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A randomized controlled trial

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Published in:
Pediatric Allergy and Immunology

DOI:
[10.1111/pai.13018](https://doi.org/10.1111/pai.13018)

Publication date:
2019

Document version
Peer reviewed version

Citation for published version (APA):
Schmidt, R. M., Laursen, R. P., Bruun, S., Larnkjær, A., Mølgaard, C., Michaelsen, K. F., & Høst, A. (2019). Probiotics in late infancy reduce the incidence of eczema: A randomized controlled trial. *Pediatric Allergy and Immunology*, 30(3), 335-340. <https://doi.org/10.1111/pai.13018>



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Article type : Original

Title page

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

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Running title: Probiotic prevention of allergic disease

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/PAI.13018](https://doi.org/10.1111/PAI.13018)

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Count: 2,433 words and 2 tables

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Abstract page

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30 Schmidt RM, Laursen RP, Bruun S, Larnkjær A, Mølgaard C, Michaelsen KF, Høst A.

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Pediatr Allergy Immunol

33

34 ABSTRACT

35 *Background*

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

39 *Methods*

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

45 *Results*

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

52 *Conclusions*

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

57

58 *Clinical Trial Registration*

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

61

62

Keywords

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

65 **INTRODUCTION**

66 Allergic diseases in childhood consist of eczema, asthma, rhino-conjunctivitis and food allergies. In
67 2014 it was estimated, that 11.6% of children under the age of 18 years suffer from eczema, 8.4%
68 from rhinitis, 10% from respiratory allergies, and 5.4% from food allergies in the United States (1).
69 In a 2015 estimate, one third of children in Denmark and Sweden was affected by at least one
70 allergic disease at 5 years of age (2). Allergic diseases present a considerable health and economic
71 burden; and might diminish the quality of life (3, 4), making the prevention of the development of
72 these diseases an important task.

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

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

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

87 Overall, studies on probiotics have shown conflicting results, and the heterogeneity of studies on
88 probiotics and the development of allergic diseases is a pitfall in the interpretation of the results
89 (10).

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

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

98

99 **METHODS**

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

103 *Participants*

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

118

119 *Intervention*

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

130 *Endpoint measures*

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

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

146 Results are expressed as Phadia Arbitrary Units (PAU)/L indicating the degree of sensitization, and
147 values ≥ 0.35 PAU/L were considered positive, i.e. the child was classified as sensitized.

148 Furthermore, specific IgE levels against a panel of food and inhalant allergens were determined
149 (ImmunoCAP ISAC™, Thermo-Fischer Scientific, Denmark) in sensitized children.

150

151 *Statistics*

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

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

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

164 Outcome incidences were compared by chi² test, $p < 0.05$ was considered significant. Statistical
165 analyses were performed using STATA IC/14.2 (Texas, USA).

166

167 *Ethics*

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

170

171 **RESULTS**

172 *Participants*

173 A total of 290 children were randomized, 144 to the intervention group and 146 to the placebo
174 group. A detailed flowchart of the study recruitment is presented elsewhere (11). In summary, five
175 children dropped out after randomization, but prior to baseline examination (1 from the probiotic
176 and 4 from the placebo group). The remaining 285 children had a mean age of 10.1 months (SD
177 0.7) at baseline examination and intervention start. Baseline characteristics were equally distributed
178 in the two groups (Table 1). Of the 285 children, 25 (8.8%) dropped out during the intervention, 13
179 from the probiotic and 12 from the placebo group. Mean age at follow-up was 16.1 months (SD
180 0.9).

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

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

186

187 *Allergic disease*

188 Regarding allergic disease, no children were diagnosed with asthma, rhinitis, or conjunctivitis at
189 baseline, whereas a total of 19 children were diagnosed with eczema, 11 in the probiotic and 8 in
190 the placebo group. The follow-up groups for asthma, rhinitis, and conjunctivitis therefore comprised
191 260 children (130 in each group), and the follow-up groups for eczema and any allergic disease
192 comprised 241 children (119 in the probiotics and 122 in the placebo group).

193 As shown in Table 2, a total of 19 children developed eczema during the intervention; 5 (4.2%) in
194 the probiotic group and 14 (11.5%) in the placebo group ($p = 0.036$), corresponding to a relative
195 risk of 0.37 (95% CI 0.14-0.98). The incidence of the other allergic diseases did not differ across
196 groups. Regarding the composite endpoint “any allergic disease”, 9 (7.6%) in the probiotic group
197 and 23 (18.9%) in the placebo group were affected ($p = 0.010$), in both groups driven by eczema
198 (55.5% and 60.9% in the probiotics and placebo group, respectively).

199

200 *Sensitization*

201 A total of 153 children had both baseline and follow-up IgE measured; 80 in the probiotic and 73 in
202 the placebo group. Of these, 13 were sensitized at baseline; 6 (7.5%) in the probiotic, and 7 (9.6%)
203 in the placebo group, and the follow-up group therefore comprised 140 children; 74 in the probiotic
204 and 66 in the placebo group. During the intervention, two in each group developed sensitization (p
205 $= 0.910$).

206

207 *Food reactions*

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

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

213

214 **DISCUSSION**

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

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

228 The high detection rate (> 90%) of LGG/BB-12 in fecal samples of the probiotic group indicates a
229 high level of compliance. However, BB-12 was also detected in 31% of the placebo group fecal
230 samples at follow-up. This could be due to the BB-12 primer being subspecies and not strain
231 specific, suggesting detection of endogenous *Bifidobacterium animalis* subsp. *lactis* or due to prior
232 ingestion of related strains through infant formula (14). From baseline to an age of 12.8 months (SD
233 1.4), 91 children in the placebo group used infant formulas, and of these, 26 had used formulas
234 containing probiotics (11). Wider dietary restrictions were considered during planning of the study,
235 i.e. prohibiting the use of infant formulas containing pre- and/or probiotics, but there was concern,
236 that it would result in difficulties recruiting participants, since a majority of currently available
237 infant formulas in Denmark contain pre- and/or probiotics.

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

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

242 LGG used in combination with other probiotics has also yielded conflicting results. Regarding
243 maternal administration, Dotterud *et al.* in 2010 used administration of three strains of probiotics,
244 LGG, BB-12 and *Lactobacillus acidophilus* LA5, pre- and postnatally. The cumulative incidence of
245 eczema at the age of two and 6 years was reduced (23, 26). Supporting this, Rautava *et al.* in 2012
246 observed a protective effect of a combination of *Bifidobacterium longum* and LGG or a
247 combination of *Bifidobacterium longum* and *Lactobacillus paracasei* on the development of
248 eczema, when administered to the mother in pregnancy and during breastfeeding (20).

249 Administration of LGG and *Bifidobacterium longum* (BL999) directly to the child in infant formula
250 from birth until 6 months of age was examined by Soh *et al.* in 2009, and no preventive effect on
251 the development of eczema at two years of age was observed (21).

252 The use of LGG as a single strain of bacteria in relation to allergic diseases has been investigated
253 several times. Kalliomäki *et al.* in 2001 (16) observed a protective effect of LGG on the incidence
254 of eczema when given prenatally to the mother and after birth to the infant, whilst no effect on the
255 development of other allergic diseases was observed. Wickens *et al.* in 2008 had similar findings
256 for *Lactobacillus rhamnosus* strain HN001 including a protective effect up to 4 years of age (22,
257 29). In two long term follow-up studies, Kalliomäki *et al.* observed that the preventive effect
258 extended to 4 and 7 years of age, respectively (27-28). Yet, Kopp *et al.* in 2008 (18) and Ou *et al.* in
259 2012 (30) did not reproduce these findings at follow-up at 2 years of age (Kopp *et al.*) and at 6, 18,
260 and 36 months of age (Ou *et al.*).

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

265 Regarding sensitization, our null-findings are in line with findings from previous studies (15-16, 18-
266 21, 24, 30). This is also the case with food reactions, where we observed no differences between the
267 two groups. Kukkonen *et al.* observed no differences on the development of food allergies between
268 probiotics and placebo groups, providing the probiotics for the mother 2-4 weeks prior to delivery
269 and to the infant for 6 months thereafter (19). Cuello-Garcia *et al.* did not find evidence in a

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

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

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

288

289 **AUTHORS' CONTRIBUTIONS**

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

295

296 **CONFLICTS OF INTEREST**

297 The study was funded by Innovation Fund Denmark, University of Copenhagen, and Chr. Hansen
298 A/S. Drs Mølgaard and Michaelsen received a grant from Chr. Hansen A/S for the current study and
299 for another study with probiotics in Ugandan children with severe acute malnutrition. Chr. Hansen
300 A/S had no involvement in analyses of data. The other authors report no conflicts of interest
301 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 chi² 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)	