

Treatment and secondary prevention effects of the probiotics *Lactobacillus paracasei* or *Bifidobacterium lactis* on early infant eczema: randomized controlled trial with follow-up until age 3 years

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Clinical & Experimental Allergy

Summary

Background Allergic disease has been associated with altered intestinal microbiota. Therefore, probiotics have been suggested as a potential treatment for eczema.

Objective We investigated whether dietary supplementation of infants with eczema at age 3–6 months with *Lactobacillus paracasei* CNCM I-2116 or *Bifidobacterium lactis* CNCM I-3446 had a treatment effect or altered allergic disease progression.

Methods Primary outcome included eczema severity (SCORing Atopic Dermatitis, SCORAD) 3 months post-randomization. Secondary: SCORAD (other visits); infant dermatitis quality of life (IDQoL); gastrointestinal permeability; urinary eosinophilic protein X; allergen-sensitization; allergic symptoms (age 12, 18, 36 months). A total of 208 infants aged 3–6 months with physician-diagnosed eczema were recruited; 137/208 (SCORAD ≥ 10 , consuming ≥ 200 mL standard formula/day) were randomized to daily supplements containing *L. paracasei* or *B. lactis* or placebo for a 3-month period, while receiving extensively hydrolysed whey-formula (dairy-free diet). There were two open observational groups, one group exclusively breastfed ($n = 22$) and the other, standard formula-fed ($n = 49$). Trial number: ISRCTN41490500.

Results Eczema severity decreased significantly over time in all groups. No significant difference was observed between randomized groups after 12-week treatment-period (SCORAD-score pre-/post-intervention: *B. lactis* 25.9 [95% CI: 22.8–29.2] to 12.8 [9.4–16.6]; *L. paracasei* 25.4 [22.1–29] to 12.5 [9.2–16.4]; placebo 26.9 [23.4–30.6] to 11.8 [9.6–14.3]; $P = 0.7$). Results were similar when analysis was controlled for allergen-sensitization, or when only sensitized infants were analysed. No differences were found for secondary outcomes. No difference was observed in SCORAD-score between randomized and observational groups.

Conclusion and Clinical Relevance We found no benefit from supplementation with *B. lactis* or *L. paracasei* in the treatment of eczema, when given as an adjunct to basic topical treatment, and no effect on the progression of allergic disease from age 1 to 3 years.

Keywords allergic disease, eczema, probiotics, quality of life, randomized double-blind placebo-controlled trial

Submitted 20 June 2011; revised 25 July 2011; accepted 12 August 2011

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Introduction

The intestinal microbiota plays a key role in immune homeostasis, and distinct patterns of gut microbiota

composition may be associated with established and developing allergic disease [1–4]. In light of this, probiotic bacteria (live micro-organisms, which, when consumed in adequate amounts, confer a health-benefit on

the host) [5] are of interest as potential treatments for allergic disease. Numerous probiotic strains have been investigated *in vitro* and *in vivo* to elucidate their biological effects [6–9]. Reports suggest that they may improve gastrointestinal permeability, reduce gastrointestinal inflammation and induce beneficial immune responses [10–12]. Whilst there may be a role for probiotics in eczema prevention, treatment studies have shown highly inconsistent results [13–15]. No long-term outcomes after early life treatment of established eczema with probiotics have been reported.

We investigated the effect of supplementation with probiotics, *Lactobacillus paracasei* CNCM I-2116 or *Bifidobacterium lactis* CNCM I-3446, as an adjunct to dairy elimination diet on eczema severity in infants, and aimed to elucidate whether these strains affected infant dermatitis quality of life (IDQoL), atopic sensitization, a marker of inflammation, gastrointestinal permeability and progression of allergic disease up until age 3 years.

Methods

Participants

We recruited infants with physician-diagnosed eczema [16] from community clinics (age 3–6 months, good general health, normal growth; exclusion criteria: ≤ 34 weeks gestation, congenital abnormality, chronic disease, currently taking oral antibiotics, currently on soya/extensively hydrolysed formula). At baseline visit, the eczema diagnosis was confirmed and standardized skin-treatment was commenced for a run-in period of 2 weeks (1% hydrocortisone ointment 2 $\times$ /day, emol-

lients/moisturizers 2–4 $\times$ /day, bath emollient). Following this, infants were randomized if SCORing Atopic Dermatitis index (SCORAD index) [17] was ≥ 10 and they were consuming ≥ 200 mL standard formula/day. Exclusively breastfed infants joined the breastfeeding open observational group. Some parents declined to use a hydrolysed formula; these infants were followed up in a standard-formula-fed open observational group (Fig. 1). The study was conducted under Registered trial number ISRCTN41490500. All aspects of the study were approved by the Local Research Ethics Committee. Informed consent was obtained from all parents.

Interventions

Randomized infants were commenced on a dairy elimination diet (standard formula changed to extensively hydrolysed whey-formula, in keeping with common clinical practice) and they received a daily supplement-sachet containing 10^{10} colony-forming units of either lyophilized powdered *L. paracasei* CNCM I-2116 or *B. lactis* CNCM I-3446 or a sachet containing placebo (Maltodextrin) for a 3-month period. Other fermented or probiotic-containing products were avoided during this period.

Outcomes

Primary. SCORAD index was calculated after a 12-week intervention.

Secondary. SCORAD index was calculated (all other visits) that included: IDQoL-score [18], gastrointestinal

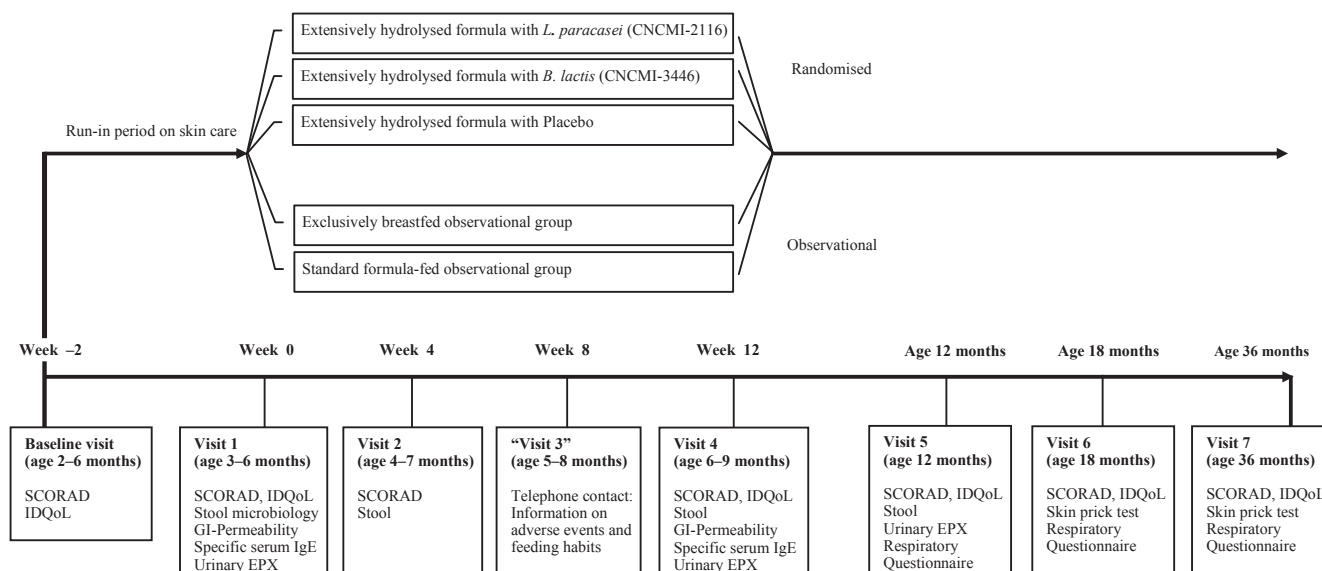


Fig. 1. Study visits and timelines.

permeability, urinary eosinophilic protein X (U-EPX), presence/absence of study strains in stools, prevalence of sensitization; symptoms of allergic disease at age 12, 18 and 36 months.

Clinical outcome measurements

Eczema severity. The SCORAD index (described elsewhere [17]) was used to document eczema severity; three trained researchers performed all assessments for the study (C. G., G. K. and K. J.).

Infant dermatitis quality of life index [18]. Ten-item questionnaire (Likert-scale) was used for assessing main domains of an infant's life.

Respiratory and allergic symptoms. Respiratory and allergic symptoms were assessed (age 12, 18, 36 months) using an interviewer-administered validated respiratory questionnaire [19].

Allergic sensitization. Specific serum IgE (cows' milk, egg, peanut, house-dust-mite, cat, dog; ImmunoCAPTM, Phadia, Uppsala, Sweden) was measured immediately pre- and post-intervention. Sensitization was defined as specific IgE >0.35 kU/L.

Skin-prick-testing was performed to cows' milk, egg, wheat, soya, dust mite, cat, dog, grass pollen (age 18, 36 months). Sensitization was defined as weal measurement ≥ 3 mm.

Urinary eosinophilic protein X. Urinary eosinophilic protein X was measured by immunofluorometric assay (detection limit <0.5 $\mu\text{g/L}$; Phadia, Uppsala, Sweden). As total urine volumes were unknown, we measured urinary creatinine and expressed results as U-EPX/creatinine ratio ($\mu\text{g}/\text{mmol}$).

Gastrointestinal permeability testing. Lactulose/mannitol dual sugar test was used according to abbreviated protocol [20]: infants received the test solution (5 g lactulose, 1 g mannitol in 50 mL water) after ≥ 3 -h fast after which, only water for next two hours. Urine sample was collected after ≥ 60 min; lactulose and mannitol were quantified by high performance liquid chromatography with pulsed amperometric detection [21]; lactulose/mannitol excretion-ratio was calculated.

Detection of probiotic study strains in faeces. Bacterial DNA was extracted from fecal samples, target bacterial sequences were amplified by PCR and bacterial profiles were produced using denaturing gradient gel electrophoresis [22]. Profiles of lactic acid bacteria in samples were obtained using group-specific

primers (lactobacilli: *Lac*-Primers [22]; bifidobacteria: *Tal*-Primers [23]). Each study species was considered *present* if it was not detected prior to commencement of the study-diet, but detected after 4 and 12 weeks of supplementation with the probiotic strains.

Adverse events. The adverse events were recorded by type, seriousness and relation to study product.

Sample size

Sample size calculation was based on SCORAD index. A previous report [24] suggested a high correlation between baseline and outcome (0.76–0.93), with a standard deviation in SCORAD increments of 7.65. A treatment effect of five SCORAD points was considered clinically relevant. To achieve 80% power, choosing $\alpha = 0.05$ (two-sided), 38 patients per diet-group were required. Forty-two infants were recruited per treatment group to allow for 10% dropout.

Randomization

The minimization method was applied to allocate subjects to study groups applying breastfeeding, family history and initial SCORAD index as stratification factors (TrialBalance[®] randomization programme; Nestec, Lausanne, Switzerland). The powders in study sachets were indistinguishable in taste and appearance (masking had been performed at source by supplier). Study visits were identical for all infants (Fig. 1).

Statistical methods

Formal hypothesis testing was only possible for randomized groups. Results are presented separately for randomized and observational groups. Primary analysis was intention-to-treat. Skin treatment was included in the analysis as follows: (1) combination of topical steroids (TS), emollients ($\geq 2\times/\text{day}$) and bath emollient (yes/no); (2) skin-care intensity (emollients vs. mild TS vs. moderately potent TS/calcineurin inhibitors).

Data were normalized, where possible, and are presented as geometric mean with 95% confidence intervals (G. M., 95% CI) if log-normalized, and if square-root transformed, as de-transformed mean (SQM) with 95% CI. We used ANCOVA to correct for the outcome values at randomization, breastfeeding and family history. For dichotomous outcomes, logistic regression and multiple logistic regression analyses were used. The statistical packages SAS (8.1) and SPSS (15.0) (SPSS Inc., Chicago, IL, USA) were used for data analysis.

Results

Participant flow

Of 328 referred infants, 208 were eligible for inclusion in the study after the run-in period and allocation to treatment groups (Fig. 2). There were no significant differences between randomized groups in age, gender, parental education/age, ethnicity, eczema severity, prevalence of partial breastfeeding and atopic family history

(Table 1). During the run-in period, both eczema severity and QoL improved significantly (SCORAD, SQM [95% CI]: 29.9 [28.2–31.7] to 24.7 [23.2–26.2]; IDQoL, SQM [95% CI]: 5.7 [5.1–6.3] to 4.3 [3.8–4.7], baseline and after run-in respectively, both $P < 0.001$).

Primary outcome

The SCORAD score decreased significantly over time (Fig. 3, Table 2). There was no significant difference in

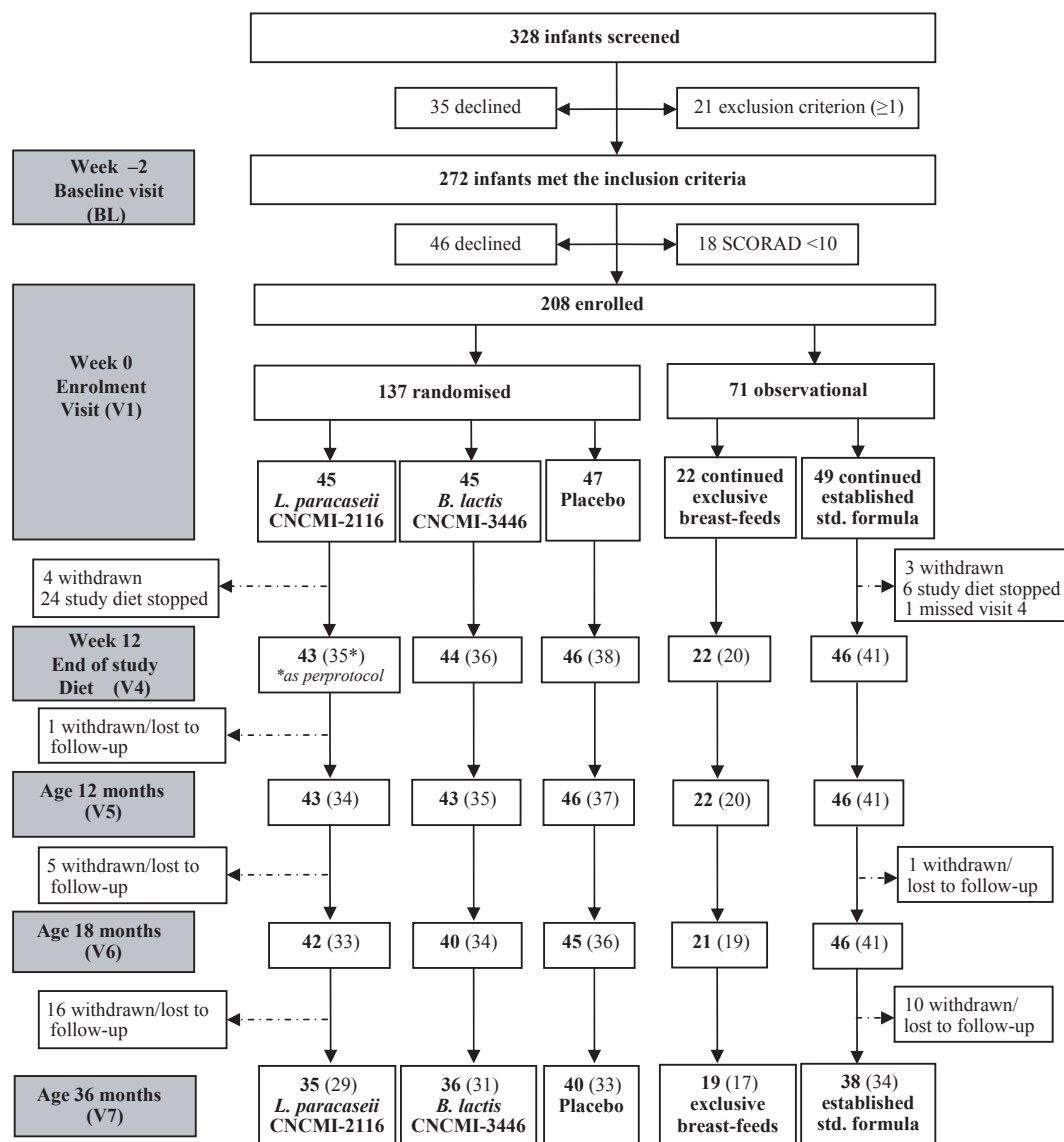


Fig. 2. Schematic representation of trial profile. Completion of intervention as per protocol (PP; defined as completing supplementation period and attending Visit 1 to/including Visit 4): randomized group (R), 109/137 (80%); formula-fed observational (FF), 41/49 (84%); breastfed observational (BF), 20/22 (91%). Reasons for not completing as per protocol (not PP): formula side effects (R: 20/137, FF: 0/49); non-compliance with diet (R: 5/137, FF: 5/49, BF: 1/22); withdrawn/lost (R: 4/137, FF: 3/49, BF: 0/22); missed post-intervention visit (R: 2/137, FF: 0/49, BF: 1/22). PP vs. not PP: no significant differences at enrolment for: parental age/education/ethnicity/history of atopy; infant gender/eczema severity (SCORing Atopic Dermatitis, SCORAD)/sensitization.

Table 1. Demographic details of the cohort

	Whole cohort (<i>n</i> = 208)	<i>Lactobacillus</i> <i>paracasei</i> (<i>n</i> = 45)	<i>Bifidobacterium</i> <i>lactis</i> (<i>n</i> = 45)	Placebo (<i>n</i> = 47)	<i>P</i> -value (randomized groups)	Breastfed observational (<i>n</i> = 22)	Formula-fed observational (<i>n</i> = 49)
Gender (male, <i>n</i> [%])*	121 [58]	28 [62]	24 [53]	28 [60]	0.68	16 [73]	25 [51]
Parental age (years, mean [SD])†							
Mother	31 [5]	30 [7]	31 [5]	32 [5]	0.22	33 [4]	31 [5]
Father	34 [6]	33 [6]	34 [7]	34 [6]	0.96	37 [6]	35 [6]
Maternal education (<i>n</i> [%])*							
No formal education	14 [7]	4 [10]	3 [8]	4 [9]	0.69	–	3 [7]
Secondary school qualification	52 [27]	11 [26]	12 [30]	14 [30]		4 [19]	11 [24]
Non-university professional training	37 [19]	12 [29]	10 [25]	6 [13]	–	2 [10]	7 [15]
College/university/higher degree	92 [47]	15 [36]	15 [38]	22 [48]		15 [71]	25 [54]
Did not wish to answer	13	3	5	1		1	3
Ethnic background*							
Caucasian (<i>n</i> [%])	164 [79]	36 [80]	40 [89]	32 [68]	0.1	17 [77]	39 [80]
Asian	18 [9]	3 [7]	3 [7]	6 [13]		3 [14]	3 [6]
African/Afro-Caribbean	7 [3]	3 [7]	–	1 [2]		–	3 [6]
Other (Oriental, mixed race)	19 [9]	3 [7]	2 [4]	8 [14]		2 [9]	4 [8]
Family history of allergic disease*							
0 (no family history)	73 [35]	16 [36]	15 [33]	16 [34]	0.9	8 [36]	18 [37]
1 (first degree relative)	82 [39]	15 [33]	18 [40]	20 [43]		6 [27]	23 [47]
≥2 (first degree relatives)	53 [25]	14 [31]	12 [27]	11 [24]		8 [36]	8 [16]
Baseline visit							
Age (weeks, median [IQR])†	19 [15–23]	19 [16–23]	20.5 [17–23]	20 [16–23]	0.81	15 [13–19.5]	19 [15–21.5]
AD duration (weeks, median [IQR])†	12 [8–16]	12 [9–16]	15 [9–18.5]	11 [5–16]	0.14	8.5 [3.5–12]	10 [7.5–15]
SCORAD (SQM [95% CI])†	29.5 [28.2–31.7]	27.4 [24–31.1]	33.7 [29.3–38.4]	32.5 [28.4–36.9]	0.07	28.7 [23.9–33.6]	27.2 [24–30.4]
IDQoL (SQM [95% CI])†	5.7 [5.1–6.3]	5.7 [4.5–7.2]	7.2 [3.5–9.1]	6.5 [5.2–8.1]	0.41	4.9 [3.4–6.6]	4.4 [3.5–5.4]
Enrolment visit							
Partial breastfeeding (yes, <i>n</i> [%])*	41 [20]	12 [27]	13 [29]	11 [23]	0.83	–	14 [29]
Age (weeks, median [IQR])†	21.5 [18–25]	23 [10–25.5]	23 [19–26]	21.5 [18.5–26]	0.66	19 [17–23]	21 [18.5–25]
SCORAD (SQM [95% CI])†	24.7 [23.2–26.2]	25.4 [22.1–29]	25.9 [22.8–29.2]	26.9 [23.4–30.6]	0.82	23.8 [19–29.1]	21.2 [18.7–23.9]
IDQoL (SQM [95% CI])†	4.3 [3.8–4.7]	4.8 [3.8–6.1]	5.2 [4.3–6.2]	4.2 [3.3–5.2]	0.34	3.8 [2.6–5.3]	3.2 [2.6–4.0]

*†Statistical methods: 1 = Chi-square; 2 = ANOVA; AD, atopic dermatitis, doctor-diagnosed at screening visit (see text).

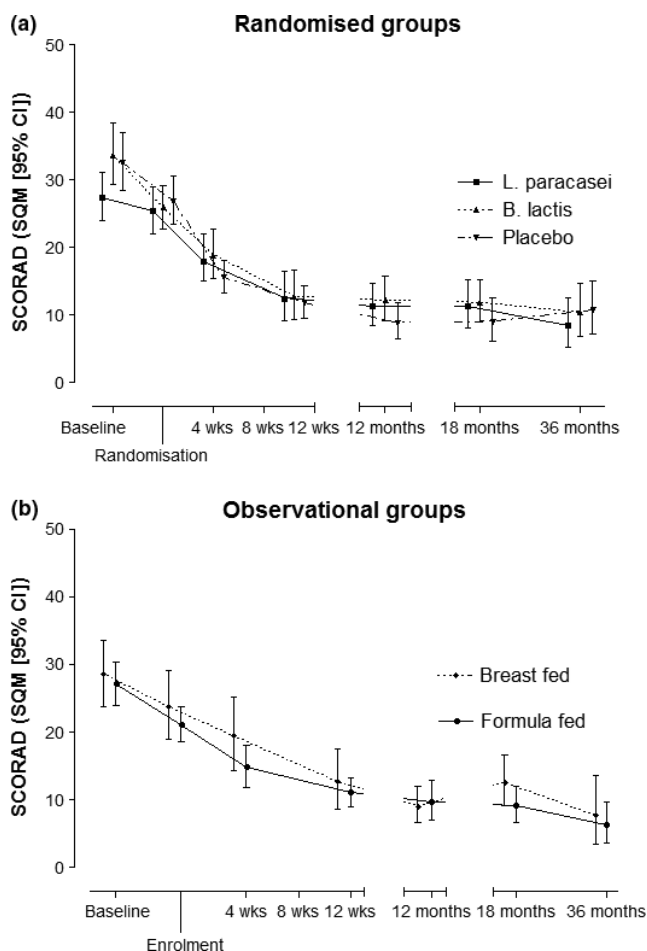


Fig. 3. (a) Randomized groups; (b) observational groups. Differences in eczema severity between study groups. Decrease in SCORing Atopic Dermatitis (SCORAD) over time in the five different groups (intention to treat, uncorrected data shown). Overall significant change in SCORAD over time, $P < 0.001$. No difference in pattern of change over time, $P = 0.76$.

the magnitude of reduction among the three groups from randomization to the end of intervention/supplementation. There was no significant treatment effect when the analysis was controlled for allergic sensitization, or when only sensitized infants were included. No difference between groups was observed when only infants were included, who completed the study as per protocol, or who completed as per protocol and had microbiological evidence of the study strains in their stool.

Secondary outcomes

Eczema severity. There was no significant difference in SCORAD score between randomized groups at any time-point up to age 3 years (Fig. 3a, Table 2). No steroid sparing effect, evaluated for frequency of use and

potency of TS, was observed between groups. In addition, eczema severity did not appear to differ in the observational groups (Fig. 3a and b).

Infant quality of life. Quality of life improved in all groups over time and no significant difference between randomized groups up to age 3 years was observed.

Allergic sensitization. Paired serum samples (pre-/post-intervention) were available from 62/137 (45%) randomized infants. The overall prevalence of allergen sensitization was 58% at randomization and 60% post-intervention. There was no significant effect of probiotic treatments on the prevalence of allergen sensitization post-intervention.

Presence/absence of study strains. At enrolment, either study species was detected in only 3/208 infants. During supplementation (infants with complete sample-series), *L. paracasei* was present in 26/42 (65%) and *B. lactis* in 22/42 (52%). At age 1 year, none had evidence of *L. paracasei* in the faeces (3–6 months post-supplementation); *B. lactis* was detected in three infants (two in *B. lactis* group).

Urinary eosinophilic protein X. We found no correlation between U-EPX-levels and eczema severity (SCORAD), and no significant difference between treatment groups at any visit.

Gastrointestinal permeability. Results from infants who were administered the L/M-permeability test as per test protocol were analysed. No significant differences between groups were found.

Symptoms suggestive of allergic disease at age 1–3 years

There was no difference between randomized groups in numbers of children with doctor-diagnosed asthma or prevalence of wheeze at age 12, 18 or 36 months. There was no difference in the prevalence of other respiratory or nasal symptoms at age 1 year. Infants in the placebo group had the lowest prevalence of eye symptoms (itching/redness) at age 12 months (itching: 7% vs. 27% *B. lactis* and 33% *L. paracasei*, $P = 0.008$; redness: 4% vs. 23% and 28% respectively, $P = 0.009$). This effect did not persist at later visits.

Adverse events. At randomization, infants were switched to extensively hydrolysed formula together with either probiotic- or placebo-sachet. At the 4-week visit, 42/137 (30.7%) parents reported some difficulties (e.g. green loose stools, increased vomiting, feed-refusal

Table 2. Primary and secondary outcome data

	N available for analysis (all groups)	Visit	<i>L. paracasei</i>	<i>B. lactis</i>	Placebo	P-value (randomized groups)*	Breastfed observational	Formula-fed observational
Primary outcome								
SCORAD-score (SQM [95% CI])	208	1	25.4 [22.1–29]	25.9 [22.8–29.2]	26.9 [23.4–30.6]	0.8	23.8 [19–29.1]	21.2 [18.7–23.9] [†]
	200	4	12.5 [9.2–16.4]	12.8 [9.4–16.6]	11.8 [9.6–14.3]	0.7	12.7 [8.6–17.5]	11.1 [9.0–13.3]
Secondary outcomes								
SCORAD-score (SQM [95% CI])	205	2	18.0 [15.0–22.1]	18.9 [15.5–22.7]	15.6 [13.3–18.1]	0.063	19.5 [14.4–25.3]	14.9 [11.9–18.1]
	198	5	11.4 [8.5–14.7]	12.2 [9.1–15.7]	8.9 [6.5–11.8]	0.96	9.1 [6.6–12.1]	9.8 [7.1–12.9]
	193	6	11.4 [8.1–15.3]	11.9 [9.0–15.2]	9.1 [6.2–12.5]	0.31	12.6 [9.2–16.6]	9.2 [6.6–12.1]
	165	7	8.5 [5.3–12.5]	10.4 [6.8–14.7]	10.8 [7.3–15.0]	0.79	7.7 [3.4–13.7]	6.4 [3.7–9.7]
IDQoL-score (SQM [95% CI])	208	1	4.8 [3.8–6.1]	5.2 [4.3–6.2]	4.2 [3.3–5.2]	0.34	3.8 [2.6–5.3]	3.2 [2.6–4.0]
	200	4	2.9 [2.0–4.0]	3.3 [2.4–4.3]	2.0 [1.6–2.6]	0.24	3.1 [1.8–4.8]	2.8 [2.1–3.6]
	200	5	2.7 [2.0–3.6]	2.7 [1.9–3.6]	1.3 [0.8–1.9]	0.07	1.8 [1.2–2.5]	2.5 [1.8–3.3]
	194	6	2.4 [1.6–3.3]	2.7 [1.8–3.7]	1.3 [0.8–1.9]	0.06	2.3 [1.4–3.5]	2.0 [1.3–2.9]
	167	7	1.3 [0.8–2.1]	1.6 [1.0–2.4]	1.3 [0.9–1.9]	0.85	1.2 [0.6–2.0]	1.5 [0.9–2.4]
Skin-care intensity/potency (<i>n</i>/<i>n</i> samples in group [%])								
Using standard skin care [†]	202	4	15/44 [34]	14/44 [32]	21/47 [45]	0.4	7/21 [33]	16/46 [35]
Potency								
Emollients, no TS	202	4	12/44 [27]	19/44 [43]	18/47 [38]	0.63	6/21 [29]	19/46 [41]
Mild TS	202	4	30/44 [68]	24/44 [55]	29/47 [62]		13/21 [62]	26/46 [57]
≥Moderate/potent TS		4	2/44 [5]	1/44 [2]	0		2/21 [10]	1/46 [2]
Sensitization [‡] (spec IgE >0.35 kU/L)	119	1	16/24 [67%]	18/35 [51%]	17/28 [61%]	0.49	5/9 [56%]	10/23 [67%]
(<i>n</i> / <i>n</i> samples in group [%])	109	4	19/29 [65%]	18/29 [62%]	14/24 [58%]	0.87	5/8 [63%]	11/19 [58%]
Sensitization (≥1 skin test positive)	185	6	18/40 [45%]	18/40 [45%]	16/44 [36%]	0.65	8/18 [44%]	13/43 [30%]
(<i>n</i> / <i>n</i> samples in group [%])	156	7	15/34 [44%]	21/36 [58%]	15/37 [40%]	0.28	6/15 [40%]	7/33 [21%]
Urinary EPX/creatinine	200	1	29.2 [19.1–44.5]	18.1 [11.0–30.0]	15.4 [9.6–24.7]	0.13	4.6 [1.8–11.4]	12.8 [7.8–20.9]
(µg/mmol, GM [95% CI])	181	4	12.7 [7.2–22.4]	13.3 [7.7–23.1]	9.6 [5.2–17.9]	0.45	8.2 [2.9–23.2]	11.4 [7.0–18.7]
	176	5	20.0 [12.7–31.5]	15.1 [9.2–24.7]	12.3 [7.2–21.0]	0.83	18.5 [8.9–38.4]	17.6 [12.1–25.4]
GI-Permeability [†]	93	1	0.17 [0.13–0.22]	0.13 [0.10–0.18]	0.14 [0.10–0.20]	0.45	0.16 [0.09–0.30]	0.14 [0.10–0.19]
(L/M ratio, GM [95% CI])	78	4	0.13 [0.09–0.17]	0.17 [0.12–0.24]	0.18 [0.11–0.28]	0.13	0.20 [0.002–26.0]	0.13 [0.09–0.18]
Symptoms of allergic disease^{**}								
(<i>n</i> / <i>n</i> samples in group [%])	200	5	16/43 [37]	14/43 [33]	16/46 [35]	0.99	7/22 [32]	14/46 [30]
Wheeze ever	193	6	17/42 [40]	10/39 [26]	17/45 [38]	0.71	10/21 [18]	14/56 [30]
	168	7	12/35 [34]	10/36 [28]	18/40 [45]	0.20	9/19 [47]	12/38 [32]
Rhino-conjunctivitis symptoms								
Frequently itchy nose []	200	5	10/43 [23]	6/43 [14]	10/46 [22]	0.5	6/22 [27]	9/46 [20]

(continued)

Table 2 (continued)

	N available for analysis (all groups)	Visit	<i>L. paracasei</i>	<i>B. lactis</i>	Placebo	P-value (randomized groups) [*]	Breastfed observational	Formula-fed observational
'Frequently itchy eyes'	199	5	14/43 [33]	11/43 [27]	3/46 [7]	0.008	7/22 [32]	6/45 [13]
'Frequently red eyes'	199	5	12/43 [28]	10/43 [23]	2/46 [4]	0.009	8/22 [36]	6/45 [13]
Food allergy diagnosis	200	5	7/43 [16]	8/43 [19]	6/46 [13]	0.77	6/22 [27]	2/4

Definition of values shown for each primary/secondary outcome in first column (*n* = number or value unit [% or 95% CI]). *Statistical analysis*: hypotheses as detailed in methods.

^{*}Results of intention-to-treat analysis including ONLY the randomized groups. (NB: as two open observational groups were included, no formal conclusions could be drawn and no analysis was performed.)

ANCOVA corrected for breastfeeding at enrolment, family history of allergic disease, concomitant TS use, pre-intervention values of SCORAD, IDQoL, EPX, GI-permeability, respectively. Pre-intervention scores/levels were the only significant determinants for later values.

[†]Placebo vs. *Bifidobacterium lactis*/*Lactobacillus paracasei* *P* = 0.07; placebo vs. *B. lactis*/*L. paracasei*/Formula obs. *P* = 0.06.

[‡]Presented numbers of infants receiving combination of topical steroids (TS), emollients ($\geq 2 \times$ /day) and bath emollient.

[§]Sensitized (specific IgE >0.35 kU/L to any allergen); sensitization data shown on all infants from whom ≥ 1 blood sample (Visit 1 and/or Visit 4) or ≥ 1 allergy skin-prick test result (Visit 6 and/or Visit 7) was available. Chi-square analysis on paired samples [*n* = 85] and unpaired samples [both NS].

[¶]Samples included if test completed as per test protocol (urine collected ≥ 75 min post-drink, no food during test period, 50 mL test solution given).

^{**}As per respiratory questionnaire; diagnosis = doctor confirmed.

or colic) related to the change in formula and 24/137 (17.5%) stopped the study formula.

Discussion

We have shown that the two probiotic study strains, *B. lactis* CNCM I-3446 and *L. paracasei* CNCM I-2116, do not provide any additional benefit to topical treatment of eczema in young infants. Our findings contradict previous reports on probiotic treatment efficacy in infant eczema. Unlike others, we could not demonstrate a beneficial effect of *B. lactis* as an eczema treatment adjunct [11], and we have established that there is no treatment role for *L. paracasei* in our study conditions. In addition, we can report that neither strain affects the resolution of eczema nor prevents progression of allergic disease. To our knowledge, we are the first to report on longer term follow-up of an early-life probiotic treatment intervention, although longer follow-up on probiotic primary prevention trials has been reported [25].

We recruited very young infants to minimize confounding effects of age, natural history of eczema and feeding practice. No infant had symptomatic IgE-mediated food allergy or gastrointestinal symptoms at enrolment and all had chronic eczema (>6 weeks), which significantly improved, but persisted after a run-in period on standardized skin treatment. Considering key factors which might influence outcomes, randomization was stratified according to eczema severity, atopic family history and partial/no breastfeeding.

Limitations

The study design does not allow formal hypothesis testing to assess the effect of extensively hydrolysed whey formula vs. standard formula. The open design of the observational group may predispose to selection bias and explain apparently lower SCORAD scores in these groups. This did not affect the main study objective – to establish the effectiveness of two probiotic strains compared with placebo in the treatment of infantile eczema.

Stratification according to sensitization would have been desirable as sensitization was common (58%) and recent studies show that probiotic effects may be limited to sensitized patients [26]. This information was not available when the study was designed, but should be considered for future probiotic trials. However, the outcomes remained similar when we included only sensitized infants into a *post hoc* statistical analysis, although the numbers were smaller and statistical power was reduced.

We confirmed the presence of both study strains in stool samples during supplementation using reliable

nucleic acid-based methods documenting prior exposure, subsequent transient persistence of the study strains and compliance with the intervention. The technique detects bacterial DNA and does not allow conclusions regarding strain viability; however, several studies have previously shown that both study probiotics survive gastrointestinal transit [27, 28].

In keeping with its natural history [29], eczema severity decreased significantly with time in all groups, and in parallel, IDQoL improved. We found no significant probiotic effect on clinical and laboratory outcomes at any time-point, nor evidence that the studied probiotics alter the natural history of allergic disease or play a role in secondary prevention of respiratory allergy.

Lactobacillus paracasei was chosen for this trial based on the promising *in vitro* and *in vivo* murine experimental results [7]. It elicited favourable cytokine responses (e.g. inhibition of pro-inflammatory cytokines IL-4, IL-5 and IFN- γ) and induced T-regulatory cell-like population, which produced substantial levels of IL-10 and TGF- β . As all these cytokines have been implicated in eczema-pathogenesis, we hypothesized that such effects could be translated into clinical improvement in eczema severity; this was, however, not observed.

Significant improvements of infant eczema after treatment with *B. lactis* Bb12 (also known as *B. lactis* CNCM I-3446) versus placebo were reported in two small studies; both included a highly selected, exclusively breastfed group of infants over half of whom had concomitant gastrointestinal symptoms suggestive of cow's milk allergy [11, 24]. We could not confirm the beneficial findings using the same strain although the number of infants was higher in our study. Study populations differed, however, as infants in our study had established eczema, no active intestinal symptoms and were consuming at least 200 mL standard formula/day at enrolment.

Several other studies have reported beneficial probiotic treatment effects (various strains, used singly or in combination) on eczema severity in infancy [10, 24, 26, 30–32]. In two of these, a significant effect was only evident in subgroup analyses including sensitized children only [26, 32]; in another, the effect was present in all, but stronger in sensitized children [31]; *post hoc* analysis did not confirm this association in our study. The contradictory findings may be due to different mechanisms of action of selected probiotic strains, the combinations used, or selection of different study populations with possibly different underlying immune responses. Several studies showing no beneficial effect of probiotics on established eczema have been published [33–35]. Notably, *Lactobacillus rhamnosus* GG did not elicit beneficial effects in two studies in contrast to earlier published work [24].

The controversy around probiotic eczema treatment extends to two recent meta-analyses reporting different – opposing – conclusions [14, 15]. A likely reason for the inconsistent findings is the obvious heterogeneity of published trials, which include different strains, combination of strains, variations in age, cultural and genetic backgrounds and allergic co-morbidities, making direct comparison and data-interpretation difficult.

We chose young infants aged 3–6 months, as we hypothesized that the window of opportunity for achieving an immune-modulating effect via the intestinal microbiota was in early life. It is possible that even at this young age, irreversible immunological events had taken place and that our intervention, although early, was in fact too late. The fact that 60% of infants were already allergen-sensitized would underpin this assumption.

If probiotics are considered further for the treatment of eczema, the precise immunological effects in humans and the variation of effects between individuals need to be understood better for optimal strain selection. Recipients may need to be selected based on phenotypic and possibly genotypic characteristics.

In conclusion, we found no benefit from early life supplementation with *B. lactis* or *L. paracasei* in the treatment of eczema, when given as an adjunct to basic topical treatment, and no effect on the progression of allergic disease up to age 3 years.

Acknowledgements/Declaration

Sources of support

This study was sponsored by Nestec Ltd, Lausanne, Switzerland, who supplied the study formula, the study probiotics and the majority of the funding. Measurements of U-EPX and serum specific IgE were generously provided by Pharmacia Diagnostics, Uppsala, Sweden. Analysis of samples for Lactulose/Mannitol ratio was performed in cooperation with the Department of Clinical Biochemistry, Royal Manchester Children's Hospital, Manchester, UK and funded by the North West Lung Research Centre Endowment Fund.

Author contributions

C.G. established the clinical trial, recruited and followed up patients, undertook some of the fecal analysis, contributed to study analysis and wrote the manuscript with contribution from all authors. C.C. performed the HPLC analysis of urine samples as part of intestinal permeability testing. C.P. undertook the U-EPX assays. G.T. and K.M. analysed fecal samples for presence/absence of study strains, performed in the Department of Microbiology, University of Otago, Dunedin, New Zealand. G. K. and K.J. recruited patients and undertook most of

the 3-year follow-up. J.M. contributed to study design and analysis and the manuscript preparation. C.M. and A.C. contributed to study design and the manuscript preparation. A.W. designed and oversaw the running of the study and contributed to the manuscript preparation.

Conflict of interest

This study was sponsored by Nestec Ltd., Lausanne, Switzerland and this funding formed part of the salaries of C.G., G.K. and K.J. during the study (2001–2005).

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Acknowledgements

We are grateful to all the participating families, to Janice Ferguson, Consultant Dermatologist for training the research team in the use of the SCORAD Index, to Elizabeth Hadley for assisting with the intestinal permeability analysis and to Dominik Grathwohl, Biostatistician, Nestlé Research Centre, for his contribution to statistical analysis.

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