

A Randomized, Double-Blind, Placebo-Controlled Trial Assessing the Oral Administration of a Heat-Treated *Lactobacillus paracasei* Supplement in Infants with Atopic Dermatitis Receiving Topical Corticosteroid Therapy

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Keywords

Probiotic · *Lactobacillus paracasei* · Atopic dermatitis · Topical corticoid · TEWL · IDQOL · SCORAD · Objective SCORAD · IgE · CCL17

Abstract

Background/Aims: Atopic dermatitis (AD) is a common disease in infancy, for which topical steroids are the first-line therapy but have side effects. Innovative approaches are needed to reduce the burden of AD and corticosteroid usage in infants. **Methods:** The once-daily consumption of heat-treated probiotic *Lactobacillus paracasei* GM-080 or placebo for 16 weeks as supplementary approach to topical treatment with fluticasone propionate cream was compared in AD infants aged 4–30 months. Outcomes were SCORAD and

its subscores, TEWL, Infants' Dermatitis Quality of Life Index (IDQOL), corticoid "sparing effect," CCL17/TARC, and IgE status. **Results:** SCORAD, objective SCORAD, itching, and IDQOL decreased significantly ($p < 0.001$) over the treatment period in both treatment groups. Slight decreases (ns) were noted in TEWL in lesional and unaffected skin and CCL17 levels. There were no differences between the treatment groups. Total IgE increased over the treatment period in both groups, with significantly higher increase in the heat-treated probiotic group ($p = 0.038$). There was no evidence of a corticoid "sparing effect" by the probiotic. **Conclusions:** In this design, the probiotic *L. paracasei* was not beneficial as a complementary approach to topical corticosteroids in infants with AD. However, slight beneficial effects may have been masked by the moderate potency corticoid.

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Introduction

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases, affecting more than 20% of the population at some time in their life. It is usually the first manifestation of atopic disease in infancy [1]. While the underlying pathogenic mechanisms of AD are still not fully understood, two hallmarks are an immune dysfunction resulting in IgE sensitization to allergens and a defect in the epithelial barrier [1].

Infants with AD may develop red, oozing, crusted rashes on the face, scalp, diaper area, hands, arms, feet or legs. Large areas of the body may be affected. In mild and moderate AD, the rashes may be limited to a few spots and have chronic recurrence. Pruritus is the major subjective symptom and scratching worsens the clinical picture. The pruritus is usually worse at night, disrupting sleep and impacting the quality of life [2, 3]. Health economics studies have shown that AD in infants is time and resource demanding for families [4]. This has highlighted the need for novel strategies to reduce the burden of the disease such as natural food ingredients which could lessen the symptoms and/or increase the quality of life.

In recent years, the use of probiotics has been investigated for beneficial effects in AD [5]. The rationale for this approach is based on the well-recognized effects of specific probiotics on cellular immune responses [6]. As part of the “hygiene hypothesis” it is speculated that the recent rise in allergic diseases may be linked to fewer bacterial encounters in progressively cleaner environments, with a substantial effect on the mucosal immunity conferred in part by the normal intestinal microbial flora [7]. Evidence has emerged that exposure to these microbial agents early in life could play an important role in maturation of type 1 T-helper cell immune responses and could balance the development of allergic type 2 T-helper cell responses and allergic (IgE) antibody production [7, 8].

In this study, the efficacy of the consumption of the heat-inactivated probiotic *Lactobacillus paracasei* (strain GM-080) was compared to a placebo for the adjuvant treatment of AD in infants. It was hypothesized that this would have a beneficial effect on the clinical symptoms of AD and help limit the quantity of corticosteroids needed as concomitant treatment (“sparing effect”). GM-080 was chosen as the candidate strain as it has been shown to decrease other allergic symptoms such as allergic rhinitis with both the live and the heat-treated form of the probiotic [9, 10].

Materials and Methods

Study Design and Subjects

This multicenter, randomized, double-blind, parallel-group, placebo-controlled clinical trial was conducted in five centers in Taiwan from July 2014 to September 2016.

Infants aged 4–30 months at the time of inclusion, with moderate or severe AD and a SCORAD ≥ 20 were eligible. The major exclusion criteria were: SCORAD < 20 ; infants requiring systemic treatment for their skin condition; infants or mothers introducing other probiotics into their diet during the 16-week study or the month before study start; infants having other inflammatory skin conditions; infants having a congenital illness or malformation that may have affected normal growth; infants using topical calcineurin inhibitors; infants undergoing UV light therapy or wet dressing or any topical treatment (lotion, skin moisturizer allowed but monitored) for their eczema other than the one provided during the study or in the month before study start; or infants that had participated in another clinical trial in the month before study start.

There were no specific dietary restrictions, and infants could be included that were exclusively or partially breastfed, exclusively infant formula fed, in the weaning period, or fed regular table food. Acceptable concomitant medications were oral antihistamines, acetaminophen or paracetamol, ibuprofen, nasal sprays indicated for colds, homeopathic cough syrup, and the topical corticoids (fluticasone propionate cream) provided to the parents/caregivers. Records and diaries were kept of diet, concomitant medications, and adverse effects (AEs).

Treatments, Blinding, and Randomization

The GM080 product was heat-treated *L. paracasei* ($1 \times 1,010$ equivalent CFU), powder packed in sachets. The placebo was maltodextrin. The two ingredients were blinded and the GM080 product was similar in color, odor, taste, and texture to the placebo. After enrolment, each subject was randomized and assigned to a treatment group.

Each subject was fed with the assigned study product (GM080 or placebo) once daily (in the morning) for a period of 16 weeks, diluted before administration in water, milk, formula, other beverages, or liquid food.

Randomization was implemented using Trial Balance software with a ratio of 1:1 for the active/placebo groups, stratified by age (4–18 and > 18 –30 months) and baseline SCORAD (20–24 = mild, 25–50 = moderate, > 50 = severe) to ensure equal distribution between the treatment groups.

Procedures

Subjects were treated for a period of 16 weeks. Assessments and measures were made at baseline and after 4, 10, and 16 weeks of treatment.

The severity of atopic eczema was assessed using the SCORAD method [11]. To calculate the index, three subscores are taken into account: the intensity of the symptoms erythema, edema/papulation, excoriations, lichenification, and dryness are scored as separate categories by the investigator using a 4-point scale (0 = none to 3 = severe) and summed (subscore intensity); the subjective manifestations itching and sleep loss are estimated over the last 3 days by parent/caregivers using a Visual Analogue Scale (VAS) (subscore subjective symptoms); and the rule of nines (estimation

of the total body surface affected) is used to estimate extent (subscore extent). The overall SCORAD is then calculated according to the formula: $\text{extent}/5 + 7 \times \text{intensity total}/2 + \text{subjective score}$. The “objective” SCORAD is calculated separately as the $\text{extent}/5 + 7 \times \text{intensity}/2 + 10$ bonus points if severe disfiguring eczema is on the face or hands.

TEWL was measured using the VapoMeter from Delfin Technologies. The VapoMeter is equipped with a closed cylindrical chamber that contains sensors for relative humidity and temperature. The relative humidity (RH%) increased in the chamber is measured to calculate TEWL value. CCL17/TARC was measured using a duo set kit from R&D Biosystems.

The parent/caregiver was asked to complete the Infants’ Dermatitis Quality of Life Index (IDQOL) [4]. The IDQOL includes dermatitis severity (0 = none to 4 = extremely severe) and 10 questions related to the life quality index.

The quantity of topical corticoids used was documented by recording weights of dispensed and returned tubes. The parents/caregivers were instructed how to use fingertip units to manage corticoid application and the number of units used was to be recorded in a diary. They were told to use the topical corticoid only if needed, preferably no more than once per day.

Blood (up to 6 mL) was collected at baseline and at the end of treatment (week 16) for measurement of total and specific IgE to egg, house dust mites, milk, molds, and peanuts, as well as for hematology and clinical chemistry values. Hematological and biochemical parameters included hematocrit, hemoglobin, RBC count, platelet count, erythrocyte mean corpuscular volume and corpuscular hemoglobin, WBC count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), aspartate aminotransferase, alanine aminotransferase, albumin, total protein, total bilirubin, alkaline phosphatase, lactate dehydrogenase, serum creatinine, blood urea nitrogen, glucose, C-reactive protein, calcium, phosphorus, sodium, potassium, chloride, and magnesium.

Statistical Methods

The primary outcome was overall SCORAD. Secondary outcomes included “objective” SCORAD as well as the subscore extent, intensity, subjective symptoms, TEWL in the affected areas, IDQOL, corticoid “sparing effect,” IgE status, and CCL17/TARC.

The null hypothesis “the effect of GM080 is equal to the effect of placebo” versus the alternative hypothesis “the effect of GM080 is unequal to the effect of placebo” was tested for the outcome measures using a linear mixed-effect model for repeated measures or a generalized estimating equation approach for repeated measurements (details can be found in online supplementary information “statistical methods” (for all online suppl. material, see www.karger.com/doi/10.1159/499436).

In addition, the amount of corticoid used every 4 weeks and the change in use from the reference period (baseline to 4 weeks) were analyzed descriptively. Comparison of treatment groups as changes from reference period was performed using a Wilcoxon rank-sum test.

The change from baseline of level of total IgE and changes in eosinophils (%) and basophils (%) were compared between treatment groups using a Wilcoxon rank-sum test. Differences in TEWL in unaffected skin versus lesional areas were compared using a paired *t* test.

All statistical tests were conducted two-sided with a significance level of 5%. The strength of association between outcome measures was determined using Pearson’s *r* correlations.

Sample Size

Background information was obtained from Weston et al. [12] to determine a clinically meaningful effect of size and variation. In their trial, the authors assessed the effect of consumption of *Lactobacillus fermentum* in a similar population. Using these data and the formulas in Hozo et al. [13], the estimated mean change and SD for SCORAD were 5.73 and 12.21, respectively. Assuming a moderate correlation of 0.6 between the repeated SCORAD measurements, 80% power and a two-sided 5% level of significance, inclusion of 50 subjects per treatment arm was deemed necessary. With an expected dropout rate of 20%, the target of a minimum number of subjects to be randomized per group was 63. Therefore, a minimum total number of 126 subjects was planned to be enrolled and randomized. Since the dropout rate was lower than 20% in this study, more than the minimum number of subjects were available for analysis in both groups.

Results

Demographic and Baseline Data

Altogether, 126 subjects were enrolled, with 64 assigned to the GM080 and 62 to the placebo group. Two subjects in the GM080 group and 1 subject in the placebo group were excluded from the full analysis set (FAS) because no postbaseline measurements were made. The mean age ($\pm$ SD) was 13.48 ($\pm$ 7.72) and 14.62 ($\pm$ 7.88) months in the GM080 and placebo groups, respectively. There were more male babies in both groups (44 males and 18 females in the GM080 group, 37 males and 24 females in the placebo group). Other characteristics such as gestational age, anthropometrics, number of siblings, age of mother at birth, and smoke exposure during and after pregnancy were well-balanced between the groups (data not shown).

Seventeen (26.6%) and 18 (29.0%) of the subjects in the GM080 and placebo groups, respectively, reported prior treatment with glucocorticoids.

Forty-seven and 55 subjects in the GM080 and placebo groups, respectively, were eligible for the Per Protocol Set (PPS) analysis. There was 1 subject in each group who discontinued prematurely due to worsening of the AD, 1 in the GM080 and 2 in the placebo group who discontinued due to noncompliance, and 1 subject in the GM080 group who withdrew due to a lack of efficacy that was reflected in the SCORAD score. Three additional subjects withdrew from the GM080 group without explanation. Other exclusions from the FAS were mainly in the GM080 arm and were due to the use of prohibited medication and

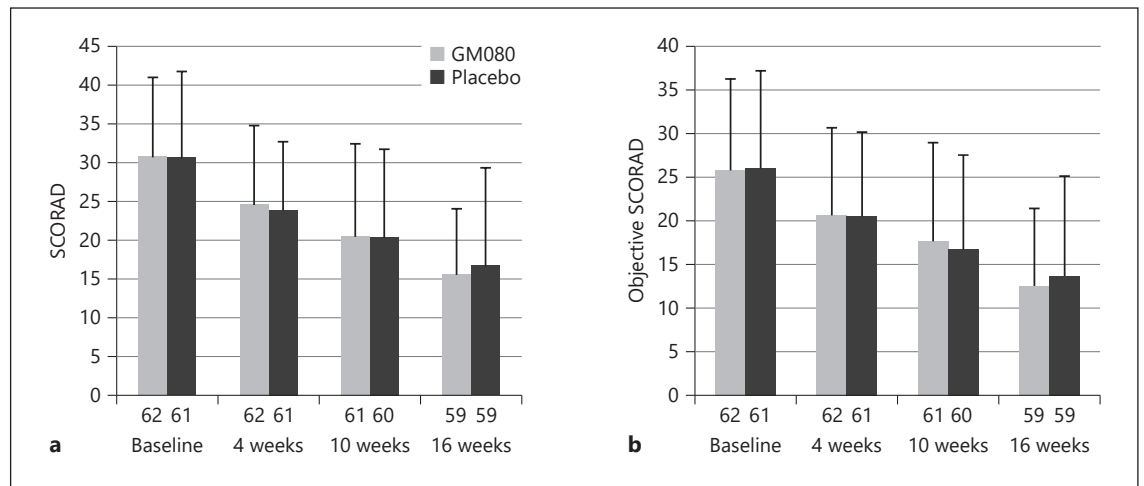


Fig. 1. Effect of adjuvant treatment with *L. paracasei* GM-080 or placebo over 16 weeks on SCORAD (a) and objective SCORAD (b) in infants receiving topical fluticasone propionate as needed for therapy of atopic dermatitis. Values are mean $\pm$ SD. Sample size (*n*) is listed below the bar for each group.

deviations in the visit schedule. For the FAS analysis, the impact of prohibited medication was considered negligible based on results of a sensitivity analysis.

Overall, results using FAS were confirmed by PPS, underscoring that imbalances between the arms did not affect interpretation of the results. Unless noted otherwise, results reported here are from the FAS analysis. Compliance was generally good in both groups with no differences in the two treatment arms.

Clinical Scores

SCORAD and objective SCORAD values were well-balanced at baseline between the treatment arms and decreased significantly ($p < 0.001$) over the treatment period in a similar manner in both GM080 and placebo groups (Fig. 1). The main improvement in SCORAD was presumed to be related to the corticosteroid treatment, and no additional treatment effect was observed between the GM080 and placebo groups. Using investigator site as a covariate, there was a significant difference in the baseline SCORAD scores between the sites ($p < 0.001$), meaning that the mean severity of the subjects' AD at inclusion was different depending on site. However, no influence of the investigator site on the treatment effect was found.

The SCORAD subscore extent, intensity, and subjective symptoms all decreased significantly ($p < 0.001$) across the study, with no differences between the treatment groups (data not shown). The contribution of the individual symptoms erythema, edema/papulation, oozing/crust, excoriations, and dryness intensity to the

SCORAD intensity score can be seen in Figure 2. Dryness and erythema were the most commonly documented symptoms at baseline. An improvement in the individual symptoms over the study duration was noted for all of the symptoms except lichenification. For the latter, there were too few scores < 0 to allow interpretation. Higher percentages of subjects reported lower scores for itching over the treatment period in both treatment groups (Table 1). At baseline, only 6.5% of subjects in the GM080 arm and 3.3% in the placebo arm reported an absence of itching. At the end of 16-week treatment period, the corresponding percentages of subjects reporting no itching in the GM080 and placebo groups were 23.7 and 27.1%, respectively. No significant differences between the treatment groups were noted for any of the symptoms. When patients were stratified by severity of AD based on SCORAD, no difference was observed between the placebo and the supplemented group.

The correlation between overall SCORAD and the subscore intensity was higher ($r = 0.86$) than for the subscores subjective symptoms ($r = 0.64$) or extent ($r = 0.39$).

TEWL

TEWL was higher in lesional skin compared to unaffected skin at all body regions and time points (Fig. 3) ($p < 0.001$ for combined data of treatment groups).

A slight overall reduction in TEWL was observed during the study period in both lesional and unaffected skin. With the exception of the lesional skin on the arms ($p = 0.043$), the TEWL decrease over the study course was not

Fig. 2. Effect of treatment with *L. paracasei* GM-080 or placebo over 16 weeks on percent change in clinical scoring of individual symptoms in infants receiving topical fluticasone propionate as needed for therapy of atopic dermatitis. Scoring was done for each symptom using the scale 0 = absence, 1 = mild, 2 = moderate, 3 = severe. Sample sizes: baseline, GM080 *n* = 62, placebo *n* = 61; 4 weeks, GM080 *n* = 62, placebo *n* = 61; 10 weeks, GM080 *n* = 61, placebo *n* = 60; 16 weeks, GM080 *n* = 59, placebo *n* = 59.

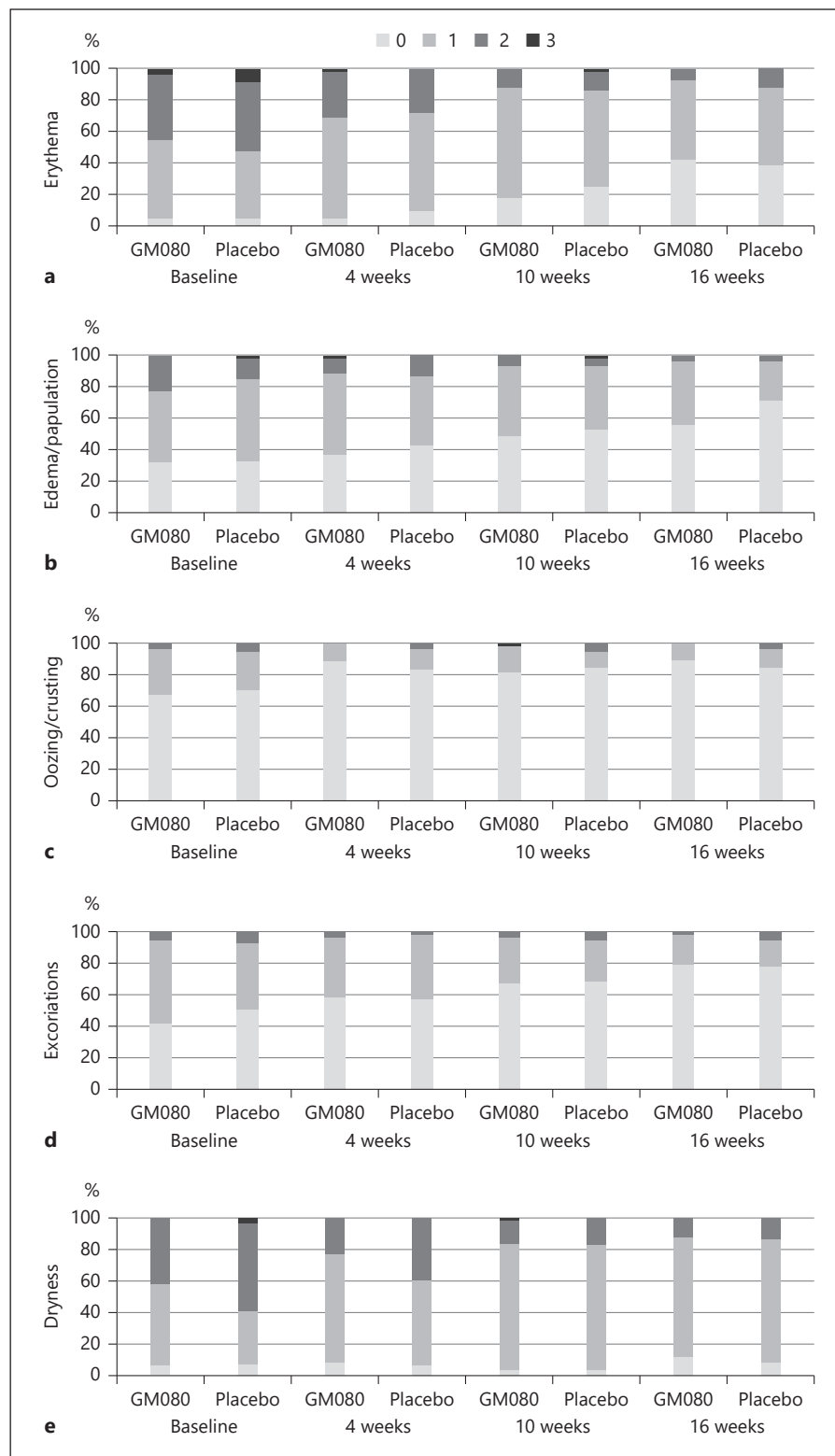


Table 1. VAS score for pruritus listed as percent of subjects at each visit

VAS score	Baseline		4 weeks		10 weeks		16 weeks	
	GM080	placebo	GM080	placebo	GM080	placebo	GM080	placebo
0	6.5	3.3	9.7	11.5	16.4	15.0	23.7	27.1
1	6.5	11.5	6.5	14.8	9.8	15.0	20.3	20.3
2	6.5	13.1	16.1	9.8	27.9	31.7	18.6	18.6
3	22.6	16.4	22.6	23.0	18.0	11.7	10.2	5.1
4	9.7	13.1	8.1	13.1	8.2	6.7	11.9	6.8
5	9.7	14.8	12.9	14.8	4.9	5.0	8.5	8.5
6	9.7	3.3	11.3	1.6	3.3	10.0	3.4	5.1
7	4.8	3.3	4.8	8.2	6.6	0.0	0.0	1.7
8	14.5	18.0	6.5	3.3	4.9	1.7	3.4	3.4
9	4.8	1.6	0.0	0.0	0.0	0.0	0.0	1.7
10	4.8	1.6	1.6	0.0	0.0	3.3	0.0	1.7

significant in any location or treatment group. Even though there were no significant differences between the treatment groups in the reduction of TEWL during the study, trends in favor of GM080 were seen for the legs ($p = 0.087$) and trunk ($p = 0.054$) in the lesional skin (Fig. 3b, c) and the legs ($p = 0.069$) in the unaffected skin (Fig. 3f).

There was only a weak to moderate positive correlation between overall SCORAD and TEWL values in the separate body areas ($r = 0.32$ – 0.41). Moderate positive correlations were noted between the TEWL values in the different body areas ($r = 0.53$ – 0.69).

IDQOL

The IDQOL decreased across the study ($p < 0.001$) in both treatment groups, reflecting an improvement in quality of life. Over the study period, the mean of the index decreased from $8.59 (\pm 3.53)$ to $4.95 (\pm 3.23)$ in the GM080 group and from $7.18 (\pm 4.17)$ to $4.58 (\pm 3.81)$ in the placebo group. No difference was found between the treatment groups.

In the opinion of parents/caregivers, dermatitis severity decreased across visits in both treatment arms, with no difference in the treatment effect between groups. With few exceptions, the subscores of the index related to subjective symptoms, mood, sleeping patterns, activities, and treatment-associated problems reflected an improvement over the treatment period irrespective of treatment group.

The IDQOL did not show a good correlation with any of the other outcomes. The best correlation was with the SCORAD subscore subjective symptoms ($r = 0.43$). The correlation with the overall SCORAD was very weak ($r = 0.16$). Interestingly, a negative correlation was noted for the subscore extent ($r = -0.40$).

Sparing Effect of Glucocorticosteroids

There was no evidence of a corticoid “sparing effect” under treatment with GM080. The amount of topical fluticasone propionate cream used per month did not change across visits and was not different between the treatment groups (Table 2).

Association of IgE Atopic Status, CCL17/TARC, and SCORAD

At baseline, 66.1% of the subjects receiving GM080 and 61.4% of the subjects receiving placebo were classified as having IgE-associated AD based on threshold values of 100 kU/L for total IgE and/or a threshold of 0.35 kU/L for specific IgE. Corresponding values for threshold values of 100 kU/L for total IgE and/or a threshold of 0.70 kU/L for specific IgE were 57.6 and 54.4%, respectively. Classification according to antigen-specific IgEs can be found in supplementary information (online suppl. Table 1). Considering all infants in both groups, a large number showed specific IgE on egg (approximately 43%) and milk (approximately 40%), followed by peanuts (20%), dust

Fig. 3. Effect of treatment with *L. paracasei* GM-080 or placebo over 16 weeks on change in TEWL values in lesional and unaffected skin in different body regions of infants receiving topical

fluticasone propionate as needed for therapy of atopic dermatitis. Values are mean $\pm$ SD. Sample size (n) is listed below the bar for each group.

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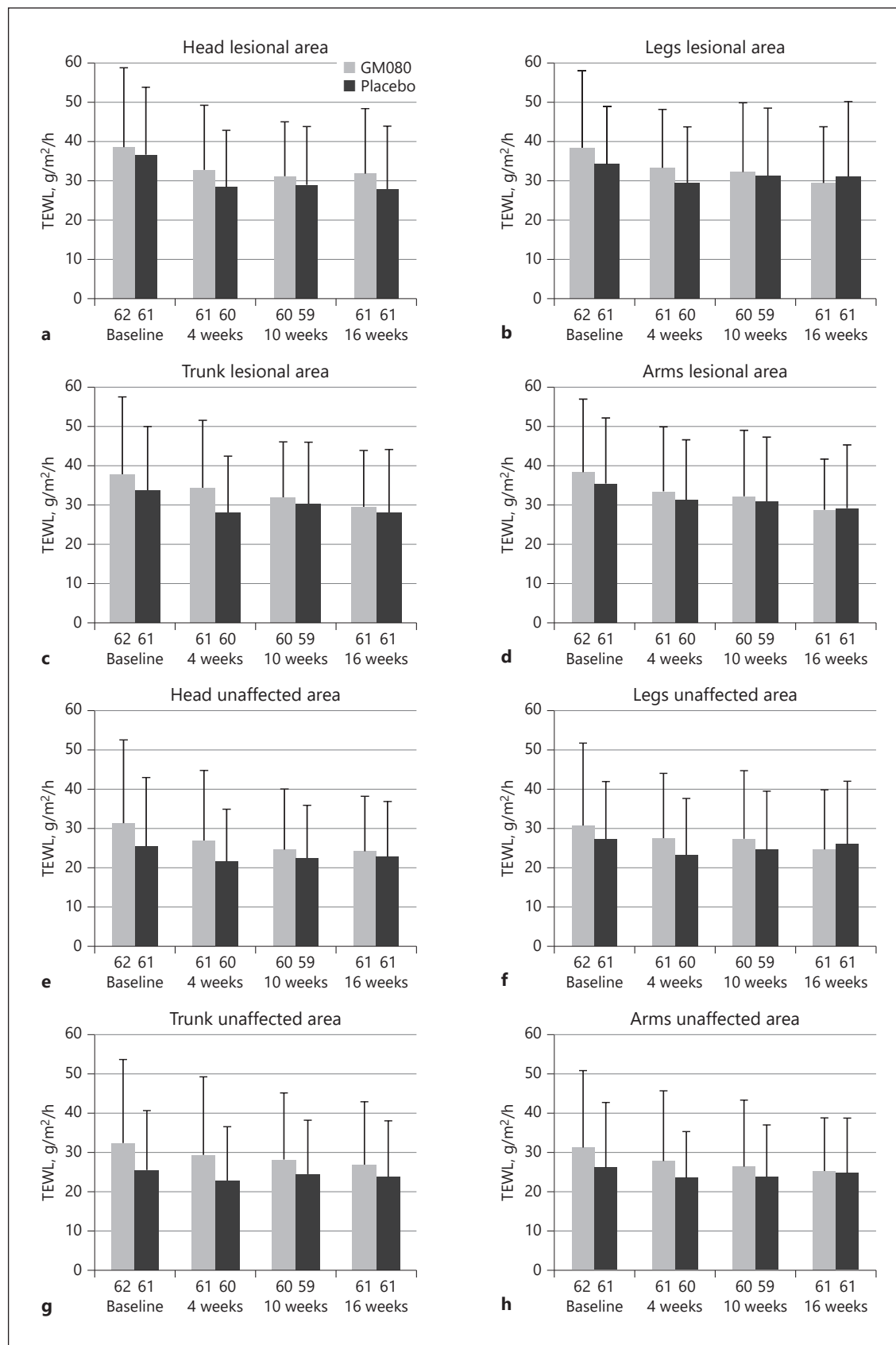


Table 2. Amount of topical fluticasone propionate cream used per month (g/month) and change between visits

Period and statistic	GM080	Placebo	GM080 change to previous period	Placebo change to previous period
Between baseline and 4 weeks				
Mean $\pm$ SD	1.78 $\pm$ 2.04	1.60 $\pm$ 1.73		
Geometric mean	0.25	0.26		
95% CI of geom. mean	0.11; 0.58	0.11; 0.58		
Between 4 and 10 weeks				
Mean $\pm$ SD	1.61 $\pm$ 1.78	1.37 $\pm$ 1.69	-0.14 $\pm$ 1.43	-0.23 $\pm$ 1.26
Geometric mean	0.29	0.23	1.21	0.90
95% CI of geom. mean	0.13; 0.65	0.10; 0.51	0.54; 2.67	0.50; 1.61
Between 10 and 16 weeks				
Mean $\pm$ SD	1.43 $\pm$ 1.60	1.62 $\pm$ 2.27	-0.29 $\pm$ 1.45	-0.01 $\pm$ 1.89
Geometric mean	0.28	0.27	1.22	0.96
95% CI of geom. mean	0.13; 0.61	0.12; 0.59	0.56; 2.64	0.44; 2.11

mites (12%), and molds (1.8%). Inclusion of IgE as a co-variate in the mixed-effect model on SCORAD did not change the primary result. The response to treatment on overall SCORAD was the same within each atopic status.

At the end of the 16-week treatment, total IgE levels (kU/L) (mean $\pm$ SD) increased in the GM080 treatment group from 151.08 $\pm$ 260.11 to 212.96 $\pm$ 365.88 and in the placebo group from 139.63 $\pm$ 243.86 to 160.57 $\pm$ 241.64. The change from baseline in total IgE level was significantly higher in the GM080 treatment group ($p = 0.038$).

The total IgE showed a weak to modest positive correlation with overall SCORAD ($r = 0.36$), objective SCORAD ($r = 0.31$), the subscore intensity ($r = 0.32$), and subjective symptoms ($r = 0.28$). There was no correlation between total IgE and the subscore extent ($r = 0.07$). Further, there was no meaningful correlation between total IgE and TEWL measured in the affected locations ($r = 0.18$ – 0.28).

Finally, the CCL17/TARC level (pg/mL), known to be associated with AD severity [14], decreased from baseline to week 16 in the GM080 treatment group from 186.58 $\pm$ 364.84 (mean $\pm$ SD) to 133.94 $\pm$ 270.42 and in the placebo group from 220.64 $\pm$ 385.42 to 128.37 $\pm$ 238.53. Taken together, this was a significant decrease for the overall population ($p = 0.017$), but no significant difference was observed between the two treatment groups ($p = 0.990$). It was not possible to show any significant association between CCL17/TARC and SCORAD.

Laboratory and Safety Data

There were no relevant differences in eosinophils or basophils from baseline values or between the treatment

groups. In both groups scattered abnormal, clinically significant laboratory values were reported at baseline and 16-week visit (data not shown).

The number of AEs was similar between the treatment groups. 71.9% of subjects in the GM080 group and 67.7% in the placebo group had at least one AE during the study, excluding diaper rash and AD. Only one of these AEs in the placebo group was classified as related (probable) to the treatment. 12.5% of subjects in the GM080 group and 17.7% in the placebo group experienced AEs related to diaper rash or AD. These led to discontinuation of 1 subject per group.

Discussion/Conclusion

Since Isolauri et al. [15] first demonstrated a positive effect of treatment with *Bifidobacterium lactis* Bb-12- and *Lactobacillus strain* GG-supplemented formulas on the SCORAD score of infants suffering from AD, there have been numerous studies investigating the potential beneficial effects of probiotics on the prevention and treatment of AD. Inconsistent results have been reported in reviews [5, 16, 17]. Whether probiotics have a beneficial effect on AD may be dependent on factors such as specific probiotic strains, timing of administration (prenatal/postnatal), duration of exposure, and dosage [5].

In the present study, it was not possible to demonstrate an additional effect of treatment with the heat-treated *L. paracasei* GM-080 on the SCORAD index or IDQOL beyond the improvements seen under topical corticosteroid therapy in children with moderate to severe AD. Nor was

there a sparing effect on corticoid use. These results are in contrast to the results of two recent studies. Wang and Wang [10] found clinical improvement in children 1–18 years old treated with *L. paracasei* GMNL-133 and *L. fermentum* GM-090 for 3 months. These authors noted a significant improvement in SCORAD scores and quality of life indices following administration of the probiotics separately and in combination compared to placebo. In a setting with concomitant corticosteroid therapy similar to the present study, Navarro et al. [18] presented strong evidence supportive of a reduction of the objective SCORAD and sparing of corticosteroid use following 12-week treatment of children aged 4–17 years suffering from moderate AD with a probiotic mixture of strains of *Bifidobacterium lactis* CECT 8145, *Bifidobacterium longum* CECT 7347, and *Lactobacillus casei* CECT9104. It is possible that the different results in similar study designs are primarily attributable to the choice of probiotic strains. Additionally, it is possible that the live probiotic strain may have worked on AD compared to the heat-treated GM080. However, other factors may also have contributed to the different outcomes. Masking of milder probiotic effects by the relatively potent corticosteroid in this study has to be considered. Interestingly, while symptoms decreased over time in both groups, possibly highlighting the natural improvement of the disease symptoms, the usage of topical fluticasone did not change over time, suggesting sustained need for topical treatment in this population. Other recent studies have shown the usefulness of different scoring systems such as the Patient-Oriented SCORAD (PO-SCORAD) to measure response to treatment [19]; such readout should be considered as an outcome in future trials.

Ideally, an improvement in skin barrier function should accompany lessening of the symptoms of AD. In the present study, TEWL was measured as an indicator of skin barrier status. As expected, the highest values were measured in the lesional skin at all visits. The TEWL values in the unaffected skin at baseline were generally higher than those previously reported in Chinese infants [20] as well as other countries and ethnicity [21, 22]. This was particularly observed in the patients assigned to the GM080 group, supporting that the barrier may be compromised even in skin appearing clinically normal in AD patients [23]. The slight decrease in TEWL from the baseline value in all four of the affected anatomical locations pointed in the direction of an improvement in barrier function. It is known that TEWL values vary among anatomical regions [24], so that differences between arms, legs, trunk and head were to be

expected. The observation of a trend towards lower values at the last visit in several body regions in the probiotic-treated group may hint at a slight beneficial effect of the probiotic. Again, the potential masking of any positive effects on skin barrier by the concomitant use of glucocorticoids must be considered. There is evidence that topical corticosteroids may further damage the skin barrier alongside of the beneficial suppression of inflammation in AD patients, and increases in TEWL in skin of patients have been observed under treatment with topical corticosteroids [25, 26]. Additionally, there is accumulating evidence that daily use of emollients may improve both TEWL and stratum corneum hydration measurements [19, 27], suggesting that topical application in our trial may have influenced the TEWL outcome in both groups. Indeed, the measurement of skin hydration in addition to TEWL and their correlation to AD severity scores [27] would have added valuable additional information.

In AD, the disrupted skin barrier is considered to be a driver for the inflammation and is thought to act as a gateway for exposure to environmental substances, promoting subsequent sensitization to specific antigens [1, 28]. Atopic sensitization leads to inflammatory reactions mediated by IgE after exposure to allergens. Around 75% of children with AD are sensitized to one or more allergens, and the predisposed child often develops AD in infancy followed by sensitization to cow's milk and eggs [1]. In the present study, the percentages of infants with total IgE-associated AD and specific IgE responses to eggs and milk follow this pattern.

In a meta-analysis of clinical trials on allergic diseases in children, probiotics were shown to be effective in reducing total serum IgE in 9 studies with a total of 1,103 atopic children [7]. In contrast, in this study treatment with heat-treated *L. paracasei* GM-080 led to a significant increase in serum IgE over the baseline value. An increase in IgE was also noted in the placebo group, but the difference was not significant. The reason for this increase is not immediately obvious but may be related to prolonged concomitant therapy with a relatively potent topical corticosteroid. One of the paradoxical effects which have been observed for corticosteroids is an increase in IL-4-stimulated production of IgE by B lymphocytes [29]. In fact, a rise in polyclonal serum IgE was shown in asthma patients following oral administration of prednisone; however, the increase did not have a negative clinical impact and was considered to reflect the broader immunomodulatory effects of corticosteroids on T lymphocytes [30].

In conclusion, there were no beneficial effects of treatment with the supplement containing heat-treated *L. paracasei* GM-080 strain for the adjuvant treatment of children with moderate to severe AD in the present study design. Additional studies are needed to clarify whether the failure to identify an effect is a true lack of potential benefit in AD or rather a milder effect which was hidden by the stronger corticosteroid effect.

Further, these results underscore the pitfalls of using markers chosen for their assumed correlations with the severity of AD. Whereas a strong correlation was seen between the overall and objective SCORAD, much weaker or negligible correlations were seen for the separate scoring of subjective symptoms, extent, IDQOL, TEWL, or CCL17/TARC. This observation is particularly important when weighting the relevance of investigator-reported clinical or laboratory outcomes versus patient-reported subjective outcomes in clinical trials and the impact on patient satisfaction and compliance with treatment regimens.

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Statement of Ethics

Written informed consent was obtained from the parents/legal guardian of each potential subject prior to any study-related activities.

The study was approved by the corresponding Institutional Ethics Committees, (IRB102-4052A3,103-1946C, 103-3082-C, CS13188, CTH-102-2-4-033 and 2014-01-01(II)).

The study was conducted according to the principles and rules laid down in the Declaration of Helsinki and was registered before the first patient enrolment on ClinicalTrials.gov Identifier: NCT02103972.

Disclosure Statement

Julie Chambard, Sophie Nutton, Jérôme Tanguy, and Carine Blanchard are employed by Nestec Ltd. The rest of the authors have no conflicts of interest in the context of this work.

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Author Contributions

Dah-Chin Yan, Chih-Hsing Hung, Leticia B. Sy, Ko-Huang Lue, I-Hsin Shih, Chin-Yi Yang, Li-Chen Chen, Hai-Lun Sun, Min-Sheng Lee, Julie Chambard, Sophie Nutton, and Carine Blanchard have participated in the design of the clinical trial, conduct of the trial, and acquisition of the data. Jérôme Tanguy, Carine Blanchard, Betsy Hughes-Formella, and Sophie Nutton have participated in the data analysis and drafting of the manuscript. All the authors have critically reviewed and approved the final version of the manuscript.

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