB-12) administered in late infancy – prior to attending daycare – on the development of allergic diseases and sensitization in terms of doctor's diagnosed allergic disease, elevated specific IgE levels, and parentally observed and reported food reactions.

METHODS

In the following, “allergic disease” covers doctor’s diagnosed asthma, allergic rhinitis, allergic conjunctivitis, and eczema; whereas “food reaction” covers parentally observed recurrent reactions to food sources.

Participants

The ProbiComp Study is a randomized, double-blind, placebo-controlled intervention trial designed to investigate the effect of probiotics on absence from daycare due to respiratory or gastrointestinal infections in infants aged 8-14 months (11). Inclusion period was August to December 2014 and August to December 2015. Infants expected to start daycare within 12 weeks after intervention start were assigned by block randomization to receive either daily probiotics or placebo for a 6 months’ period. Inclusion criteria were birthweight > 2500 g, gestational age > 36 weeks, being single-born, and expected to start in daycare at age 8-14 months between September and February. Exclusion criteria were severe chronic illness, regular medication (including proton pump inhibitors), antibiotic treatment within 4 weeks prior to baseline examination, and non-Danish speaking parents. Written, informed consent was given by parents or legal guardians of 290 participants. Baseline examination including a structured interview, anthropometric measurements, and a venous blood sample was conducted after randomization, but prior to intervention start. The procedure was repeated at the end of the intervention, 6 months later. Anthropometric measurements were weight and length, but none of these are included in the present manuscript.

Intervention

Intervention started the day following the baseline examination. The intervention group received sachets of 1.0 g maltodextrin supplemented with LGG and BB-12 each in a dose of 10^9 colony forming units (CFU), and the placebo group received maltodextrin only. LGG/BB-12 and placebo sachets were identical in appearance, smell and taste. Both LGG and BB-12 are registered trademarks of Chr. Hansen A/S (Hørsholm, Denmark) and were provided by Chr. Hansen A/S free of charge. To review the isolated effect of LGG/BB-12, fermented dairy products supplemented with probiotics were prohibited two weeks prior to and within the intervention period. Un-supplemented yogurt was allowed 1-2 times per week. There were no restrictions on the use of infant formulas, whether or not the formula contained pro- or prebiotics.

Endpoint measures

The structured interview at baseline contained questions on family and household characteristics as well as allergic disease prior to enrolment. During the intervention period of 6 months, parents were to monthly register symptoms and diagnosis of allergic disease as well as reactions to foods (milk, egg, fish, peanuts, other nuts (e.g. almonds or hazelnuts), flour products, legumes, fruit, and vegetables) in a web-based questionnaire. The questions on allergic symptoms were previously validated in a prospective birth cohort study, where infants were diagnosed with atopic eczema using five different criteria; Hanifin and Rajka, Schultz-Larsen, Danish Allergy Research Centre (DARC), doctor's diagnosed visible eczema, and (as used in the present study) the U.K. Working Party's diagnostic criteria using discriminatory features from Hanifin and Rajka in a questionnaire form (12).

Sensitization was defined using the ImmunoCAP® Phadiatop® Infant blood test (Phadia AB, Sweden), which is an in vitro qualitative and semi-quantitative assay for graded determination of specific IgE antibodies to food and inhalant allergens that are relevant in the development of atopy in younger children. The allergens included in the test are: cow's milk, hen's egg, peanut, shrimp, cat, dog, *Dermatophagoides pteronyssinus*, birch, timothy, ragweed, and *Parietaria judaica* (13).

Results are expressed as Phadia Arbitrary Units (PAU)/L indicating the degree of sensitization, and values ≥ 0.35 PAU/L were considered positive, i.e. the child was classified as sensitized. Furthermore, specific IgE levels against a panel of food and inhalant allergens were determined (ImmunoCAP ISAC™, Thermo-Fischer Scientific, Denmark) in sensitized children.

Statistics

Descriptive statistics were performed to describe the participants, their family and household characteristics. Continuous variables are presented as mean (SD) if normally distributed, otherwise as median (IQR), categorical variables as *n* (%).

The outcomes of the present analysis within the ProbiComp Study were 1) the incidence of allergic disesses during the intervention period, 2) the incidence of sensitization, i.e. ImmunoCAP®

Phadiatop® test with specific IgE ≥ 0.35 PAU/L at the end of the intervention, 3) the incidence of food reactions during the intervention period. Finally, a composite outcome in terms of "any allergic disease", i.e. asthma, rhinitis, conjunctivitis, and eczema was included and analyzed separately. A per protocol approach was chosen due to non-availability of outcome measurements among drop-outs. Furthermore, for every outcome, children already affected at baseline were excluded at follow-up, e.g. children with eczema at baseline were excluded when assessing the incidence of eczema during the intervention period.

Outcome incidences were compared by chi^2 test, $p < 0.05$ was considered significant. Statistical analyses were performed using STATA IC/14.2 (Texas, USA).

Ethics

The study was approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-4-2014-032), and registered at www.clinicaltrials.org (NCT02180581).

RESULTS

Participants

A total of 290 children were randomized, 144 to the intervention group and 146 to the placebo group. A detailed flowchart of the study recruitment is presented elsewhere (11). In summary, five children dropped out after randomization, but prior to baseline examination (1 from the probiotic and 4 from the placebo group). The remaining 285 children had a mean age of 10.1 months (SD 0.7) at baseline examination and intervention start. Baseline characteristics were equally distributed in the two groups (Table 1). Of the 285 children, 25 (8.8%) dropped out during the intervention, 13 from the probiotic and 12 from the placebo group. Mean age at follow-up was 16.1 months (SD 0.9).

Fecal samples from baseline and follow-up was obtained from 201 children, and their gut microbiota composition has recently been described in detail elsewhere (14). To summarize, LGG and BB-12 was detected in 91% and 95%, respectively, of the fecal samples from the probiotic

group, and in 2% and 31%, respectively, of the fecal samples from the placebo group at follow-up. Noteworthy, the BB-12 primer was subspecies specific, as opposed to strain specific (14).

Allergic disease

Regarding allergic disease, no children were diagnosed with asthma, rhinitis, or conjunctivitis at baseline, whereas a total of 19 children were diagnosed with eczema, 11 in the probiotic and 8 in the placebo group. The follow-up groups for asthma, rhinitis, and conjunctivitis therefore comprised 260 children (130 in each group), and the follow-up groups for eczema and any allergic disease comprised 241 children (119 in the probiotics and 122 in the placebo group).

As shown in Table 2, a total of 19 children developed eczema during the intervention; 5 (4.2%) in the probiotic group and 14 (11.5%) in the placebo group ($p = 0.036$), corresponding to a relative risk of 0.37 (95% CI 0.14-0.98). The incidence of the other allergic diseases did not differ across groups. Regarding the composite endpoint "any allergic disease", 9 (7.6%) in the probiotic group and 23 (18.9%) in the placebo group were affected ($p = 0.010$), in both groups driven by eczema (55.5% and 60.9% in the probiotics and placebo group, respectively).

Sensitization

A total of 153 children had both baseline and follow-up IgE measured; 80 in the probiotic and 73 in the placebo group. Of these, 13 were sensitized at baseline; 6 (7.5%) in the probiotic, and 7 (9.6%) in the placebo group, and the follow-up group therefore comprised 140 children; 74 in the probiotic and 66 in the placebo group. During the intervention, two in each group developed sensitization ($p = 0.910$).

Food reactions

A total of 27 children had food reactions at baseline; 13 (9.1%) in the probiotic and 14 (9.9%) in the placebo group, leaving a total of 233 children in the follow-up group; 117 in the probiotic and 116 in the placebo group. Twenty-five children presented with newfood reactions during the

intervention according to parental report, 12 (10.2%) in the probiotic and 13 (11.2%) in the placebo group ($p = 0.814$).

DISCUSSION

In this double-blind, placebo-controlled study, participants were randomized to receive either a mixture of two strains of probiotics (LGG/BB-12) or placebo in late infancy, prior to attending daycare. Despite the late start of administration (mean age 10.1 months), we observed a significantly lower incidence of eczema in the probiotic group compared to placebo during the intervention. Concerning other allergic diseases, we observed no differences in incidences between the groups, which could be due to a later onset of these diseases. Neither did we observe any differences in the incidences of sensitization or food reactions.

Whereas most other studies have included participants based on either maternal allergic disease or first degree relative with allergic disease (15-22), participants in the ProbiComp study were unselected. However, more than half of the children (in both groups) had a first degree relative with a history of allergic disease. This is in line with previous, unselected studies (23-24), and probably reflects a high frequency and awareness of allergic diseases in the population, and a greater intent to participate within families with allergic diseases.

The high detection rate (> 90%) of LGG/BB-12 in fecal samples of the probiotic group indicates a high level of compliance. However, BB-12 was also detected in 31% of the placebo group fecal samples at follow-up. This could be due to the BB-12 primer being subspecies and not strain specific, suggesting detection of endogenous *Bifidobacterium animalis* subsp. *lactis* or due to prior ingestion of related strains through infant formula (14). From baseline to an age of 12.8 months (SD 1.4), 91 children in the placebo group used infant formulas, and of these, 26 had used formulas containing probiotics (11). Wider dietary restrictions were considered during planning of the study, i.e. prohibiting the use of infant formulas containing pre- and/or probiotics, but there was concern, that it would result in difficulties recruiting participants, since a majority of currently available infant formulas in Denmark contain pre- and/or probiotics.

Regarding the use of the *combination* of LGG and BB-12, Huurre *et al.* in 2008 investigated pre- and postnatal maternal administration of a combination of LGG and BB-12. Eczema was developed

in 17.6% of the placebo group and 9.7% of the probiotics group, though not reaching statistical significance ($p = 0.131$) (25).

LGG used in combination with other probiotics has also yielded conflicting results. Regarding maternal administration, Dotterud *et al.* in 2010 used administration of three strains of probiotics, LGG, BB-12 and *Lactobacillus acidophilus* LA5, pre- and postnatally. The cumulative incidence of eczema at the age of two and 6 years was reduced (23, 26). Supporting this, Rautava *et al.* in 2012 observed a protective effect of a combination of *Bifidobacterium longum* and LGG or a combination of *Bifidobacterium longum* and *Lactobacillus paracasei* on the development of eczema, when administered to the mother in pregnancy and during breastfeeding (20). Administration of LGG and *Bifidobacterium longum* (BL999) directly to the child in infant formula from birth until 6 months of age was examined by Soh *et al.* in 2009, and no preventive effect on the development of eczema at two years of age was observed (21).

The use of LGG as a single strain of bacteria in relation to allergic diseases has been investigated several times. Kalliomäki *et al.* in 2001 (16) observed a protective effect of LGG on the incidence of eczema when given prenatally to the mother and after birth to the infant, whilst no effect on the development of other allergic diseases was observed. Wickens *et al.* in 2008 had similar findings for *Lactobacillus rhamnosus* strain HN001 including a protective effect up to 4 years of age (22, 29). In two long term follow-up studies, Kalliomäki *et al.* observed that the preventive effect extended to 4 and 7 years of age, respectively (27-28). Yet, Kopp *et al.* in 2008 (18) and Ou *et al.* in 2012 (30) did not reproduce these findings at follow-up at 2 years of age (Kopp *et al.*) and at 6, 18, and 36 months of age (Ou *et al.*).

To our knowledge, only one other study has investigated the effects of probiotics administered in late infancy on the development of allergic disease. West *et al.* used *Lactobacillus paracasei* ssp. administered during weaning, i.e. from four to 13 months of age, and observed a reduced incidence of eczema at 13 months of age (24).

Regarding sensitization, our null-findings are in line with findings from previous studies (15-16, 18-21, 24, 30). This is also the case with food reactions, where we observed no differences between the two groups. Kukkonen *et al.* observed no differences on the development of food allergies between probiotics and placebo groups, providing the probiotics for the mother 2-4 weeks prior to delivery and to the infant for 6 months thereafter (19). Cuello-Garcia *et al.* did not find evidence in a

systematic review and meta-analysis to support the effects of probiotics to reduce the risk of allergic diseases, other than eczema (7). Finally, EAACI does not support the use of probiotics in the prevention of food allergy in their guidelines (9).

A limitation of the present analysis is that sample size was based on the primary outcome of the ProbiComp study, i.e. absence from daycare due to infections (11). Despite this, we observed significant differences in the development of eczema, and regarding allergic diseases other than eczema, we probably would not have benefited from a larger sample size, since asthma, rhinitis, and conjunctivitis usually do not develop until later in childhood, and food reactions are likely to have already occurred prior to the intervention period. Furthermore, as often observed in randomized controlled trials including healthy individuals, the ProbiComp study population was self-selected and consisted of primarily well-educated, high-income families with a special interest in the study and study participation in general. This may explain the high number of participants completing the study, which is indeed a strength.

In conclusion, we observed that administration for 6 months of a combination of two strains of probiotics (LGG and BB-12) starting in late infancy prior to attending daycare, had a preventive effect on the development of doctor's diagnosed eczema, but no effects on other allergic diseases, sensitization or recurrent food reactions. The late timing of the administration of probiotics suggests an even broader window of opportunity in the prevention of eczema by use of probiotics.

AUTHORS' CONTRIBUTIONS

Ms Meineche Schmidt conducted the analyses and drafted the initial manuscript. Dr Laursen coordinated and conducted the data collection. Ms Bruun provided statistical guidance, critically reviewed and revised the manuscript. Drs Larnkjær, Mølgaard, Michaelsen, and Høst conceptualized and designed the study and critically reviewed the manuscript. All authors approved the final manuscript and revision.

CONFLICTS OF INTEREST

The study was funded by Innovation Fund Denmark, University of Copenhagen, and Chr. Hansen A/S. Drs Mølgaard and Michaelsen received a grant from Chr. Hansen A/S for the current study and for another study with probiotics in Ugandan children with severe acute malnutrition. Chr. Hansen A/S had no involvement in analyses of data. The other authors report no conflicts of interest relevant to this article.

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Table 1 – Baseline characteristics

All values are n (%) unless otherwise stated. Percentages are based on the group total.

	Probiotics	**Placebo**
N	143	142
Household characteristics		
First degree relative with allergic disease[1]	83 (58.0)	81 (57.0)
Older sibling(s)	71 (49.7)	66 (46.5)
Parental smoking, indoor	1 (0.7)	0 (0.0)
Parental smoking, outdoor	13 (9.1)	14 (9.9)
Furry pet[2]	26 (18.2)	25 (17.6)
Age at baseline, months mean (SD)	9.98 (0.81)	10.08 (0.88)
Birth characteristics		
Vaginal birth	111 (77.6)	121 (85.2)
Female sex	69 (48.3)	71 (50.0)
Birth weight, grams mean (SD)	3,543 (492)	3,532 (456)
Nutrition characteristics		
Currently breastfed	72 (50.3)	63 (44.3)
Duration of exclusive breastfeeding, months median (IQR)	4.0 (1.0-5.0)	4.0 (1.0-4.9)[3]

Use of infant formula at baseline	92 (64.3)	102 (71.8)
with probiotics	36 (25.2)	36 (25.4)
with prebiotics	50 (35.0)	60 (42.3)
No use of infant formula at baseline	6 (4.2)	6 (4.2)

1) Asthma, rhinitis, conjunctivitis, or eczema

2) E.g. cat, dog, guinea pig, rabbit

3) n = 140

Table 2 – Doctor's diagnosed allergic disease at follow-up

All values are n (%) unless otherwise stated. Percentages are based on the group total. A per protocol approach was chosen, i.e. N are study population at baseline and follow-up n are study population for the specific endpoint after exclusion of censored cases (those who withdrew prior to follow-up and those who were already diagnosed at baseline). P values are for chi^2 test.

	Probiotics	**Placebo**	**p**
N	143	142	-
Drop-out prior to follow-up	13	12	
Asthma			
Follow-up n	130	130	0.309
Diagnosed at follow-up	3 (2.3)	6 (4.6)	
Rhinitis			
Follow-up n	130	130	-
Diagnosed at follow-up	0 (0)	0 (0)	
Conjunctivitis			
Follow-up n	130	130	0.314
Diagnosed at follow-up	1 (0.8)	3 (2.3)	
Eczema			
Follow-up n	119	122	0.036
Diagnosed at follow-up	5 (4.2)	14 (11.5)	
Any allergic disease			
Follow-up n	119	122	0.010
Diagnosed at follow-up	9 (7.6)	23 (18.9)	