BJD
British Journal of Dermatology

Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial

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Accepted for publication
30 May 2010

Key words
asthma, atopic dermatitis, paediatrics, prevention, rhinitis

Conflicts of interest
T.Ø. and O.S. participated in seminars sponsored by Tine BA. All other authors declare that they have no conflict of interest.

DOI 10.1111/j.1365-2133.2010.09889.x

Summary

Background Previous reports have suggested that certain probiotics given to mothers and children at risk of atopy halves the incidence of atopic dermatitis (AD) at 2 years of age.

Objectives To examine if probiotics given to pregnant women in a nonselected population could prevent atopic sensitization or allergic diseases during the child's first 2 years.

Methods In a randomized, double-blind trial of children from a nonselected maternal population (ClinicalTrials.gov identifier: NCT00159523), women received probiotic milk or placebo from 36 weeks of gestation to 3 months postnatally during breastfeeding. The probiotic milk contained *Lactobacillus rhamnosus* GG, *L. acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12. Children with an itchy rash for more than 4 weeks were assessed for AD. At 2 years of age, all children were assessed for atopic sensitization, AD, asthma and allergic rhinoconjunctivitis. The intention-to-treat (ITT) analysis was enabled by multiple imputations.

Results Four hundred and fifteen pregnant women were computer randomized. At 2 years, 138 and 140 children in the probiotic and the placebo groups, respectively, were assessed. In the ITT analysis, the odds ratio (OR) for the cumulative incidence of AD was 0·51 in the probiotic group compared with the placebo [95% confidence interval (CI) 0·30–0·87; P = 0·013]. There were no significant effects on asthma (OR 0·68, 95% CI 0·26–1·80; P = 0·437) or atopic sensitization (OR 1·52, 95% CI 0·74–3·14; P = 0·254).

Conclusions Probiotics given to nonselected mothers reduced the cumulative incidence of AD, but had no effect on atopic sensitization.

Worldwide time trends for allergic diseases such as asthma, atopic dermatitis (AD) and allergic rhinoconjunctivitis (ARC) are increasing and high.[1] The hygiene hypothesis, proposed by Strachan in 1989,[2] has been revised, and the role of microbial exposure during infancy in the development of allergic diseases has received particular scientific interest.[3] Randomized controlled trials using various probiotic species and forms of administration have shown partly conflicting results in the prevention of AD and sensitization,[4–11] but were found to be effective in a meta-analysis.[12] All studies administered the probiotics directly to all or to the majority of the children. The mechanisms for the possible preventive effects of probiotics are unclear.[13] Because of the lack of change in atopic sensitization in these studies, and because a large proportion of all cases of AD develops in 'low-risk' groups, Williams pointed out the need for a study involving children both with and without a family history of atopy.[14] Furthermore, the increase in allergic diseases has been suggested to be greatest among those without a family history of atopy.[15] If the increase were due to the loss of protective bacterial colonization in mothers and children during infancy, it is reasonable to hypothesize that administration of probiotics would prevent allergic diseases, especially in those with a less hereditary disposition. In any such study, a limited administration of probiotic supplements to mothers during pregnancy would be preferred to more extended administration to infants, and might be more feasible as a possible public health intervention to prevent allergic disease.[16]

This study aimed to investigate whether a probiotic supplement given to pregnant women during the last 4 weeks of pregnancy up until 3 months after birth would reduce the incidence of allergic disease and allergic sensitization at

2 years of age compared with a placebo, in children both with and without a family history of atopy.

Materials and methods

Participants

The Prevention of Allergy Among Children in Trondheim (PACT) study is a large population-based intervention study in Norway focused on the impacts on childhood allergy of systematic and structured interventions to reduce tobacco exposure, increase consumption of n-3 polyunsaturated fatty acids and reduce indoor dampness.[17] These interventions were implemented as part of the recommended maternity care lifestyle counselling programme throughout the city, regardless of participation in the PACT study or not. We undertook this substudy, Probiotics in the PACT study (Pro-PACT), during the intervention period of the PACT study. Pregnant women were recruited through all seven midwives in Trondheim during pregnancy check-ups. All pregnant women were eligible for inclusion if they understood Norwegian, signed the written consent form, were planning to breastfeed during the first three postnatal months, were in week ≤ 36 of pregnancy, liked and tolerated fermented milk and were not at risk of developing pregnancy complications such as pre-eclampsia. Women were ineligible if they had been taking probiotic supplements during the last 4 weeks, or were planning to move away from Trondheim < 25 months following randomization.

Study design

Once included, pregnant women were randomized in a double-blind manner to 250 mL probiotic low fat fermented milk or 250 mL placebo skimmed fermented milk per day. The probiotic milk, Biola® (Tine BA, Oslo, Norway), contained *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium animalis* subsp. *lactis* Bb-12 (Bb-12) and *L. acidophilus* La-5 (La-5), equalling 5×10^{10} colony-forming units of LGG and Bb-12, and 5×10^{9} of La-5 per day for its entire shelf life. The heat-treated (75 °C for 4 s) placebo milk was sterile and contained no probiotic bacteria. The probiotic and placebo products were produced having equal tastes, were quality controlled, packed in neutral packaging and distributed to the mothers according to the randomization list by Tine BA.

The study milk was given for 4 months, from 36 weeks of gestation to 3 months postnatally. The children were to be breastfed during this period. The mothers recorded their daily consumption of study milk and breastfeeding activities in a diary that was collected 3 months after birth. No specific instructions were given on how to drink the milk.

Self-reported questionnaires on family history of atopy, gender, birth weight, breastfeeding, prematurity, parity, maternal age, parental smoking behaviour and pet exposure were collected, among other data, at baseline, at the age of 6 weeks, at 1 year and at 2 years.

Stool samples, cord blood and venous blood were collected from the children at the ages of 10 days, 3 months and 1 and 2 years. In addition, maternal breast milk and bacterial samples from the vaginal mucosa of the mother and from the oral mucosa of the child were collected. The results from these biological materials are to be reported in a separate paper.

The Regional Committee for Medical Research Ethics for Central Norway (Ref. 097-03) and the Norwegian Data Inspectorate approved the study (Ref. 2003/953-3 KBE/). One of the parents signed a written informed consent form. If the participants dropped out of the study, we were not supposed to ask for the reason. The trial was registered in Clinical Trials.gov (identifier NCT00159523).

Outcome measures

The primary endpoint was diagnosed atopic disease, each one assessed separately as AD, ARC or asthma, during the first 2 years of life. AD was defined according to the U.K. Working Party's diagnostic criteria for AD.[18] Children were offered and recommended an examination for AD during the study period if they had an itchy rash for more than 4 weeks. A trained nurse performed this endpoint examination. At the endpoint of 2 years of age, a paediatrician (Dr Rakel Berg) examined all children. Asthma was defined as at least three episodes of wheezing in the last 12 months combined with treatment by inhaled glucocorticoids, or signs of suspected hyper-reactivity (cough or wheeze at excitement or impaired night sleep) without concurrent upper respiratory infection. The diagnosis of ARC was a clinical decision made by the paediatrician based on a structured medical history and clinical examination. Atopic sensitization was assessed by a positive skin prick test (SPT) or elevated specific IgE (≥ 0·35 kU L^{-1}). SPTs were performed by two experienced study assistants according to the ISAAC II procedure.[19] Standardized extracts from Soluprick® allergens (ALK Abelló, Hørsholm, Denmark) were used: mite (*Dermatophagoides pteronyssinus*), mould (*Cladosporium herbarum*), cat and dog dander, birch, timothy (grass) and mugwort pollen, hen's egg white, codfish, hazelnut and peanut. For cow's milk, fresh skimmed milk was used. The reading of the tests followed standardized procedures.[20] Children were offered epicutaneous anaesthesia with EMLA™ cream (AstraZeneca Ltd, London, U.K.) prior to the venous sampling, which was carried out only once. Sera from venous blood samples were analysed for specific IgE using assays testing for the same allergens as the SPTs. The specific IgE analysis was performed in one laboratory at the University Hospital in Trondheim with the Immulite® 2000 Allergen-specific IgE system (Siemens Medical Solutions Diagnostics, Deerfield, IL, U.S.A.).

The AD severity was assessed using the Nottingham Eczema Severity Score (NESS).[21] IgE-associated AD was defined as AD with a positive SPT and/or elevated specific IgE, and non-IgE-associated AD as AD with a negative SPT and no elevated specific IgE. The children were defined as atopic sensitized if either SPT or specific IgE was positive. A family history of

atopy was defined as AD or asthma or ARC among parents or siblings.

Statistical analyses

The incidence of AD in the placebo group at 2 years of age was assumed to be 40%, and the relative reduction of AD in the probiotic group was assumed to be 40%.[10] Based on 80% power to detect a significant difference ($P = 0{\cdot}05$, two sided), 145 children were required for each study group. To compensate for an expected dropout rate of 30% in both groups, we needed to randomize 208 children in each group.

The Department of Applied Clinical Research at the Norwegian University of Science and Technology randomly assigned the participants. Participants and investigators were blinded to the group to which the participants were assigned. The computer-generated randomization list was revealed to the researchers once all of the participants had completed the endpoint examinations, including the SPTs and specific IgE analyses at 2 years of age.

Analyses adopted the intention-to-treat (ITT) principle, with all participants analysed 'as randomized' after multiple imputation (MI) for missing data. MIs were performed using the method of chained equations with 100 sets of imputations, as implemented in STATA's ICE command.[22] In the imputation model, the sets of predictor variables included compliance with the study milk, trial group, all variables in Table 1, and the outcome. The compliance was dichotomized and defined as drinking ≥ 250 mL of study milk on ≥ 50% of the study days. The participants who did not return the diary were coded as noncompliers. For each outcome variable, a separate MI dataset was created. We combined the resulting estimates with Rubin's rule by the MIM command.[23] All variables in Table 1 were considered as potential confounders and tested separately for alteration on the effect estimator in a multivariate logistic regression model.

The complete-case analysis included all participants who completed the endpoint examinations. The cumulative incidence of AD was summarized by using a Kaplan–Meier curve and the groups were compared with the log rank test. A Cox proportional hazards model was used to estimate the hazard ratio (HR). The relative risk (RR) of the cumulative incidence of AD was calculated from the probability of having AD in the probiotic group vs. the placebo group. The per-protocol analysis included the participants who completed the endpoint examinations, who were compliant with the study milk, reported breastfeeding during the child's first 3 months, and had no intake of other probiotic products during the study period. Children with and without a family history of atopy were included in two separate subgroup analyses. Throughout, the odds ratio (OR) estimates were conducted with logistic regression. The ORs, HRs and RRs for binominal data were accompanied by 95% confidence intervals (CIs). Two-sided significance tests were used, with $P < 0{\cdot}05$ considered statistically significant.

Results

Participants

From September 2003 to September 2005, 415 pregnant women were recruited to the probiotic group (n = 211) or placebo group (n = 204; Fig. 1). The clinical examinations were completed in December 2007. The mean ± SD ages of the children were 26·1 ± 2·3 months and 25·9 ± 2·2 months, respectively. During the study period, 58 children in the

Table 1 Baseline data and clinical characteristics (n = 415)

	Complete-case		Dropouts	
	Probiotic group (n = 138)	Placebo group (n = 140)	Probiotic group (n = 73)	Placebo group (n = 64)
Age, mother (years), mean ± SD	29·97 ± 3·84	29·78 ± 4·10	28·40 ± 4·22	29·53 ± 5·40
Education, mother (years), mean ± SD	15·23 ± 2·02	15·36 ± 2·08	15·25 ± 2·86	14·88 ± 2·72
Education, father (years), mean ± SD	14·87 ± 2·43	14·59 ± 2·42	14·75 ± 3·35	14·83 ± 2·60
Birth weight (g), mean ± SD	3671 ± 484·0	3595 ± 487·0	3636 ± 465·0	3600 ± 425·4
Gender (male), n (%)	72 (52·2)	57 (40·7)	24 (47·1)	18 (46·2)
Premature, n (%)	14 (10·1)	8 (5·7)	2 (4·1)	4 (10·8)
Primiparous, n (%)	74 (53·6)	92 (65·7)	43 (63·2)	37 (62·7)
Atopy in family, n (%)[a]	92 (66·7)	99 (71·2)	54 (79·4)	44 (72·1)
Smoking mother, n (%)	3 (2·2)	4 (2·9)	2 (3·2)	6 (9·8)
Smoking father, n (%)	9 (6·7)	19 (13·9)	17 (27·4)	15 (25·9)
Breastfed ≥ 3 months, n (%)	124 (90·5)	123 (88·5)	27 (84·4)	23 (82·1)
Pets at home, n (%)	46 (33·6)	47 (33·6)	19 (28·4)	19 (32·8)
Child ever used antibiotics, n (%)	57 (41·6)	59 (42·4)	Not applicable	Not applicable
Introduced fish ≤ 6 months of age, n (%)	26 (19·0)	23 (16·5)	6 (20·7)	4 (14·8)
Introduced vegetables ≤ 6 months of age, n (%)	70 (51·1)	90 (64·7)	13 (44·8)	14 (51·9)

[a]Mother, father or sibling ever had asthma or allergic rhinoconjunctivitis or eczema.

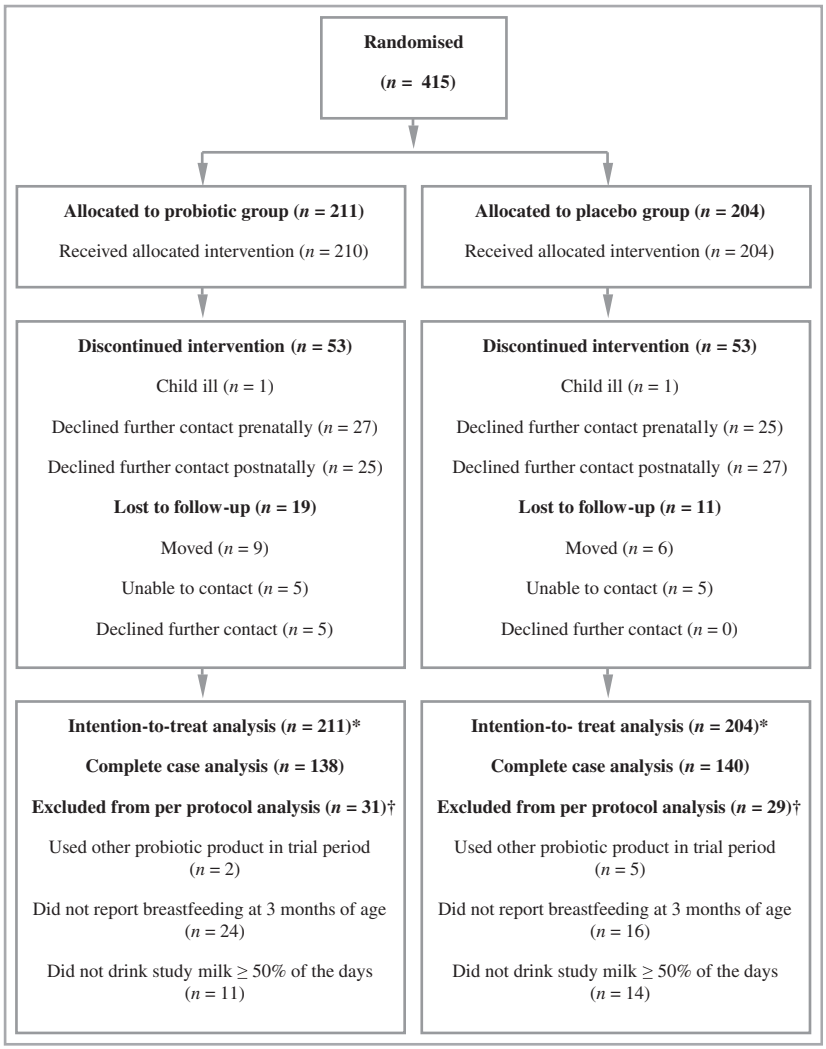

Fig 1. Flow of participants in the probiotic and placebo groups. *Intention-to-treat analysis with multiple imputations. †Used other probiotics or did not report breastfeeding at 3 months of age or did not drink study milk on ≥ 50% of the days.

placebo group and 42 children in the probiotic group attended the offered continual clinical examination of AD and determination of severity. Their mean ± SD ages were 7·7 ± 5·1 months and 7·8 ± 4·9 months, respectively. Three months after birth, 147 and 145 diaries were collected in the probiotic and placebo groups, respectively. Among those who returned the diary, the compliance rates, defined as drinking ≥ 250 mL of study milk on ≥ 50% of the days, were 90·5% and 91·7%, respectively.

The study groups were comparable regarding baseline data and clinical characteristics (Table 1). The dropout rate was 34·6% and 31·4% in the probiotic and placebo groups, respectively (Fig. 1). The dropouts in both study groups were different regarding smoking during pregnancy, both among mothers and fathers, compared with the complete-case group (Table 1). When comparing the baseline and clinical characteristics of the Pro-PACT participants (n = 415) and the intervention cohort in the PACT study (n = 2458), the mean ± SD length of education among mothers was 15·2 ± 2·28 years and 15·9 ± 2·54 years, respectively. Moreover, 28 (7·8%) and 207 (13·3%) babies were premature, 246 (60·7%) and 1320 (54·0%) mothers were primiparous, and 15 (3·7%) and 151 (6·5%) mothers smoked during pregnancy in the Pro-PACT and PACT study, respectively. We found no differences between the samples regarding maternal age, children's birthweight, atopy in the family, gender, breastfeeding, paternal smoking during pregnancy or pets at home.

Intention-to-treat analysis

The OR of the cumulative incidence of AD in the probiotic group compared with the placebo was 0·51 [95% CI 0·30–0·87; P = 0·013; number needed to treat to benefit (NNT_b) = 8; Table 2]. The effect was stronger for non-IgE-associated AD (OR 0·43, 95% CI 0·23–0·81; P = 0·009). In contrast, there was no effect on IgE-associated AD (OR 0·90, 95% CI 0·37–2·17; P = 0·812). There were no significant effects on asthma (OR 0·68, 95% CI 0·26–1·80; P = 0·437),

Table 2 Complete-case and intention-to-treat analyses. Cumulative incidence of allergic disease among 2-year-old children in the probiotic group and the placebo group

	Complete-case			Intention-to-treat
	Probiotic group, n (%)	Placebo group, n (%)	OR (95% CI)[a]	OR (95% CI)[b]
AD	29 (21·0)	48 (34·3)	0·51 (0·30–0·87)	0·51 (0·30–0·87)
IgE-associated AD[c]	9 (6·9)	10 (7·5)	0·91 (0·36–2·31)	0·90 (0·37–2·17)
Non-IgE-associated AD[d]	17 (13·0)	34 (25·6)	0·43 (0·23–0·83)	0·43 (0·23–0·81)
Asthma	8 (5·8)	12 (8·6)	0·66 (0·26–1·66)	0·68 (0·26–1·80)
Allergic rhinoconjunctivitis	1 (0·7)	1 (0·7)	Not applicable	Not applicable
Positive SPT	6 (5·0)	5 (4·2)	1·19 (0·35–4·01)	1·45 (0·46–4·59)
Specific IgE ≥ 0·35 kU L^{-1}	19 (19·6)	13 (12·7)	1·67 (0·77–3·60)	1·63 (0·78–3·40)
Atopic sensitized[e]	20 (15·3)	15 (11·3)	1·42 (0·69–2·91)	1·52 (0·74–3·14)

OR, odds ratio; CI, confidence interval; AD, atopic dermatitis; SPT, skin prick test. [a]Complete-case analysis for probiotic group (n = 138) vs. placebo group (n = 140). [b]Intention-to-treat analysis with multiple imputations for probiotic group (n = 211) vs. placebo group (n = 204). [c]Eczema with a positive SPT or specific IgE ≥ 0·35 kU L^{-1}. [d]Eczema with a negative SPT and specific IgE < 0·35 kU L^{-1}. [e]A positive SPT or specific IgE ≥ 0·35 kU L^{-1}.

ARC or atopic sensitization (OR 1·52, 95% CI 0·74–3·14; P = 0·254). When the analyses were adjusted for potential confounders the estimates of the associations did not change (data not shown).

Complete-case analysis

In the complete-case analysis, there was a significant difference in the cumulative incidence of AD between the probiotic and placebo groups (log rank, P = 0·022; Fig. 2) and the RR was 0·61 (95% CI 0·41–0·91; P = 0·013; NNT_b = 8). The HR was 0·58 in the probiotic group compared with the placebo group (95% CI 0·36–0·93; P = 0·024). According to NESS, the children with AD in the probiotic group had a significantly (P = 0·044) reduced risk of having moderate AD compared with the placebo group (Table 3).

Subgroup analyses regarding family history of atopy disclosed different effects of probiotics on AD (P = 0·004 for interaction; Table 4). Among those with a family history of atopy in the probiotic group (n = 92) and the placebo group (n = 99), the effect on AD was nonsignificant (OR 0·65, 95% CI 0·36–1·17; P = 0·152) regardless of whether the AD was non-IgE associated (OR 0·58, 95% CI 0·29–1·16; P = 0·123) or IgE associated (OR 0·99, 95% CI 0·38–2·57; P = 0·989). Among the participants without a family history of atopy in the probiotic (n = 46) and the placebo (n = 41) groups, however, the effect on AD was statistically significant (OR 0·09, 95% CI 0·01–0·77; P = 0·028; NNT_b = 6). This effect was found among those with non-IgE-associated AD, as none of those without a family history of atopy had an IgE-associated AD. In a separate subgroup analysis regarding family history of AD in the probiotic (n = 55) and placebo (n = 58) groups, the effect on AD was comparable in those with and without a family history of AD (P = 0·705 for interaction).

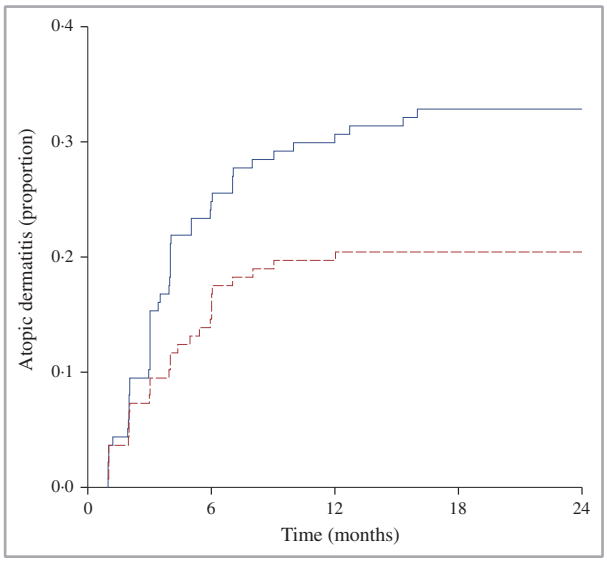

Fig 2. Complete-case analysis. Kaplan–Meier plot showing the cumulative incidence of atopic dermatitis in the probiotic group (broken red line; n = 137) and the placebo group (blue line; n = 137). Log rank; P = 0·022.

Table 3 The severity of atopic dermatitis up until 2 years of age according to the Nottingham Eczema Severity Score (n = 77)

	Probiotic group, n (%)	Placebo group, n (%)	OR (95% CI)[a]
Mild	23 (79)	28 (58)	2·74 (0·94–7·51)
Moderate	5 (17)	19 (40)	0·32 (0·10–0·98)
Severe	1 (3)	1 (2)	Not applicable

OR, odds ratio; CI, confidence interval. [a]For probiotic group vs. placebo group.

Table 4 Subgroup analysis stratified on family history of atopy. Cumulative incidence of atopic dermatitis among 2-year-old children in the probiotic group and the placebo group

	Family history of atopy			No family history of atopy		
	Probiotic group (n = 92), n (%)	Placebo group (n = 99), n (%)	OR (95% CI)[a]	Probiotic group (n = 46), n (%)	Placebo group (n = 41), n (%)	OR (95% CI)[a]
AD	28 (30·4)	40 (40·4)	0·65 (0·36–1·17)	1 (2·2)	8 (19·5)	0·09 (0·01–0·77)
IgE-associated AD[b]	9 (10·5)	10 (10·5)	0·99 (0·38–2·57)	0 (0·0)	0 (0·0)	Not applicable
Non-IgE-associated AD[c]	16 (18·6)	27 (28·4)	0·58 (0·29–1·16)	1 (2·2)	7 (18·4)	0·10 (0·01–0·86)

OR, odds ratio; CI, confidence interval; AD, atopic dermatitis. [a]For probiotic group vs. placebo group. [b]Eczema with a positive skin prick test (SPT) or specific IgE ≥ 0·35 kU L^{-1}. [c]Eczema with a negative SPT and specific IgE < 0·35 kU L^{-1}. Data on SPT or specific IgE were available for 131 and 133 participants in the probiotic group and placebo group, respectively.

Per-protocol analysis

A corresponding significant difference in AD was found in the per-protocol analysis (OR 0·47, 95% CI 0·26–0·85; P = 0·013; NNT_b = 7) and in non-IgE-associated AD (OR 0·37, 95% CI 0·18–0·77; P = 0·008). The difference in IgE-associated AD was minor and nonsignificant (OR 0·92, 95% CI 0·36–2·36; P = 0·861).

Adverse events

No adverse events were reported.

Discussion

The administration of the probiotic bacteria LGG, Bb-12 and La-5 to nonselected women for only 4 months significantly reduced the cumulative incidence of AD among their children at 2 years of age according to both the ITT analysis and the complete-case analysis. When the analyses were adjusted for potential confounders, the association between probiotics and AD was unchanged. This effect was evident for non-IgE-associated AD, and not for IgE-associated AD, corresponding to no preventive effect on atopic sensitization. We found no reduction in the incidence of asthma or ARC at 2 years. In the complete-case analysis the estimates of HR, accounting for person-time before disease onset, and of RR in the cumulative incidence of AD at 2 years, corresponded to each other. According to NESS, the severity of AD was reduced in the probiotic group compared with the placebo group. In a subgroup analysis the preventive effect on AD was evident in children without a family history of atopy and was statistically nonsignificant in those with a positive family history.

Previous trials on preventive effects of probiotics have been conducted in children with a family history of atopy,[5–11,24] with one exception.[4] Furthermore, these previous trials mainly gave probiotics to both the pregnant mother and her child. As suggested by Kalliomäki *et al.*,[25] we applied potentially successful probiotic strains in combinations to prevent allergic diseases. Even though we enrolled nonselected pregnant women and administered probiotics to the mother only, the preventive effect on AD was consistent with previous studies.[4,5,8,10,11] The lack of a preventive effect on AD in some studies could be explained by the use of different probiotic strains and different doses, but also by differences in the children's hereditary disposition and environmental factors. As shown in this study, the preventive effect was weaker in children with a hereditary disposition and it may not be sufficient just to use probiotics for the prevention of AD in a group of children at a particularly high risk of AD. Our study indicates that AD in children without a family history of atopy may differ in its aetiology from classical AD, thus being more susceptible to prevention with probiotics. In two studies, the effects on IgE-associated AD were shown to be similar compared with the effects on AD.[5,8] In another study, a protective effect on IgE-associated AD and atopic sensitization, but not on AD, was found.[9] In contrast, we found no effect on IgE-associated AD. One study suggested that postnatal probiotic administration to infants was associated with an increased likelihood of atopic sensitization at 1 year of age,[7] but the increase in sensitization was no longer apparent in the second year of life.[26] We did not find a statistically significant effect on atopic sensitization estimated by using SPTs or specific IgE, as consistent with previous studies.[4–6,8,10,11,24–28]

Overall, documentation of the preventive effects of some probiotics on AD is convincing, but evidence that the preventive effects are mediated through atopic sensitization is modest. The corresponding HR and RR indicate that the mechanisms by which probiotics prevent AD is a primary phenomenon and not a postponing of its onset. Furthermore, the effects are not necessarily related to postnatal administration to the infants. Perez *et al.*[29] have shown that maternal peripheral blood mononuclear cells and breast milk contain small amounts of viable bacteria and a wide range of bacterial DNA signatures, and maternal supplementation of probiotics might influence the composition of the infants' intestinal microbiota.[16] This may be a potential mechanism by which the neonate can be influenced by maternal probiotic

administration. Maternal supplementation of probiotics also increases anti-inflammatory immunoregulatory factors in maternal breast milk and cord blood.[24,30] We found fewer and less severe cases of AD in the probiotic group compared with the placebo group, and the subgroup analysis showed the preventive effect to be strongest in the participants without a family history of atopy. No statistically significantly different effect was found when stratifying for a family history of AD. Therefore, the family history of atopy, not the history of AD *per se*, seemed more important for the probiotic effect. We hypothesize that the probiotic effect might be anti-inflammatory, leading to a less penetrant AD, especially in children with a lower hereditary disposition. This effect may be mediated either through microbial colonization or by maternal immunoregulatory factors, and may not necessarily be related to sensitization.[16,24,29,30] Early and proactive intervention with effective control of skin inflammation is advised in the management of AD, and may be effective to counter later atopic sensitization.[31] However, a follow up during the first 7 years of life of the original Kalliomäki study failed to show a reduction in atopic sensitization in the probiotic group, while the reduction in AD persisted.[25,28] In another follow-up study the allergy-preventive effects of probiotics were not sustained at the age of 5 years.[27]

The strengths of this study included the randomized, controlled design, a long duration of follow up, blinding and proper concealment of the intervention and simple, clear outcomes. The use of MI enabled us to conduct an ITT analysis, as we did not include the dropouts at endpoint examination. The MI strategy is valid upon the Missing At Random (MAR) assumption, i.e. that the missing values can be predicted unbiased from the remaining observed data. The imputation model included a wide range of baseline characteristics, compliance with the study milk, and outcome. As we imputed 100 datasets, the sampling error associated with the MI-based estimates was low; we were able to reproduce the estimates with a sensible precision when repeating the imputation procedure several times.

The reason for the dropout rate might be associated with the taste of the study milk, the demanding study protocol, and this being a preventive trial: it might have been more difficult to motivate for continued participation compared with a treatment trial. The dropout rate was similar in both the probiotic and placebo groups. We assumed dropping out of the study before 3 months of age, moving out of Trondheim and being unreachable at endpoint not to be a risk of disease modification of continued participation. Only five participants in the probiotic group declined further contact at endpoint at 2 years of age. There is a risk that these five dropouts were associated with AD and might be Missing Not At Random. However, regardless of the mechanism of missingness for these few dropouts, the estimates would not have been significantly altered. Taken altogether, we consider the MAR assumption to be appropriate given the predictors included in the MI model. The MI-based ITT analysis and complete-case analysis gave similar results for the primary outcomes, which is reassuring and may reflect the fact that the dropouts were similar to those who completed to endpoint. The complete-case estimates are therefore not likely to be biased due to the dropout rate. The nonsignificant results in asthma and ARC may be a result of insufficient statistical power.

We did not collect information on how many pregnant women were asked to participate in this study. However, we were able to compare the baseline characteristics of the participants with the PACT study population, which has been shown to be comparable with the general population in Trondheim.[17] Comparatively, the Pro-PACT study population was marginally different but in such a way that we do not believe this seriously compromises the generalizability of our findings to pregnant women under comparable conditions.

In conclusion, our study suggests that a probiotic supplement given to mothers from a nonselected population alone may be sufficient to prevent AD, but not atopic sensitization, in children at 2 years of age. The probiotics did not postpone the first appearance, indicating a primary preventive effect. The effect was most evident in children without a family history of atopy. As such, certain probiotics may be applicable as a public health intervention in populations with a high incidence of AD. Studies focusing on the mechanisms by which maternal supplementation of probiotics exerts an effect on AD during early life are clearly necessary. Such studies would also contribute to a better understanding of the pathogenesis of AD.

What's already known about this topic?

- Probiotics have been tested in the prevention of allergy in infants, but the body of evidence is not yet fully conclusive.
- There are currently no publications on the efficacy of probiotic bacteria administered to nonselected women as potential prevention of allergic disease in their offspring.

What does this study add?

- The short-term administration of probiotic bacteria to mothers reduced the cumulative incidence of atopic dermatitis in the offspring of nonselected women during the first 2 years of life, and reduced the severity of atopic dermatitis in affected children.
- The primary preventive effect was most evident in children without a family history of atopy.
- As such, certain probiotics may be applicable as a public health intervention in populations with a high incidence of atopic dermatitis.

Acknowledgments

The authors thank all of the children and their parents for cooperation in this study, the project assistants Guri Helmer-

sen and Else Bartnes, the nurse Kirsten Øyum, Dr Rakel Berg, Dr Anne Rø, research fellow Marit Saunes, all seven midwives in Trondheim and Pål Romundstad for statistical advice. This study was supported by the Norwegian University of Science and Technology, the Norwegian Research Council, Nidarosfondet and Siemens Medical Solutions Diagnostics AS. Tine BA sponsored the study, including the study milk. The Tine BA Research Manager, Stein-Erik Birkeland, and the Senior Researcher, Anette Roll Mosland, contributed to the practical accomplishment of the study. The funding sources had no role in the study design, data collection, data analysis, interpretation of the study results, or writing of the manuscript.

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