PROBIOTICS TO YOUNG CHILDREN WITH ATOPIC DERMATITIS: A RANDOMIZED PLACEBO-CONTROLLED TRIAL

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ABSTRACT: Impairment of the intestinal mucosal barrier is involved in the pathogenesis of atopic dermatitis (AD), and studies suggest that probiotics stabilize the intestinal barrier function and decrease gastrointestinal symptoms in children with AD. The purpose of this study was to evaluate the clinical and immunological changes of AD after consumption of the probiotic strains Lactobacillus acidophilus NCFM and Bifidobacterium animalis subsp. lactis Bi-07. Double-blind, randomized placebo-controlled intervention study. Fifty children (mean age 18 months) with AD received NCFM (10⁹ CFU/day), Bi-07 (10⁹ CFU/day) or placebo for 8 weeks. The immunological activity and clinical effect was evaluated by IgE, ECP, IL-10, IFN-γ, IL-31, faecal calprotectin and SCORAD index. There were no overall beneficial effects of the probiotic strains on the degree of AD measured by SCORAD index. However, a post hoc analysis showed a significant reduction in severity of AD in the Bi-07 group and together with the decreasing levels of IFN-γ and IL-10 possible beneficial effects of this probiotic strain could be of interest. There was no effect on inflammatory markers or faecal calprotectin. The significant correlation between ECP and SCORAD index suggest the use of ECP as a measure of the degree of AD in children.

KEYWORDS: Atopic Dermatitis, Children, IgE, Immune Status, Probiotics

INTRODUCTION

The prevalence of atopic dermatitis (AD) is rising, and is now affecting more than twenty percent of all children in the western societies (Horii et al., 2009; Larsen, 1996; Larsen et al., 1996), being the most common skin disease in children (Gruber et al., 2007). The physical signs of the disease like itching eczema and pruritus can cause emotional stress and skin damage and thereby reduce the quality of life for the child, but also for the family (Levy, 2007). Local and anti-inflammatory treatment with corticosteroids is the current recommended treatment for infants with AD. Parents often fear side effects of the treatment and this may lead to non-compliance (Gustafsson et al., 2000). Therefore innovative treatment strategies with probiotics are of interest.

Individuals with AD show elevated mucosal immunological responses and gastrointestinal inflammation which varies over time and impairment of the intestinal mucosal barrier appears to be strongly involved in the pathogenesis of AD. Studies suggest that probiotic supplementation stabilize the intestinal barrier function and decrease the gastrointestinal symptoms in children with AD (Rosenfeldt et al., 2003; Rosenfeldt et al., 2004). This means that the tendency to increased permeability of the intestinal mucosa which is observed in children with AD (Rokaite and Labanauskas, 2005), and possible leading to intestinal inflammation (Baumgart and Dignass, 2002), may be reduced by probiotic supplementation.

The atopic march, referring to the increase in allergic diseases observed in high income societies, suggests that the change in lifestyle is a possible environmental cause that leads to the increasing number in incidence of AD (Kalliomaki et al., 2001). According to the ‘hygiene hypothesis’ the Western lifestyle has decreased the incidence of infections in early life and thereby lead to an undesirable effect on later development of allergies (Schaub et al., 2006). Influence of nutrition, use of antibiotics and maturation of the gut microbiota is included in the newest variety of the hygiene hypothesis (Noverr and Huffnagle, 2004; Schaub et al., 2006).

Atopic dermatitis in infancy is believed to be associated with IgE-mediated food allergy, and studies have found a more pronounced beneficial effect of probiotics in the management of AD among children with a positive skin prick test and increased IgE levels (Rosenfeldt et al., 2003).
The aim of this study was to investigate the effect of two probiotic strains, *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* (B. *lactis*) Bi-07, on the severity of AD in young children. The investigation was focusing on SCORing Atopic Dermatitis (SCORAD) index, faecal calprotectin, total and specific IgE, Eosinophil Cationic Protein and selected immune regulatory cytokines (IL-10, IFN-γ and IL-31).

**METHODS**

**Study design**

The study was a double-blinded, randomized, placebo controlled intervention study, and included a total of fifty children diagnosed with atopic dermatitis. Children were between the age of 7 and 24 months. The study was approved by The Scientific Ethical Committees of Copenhagen and Frederiksberg (KF 01 271345) and registered at ClinicalTrials.gov (NCT 1007331). All children were randomized to a daily dose of either *Lactobacillus acidophilus* NCFM (ATCC 700396) (10^10 CFU), *Bifidobacterium animalis* subsp. *lactis* Bi-07 (ATCC SD5220) (10^10 CFU) or placebo for 8 weeks.

Recruitment was done via the Danish civil registration number registry, by advertising in local newspapers and via local health visitors. The recruitment period lasted from June 2007 to June 2008.

**Clinical evaluation**

The severity of AD was evaluated using the standardized SCORing Atopic Dermatitis (SCORAD) index (Stadler, 1993).

**Anthropometry**

The anthropometric measures included weight which was determined using the Tanita BWB-600 Digital Medical Scale (Arlington Heights, IL, USA), triceps and subscapularis skin folds were measured by the Harpenden skinfold calibre (Chasmors Ltd, UK), head circumference (Harlow Printing Ltd, UK) and Recumbent length was measured using a digital measuring board with movable head- and footboard (FORCE Technology, Brøndby, Denmark).

**Blood collection**

Venous blood samples were drawn before and after intervention. All samples were drawn after a minimum of 9 hours of fasting.

**Serum immunoglobulin E**

Total IgE, specific IgE for egg and milk were analysed. Serum IgE was analysed using the ImmunoCAP™ (Pharmacia Diagnostics, Uppsala, Sweden). The used cut-off limit for total IgE levels of children less than a year old was 30 kU/l, whereas 60 kU/l were used for older children. The cut-off limit of 0.35 kU/l for increased levels of IgE specific for hen’s egg and cow’s milk was chosen (ETAC Study Group, 1997).

**Eosinophil Cationic Protein**

Serum ECP was analysed by the immunoCAP™100E fluoroenzymeimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden). All analysis was run in duplicate.

**Cytokine production**

Concentrations of IL-10 and IL-31 in the supernatants were determined by enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D Systems (Abingdon, UK) and IFN-γ by ELISA kits from Biosource (Nivelles, Belgium).

**Probiotic cultures**

Capsules with the probiotic strains *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* (B. *lactis*) Bi-07 and placebo were provided by Danisco Inc (USA), and were undergoing continuous quality control of cell counts for the duration of the trial. There was no reduction in viability observed in the capsules for the duration of the trial. Capsules were stored at 5°C and filler consisted of cellulose, silicon dioxide and rice-maltodextrin. The placebo consisted of filler material only. Probiotic cultures were ingested in the dosage of 10^10 CFU/day during a period of 8 weeks.

**Stool**

A stool sample were collected at baseline and after intervention, kept in iceboxes and brought to the laboratory within 24 hours where they were stored at -80 °C until analysis.

**Faecal calprotectin**

Faecal calprotectin were analyzed by an enzyme-linked immunosorbent assay (ELISA), and preparation of the assay was done as described by the manufacturer (PhiCal™Test, Oslo, Norway).

**Detection of *L. acidophilus* and *B. lactis* in stool**

Total bacterial DNA was extracted from faecal samples using the QIAamp DNA Stool Mini kit (QIAGEN, GmbH, Germany) according to the manufacturer’s protocol. *L. acidophilus* and *B. lactis* in fecal DNA were assessed by real-time quantitative PCR (qPCR) with the use of species-specific primers. Primers for quantification of *L. acidophilus*, forward primer CTGCTGTCTTCTCCAGCATCT and reverse primer TACGTATTGATACCACGTAAT, were constructed using cpn60 genes from the Chaperonin Database (http://cpndb.cbr.nrc.ca/). Specific primers for *B. lactis*, forward primer TACC GGATGTG CTCCGT and reverse primer GCCCTGGTGG GGCCCATC, targeted 16S rRNA gene (Ribosomal Database Project, Release 10; http://rdp.cme.msu.edu). Quantification of bacteria was carried out using the 7500 Fast Real-Time PCR System (Applied Biosystems, USA). The reaction mixture (20 µl) was composed of 0.5 µM of each primer, 10 µl Power SYBR Green PCR Master Mix (Applied Biosystems, Calif., USA), and 4 µl template DNA isolated from faecal samples. The amplification program consisted of one cycle of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. Standard curves were constructed from 10-fold serial dilutions of chromosomal DNA, isolated from *L. acidophilus* NCFM and *B. lactis* (Ribosomal Database Project, Release 10; http://rdp.cme.msu.edu). Quantification of bacteria was carried out using the 7500 Fast Real-Time PCR System (Applied Biosystems, USA). The reaction mixture (20 µl) was composed of 0.5 µM of each primer, 10 µl Power SYBR Green PCR Master Mix (Applied Biosystems, Calif., USA), and 4 µl template DNA isolated from faecal samples. The amplification program consisted of one cycle of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. Standard curves were constructed from 10-fold serial dilutions of chromosomal DNA, isolated from *L. acidophilus* NCFM and *B. lactis* (Ribosomal Database Project, Release 10; http://rdp.cme.msu.edu).
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**Enterococcus faecalis** Bi-07, with the use of GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, Germany). Cell numbers of bacteria in faecal samples were expressed as Log$_{10}$ colony-forming units (Log$_{10}$ CFU) per gram stool sample.

**Statistical analysis**

The effect of the probiotic intervention were analysed for several outcomes. The primary outcome was the SCORAD index, and thus the effect of the intervention on the severity of disease, was analysed using a mixed linear model including a random effect for each child. The effect of intervention on SCORAD index was calculated with and without possible confounders. The model included the changes in SCORAD index, with baseline-values as covariates. Furthermore, mean values at baseline and after intervention with regards to SCORAD index was compared in each of the three groups using paired t-test.

The secondary outcomes were total IgE, specific IgE, ECP, faecal calprotectin and the cytokines IL-10, IFN-γ and IL-31. Analysis of IgE was done on the basis of the 45 children having levels above the detection level at baseline. The analysis of cytokines was based on a total of 46 children as 4 children were excluded due to missing data. IgE, Faecal calprotectin, ECP and cytokine levels were analysed using the non-parametric Kruskal-Wallis test. The Chi-Square, mixed mean model, measures of agreement, was used for detecting differences in gender between intervention groups. Differences in the ages of the groups were analysed using the Kruskal-Wallis test. All anthropometric measures were analysed using the 1-way ANOVA analysis. Correlations between relevant parameters were tested using the Pearson correlation coefficient. Statistical analysis were done using the software program SAS, version 9.1 (SAS Institute, Cary, USA). The level of significance was considered at p<0.05.

**RESULTS**

A total of 50 children completed the study. The mean age of the children were 18 months at inclusion, with a range from 7-24 months. Children were from the area of Copenhagen, Denmark. All children were diagnosed with atopic dermatitis by their general practitioner or dermatologist, and were at time of inclusion experiencing continued itching and pruritus. Forty-four children out of 49 (90 %) had a mother, a father or a sibling with allergy. One child was adopted and information on predisposition was therefore not available. Eighty percent of the children were at time of inclusion treated with steroid products of various types. Parents were advised to follow the guidance and treatment given by the child’s general practitioner or dermatologist.

There were no statistical significant differences in age between the intervention groups. There were a significant higher number of boys in the NCFM intervention group compared to the Bi-07 and the placebo group (Chi-Square p = 0.04). There were no significant differences in the anthropometric measures of length, head circumference, weight and the skin folds of triceps and subscapularis between the groups at baseline or after intervention. The baseline characteristics of the children are shown in table 1.

**TABLE 1. Baseline characteristics of the children of the three intervention groups (N = 50, mean ± SD). * Significant higher fraction of boys in the NCFM group (Chi-Square p = 0.0369)**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Bi-07 (N = 17)</th>
<th>NCFM (N = 17)</th>
<th>PLACEBO (N = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, z-scores</td>
<td>0.02 ± 0.99</td>
<td>-0.11 ± 1.16</td>
<td>0.09 ± 0.89</td>
</tr>
<tr>
<td>Boys</td>
<td>9</td>
<td>15*</td>
<td>8</td>
</tr>
<tr>
<td>Girls</td>
<td>8</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Length, z-scores</td>
<td>0.17 ± 0.77</td>
<td>-0.38 ± 1.20</td>
<td>0.15 ± 0.97</td>
</tr>
<tr>
<td>Head circumference, z-scores</td>
<td>-0.12 ± 0.81</td>
<td>-0.06 ± 1.28</td>
<td>0.10 ± 0.88</td>
</tr>
<tr>
<td>Weight, z-scores</td>
<td>0.13 ± 0.91</td>
<td>-0.21 ± 1.11</td>
<td>0.04 ± 1.04</td>
</tr>
<tr>
<td>Triceps, z-scores</td>
<td>0.33 ± 0.86</td>
<td>0.06 ± 1.29</td>
<td>-0.34 ± 0.68</td>
</tr>
<tr>
<td>Subscapularis, z-scores</td>
<td>0.26 ± 1.02</td>
<td>-0.09 ± 1.06</td>
<td>-0.19 ± 0.95</td>
</tr>
</tbody>
</table>

**TABLE 2. Change in SCORAD index between baseline and after intervention (N = 50, mean ± SD). ** Significant difference between baseline and after intervention in the Bi-07 group in post hoc analysis**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BASELINE ±SD</th>
<th>AFTER INTERVENTION ±SD</th>
<th>DIFFERENCE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi-07 (N = 17)</td>
<td>22.5 ± 11.0</td>
<td>15.8 ± 8.9</td>
<td>-6.7</td>
<td>0.009**</td>
</tr>
<tr>
<td>NCFM (N = 17)</td>
<td>20.6 ± 13.1</td>
<td>17.6 ± 10.5</td>
<td>-3.0</td>
<td>0.22</td>
</tr>
<tr>
<td>Placebo (N = 16)</td>
<td>20.2 ± 8.3</td>
<td>14.7 ± 9.7</td>
<td>-5.5</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**SCORAD index**

According to the SCORAD index thirty-three children was classified as having mild eczema (< 25), whereas 16 children had moderate eczema (25-50) and 2 children had severe eczema (>50) at time of inclusion. Included children had an average score of 21, indicating a mild severity of AD among the children. There was no significant difference in SCORAD index between the three intervention groups at baseline. The analysis of the changes from baseline to after intervention, with baseline-values included as covariates, did not detect an overall effect of the interventions on SCORAD index between the groups. Analyses were run both with and without the possible confounders of gender, predisposition to allergy, age and whether or not IgE levels were increased, without changes in conclusions. The mean SCORAD index from baseline to after intervention in each of the groups were analysed separately in a post hoc analysis. These data showed a significant decrease in SCORAD index in the Bi-07 group (p = 0.009), meaning that children in this group had significantly less clinical signs after the 8 weeks of a daily dose of Bi-07. There were no differences in the NCFM or the placebo group between values at baseline and after intervention. Table 2 shows the change in
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SCORAD index in each of the three intervention groups from baseline to after intervention.

All analyses of data were additionally run using the objective SCORAD, excluding the subjective signs included in the SCORAD index, as it has been argued that these subjective signs are counting too much of the total score (20%). Thus, there were no differences between analyses including SCORAD index and analysis excluding subjective measures (objective SCORAD). The two measures did, not surprisingly, correlate with Pearson correlation coefficient at 0.91. There was no significant correlation between the objective SCORAD and the subjective signs reported by the parents (data not shown).

**Serum immunoglobulin E**

A total of eight children had increased total IgE levels, when using the cut-off limit of 30 kU/l for children less that a year and 60 kU/l for older children. The cut-off limit for increased levels of IgE specific for hen’s egg and cow’s milk was chosen to 0.35 kU/l which resulted in eight children having increased IgE levels specific to egg and 7 children with increased IgE levels specific to milk at baseline. Five of the children were having increased levels to both egg and milk IgE. There were no significant differences in IgE levels between the groups at baseline, and there were no significant changes in IgE levels between baseline and after intervention in either of the groups. Results are shown in table 3.

**Faecal calprotectin, Cytokines and Eosinophil Cationic Protein**

There was no significant difference in change from baseline to after intervention between the groups with regard to faecal calprotectin, ECP, IL-10, IL-31 or IFN-γ. However, the changes IFN-γ and IL-10 from baseline to after intervention in the Bi-07 group showed a decreasing tendency (p = 0.1 and p = 0.07 respectively). Characteristics of the inflammatory parameters at baseline and after intervention can be seen in table 3.

There was a clear correlation between SCORAD index and ECP at baseline (Pearson correlation coefficient 0.497 p = 0.001) shown in figure 1.

**TABLE 3. Inflammation parameters at baseline and after intervention expressed as median values (25-75 quartile)**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BI-07 (N = 17)</th>
<th>NCFM (N = 17)</th>
<th>PLACEBO (N = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After</td>
<td>P</td>
</tr>
<tr>
<td>IgE, total (kU/l)</td>
<td>7.75 (5.2-24.4)</td>
<td>6.77 (3.1-23.7)</td>
<td>0.86</td>
</tr>
<tr>
<td>IgE egg (kU/l)</td>
<td>0.04 (0.01-0.11)</td>
<td>0.03 (0.0-0.07)</td>
<td>0.18</td>
</tr>
<tr>
<td>IgE milk (kU/l)</td>
<td>0.05 (0.03-0.10)</td>
<td>0.06 (0.0-0.07)</td>
<td>0.75</td>
</tr>
<tr>
<td>ECP (µg/l)</td>
<td>8.82 (5.05-17.6)</td>
<td>11.42 (6.09-16.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>106.5 (30.0-206.4)</td>
<td>75.2 (1.84-155.5)</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>358.4 (200.4-502.5)</td>
<td>198.7 (35.0-370.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>IL-31 (pg/ml)</td>
<td>71.0 (49.0-388.9)</td>
<td>105.8 (49.0-1664.2)</td>
<td>0.34</td>
</tr>
<tr>
<td>Faecal Calprotectin (µg/g)</td>
<td>59.1 (42.9-162.0)</td>
<td>39.9 (36.2-57.6)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**Compliance**

Compliance based on calculation of remaining capsules after intervention was in average 94% (Bi-07: 93.6%; NCFM: 95% and Placebo: 93.3%), which was corresponding to the compliance considered based on self-reported diaries. No participating children had compliance
below 72%. There was no difference in compliance between the groups (p = 0.6).

Quantification of probiotic bacteria in stool

Faecal counts of *L. acidophilus* at the start of intervention in the NCFM group and at all time points of sampling in the two other groups were below the detection limit. After administration of *L. acidophilus* the count was 6.0 – 9.0 Log$_{10}$ CFU per g stool in 15 out of 17 subjects of the NCFM intervention group. *B. lactis* was found in all samples from the Bi-07 intervention group after probiotic intake in the range between 6.3 and 9.1 Log$_{10}$ CFU per g stool.

DISCUSSION

The present study did not detect an overall beneficial effect of the 8 weeks daily intervention with the two probiotic strains *L. acidophilus* NCFM and *B. lactis* Bi-07 on the severity of AD in children less than 2 years with regard to clinical or immunological parameters. However, a post hoc analysis showed a significant reduction in the severity of AD measured by SCORAD index in the Bi-07 intervention group.

The cytokine IFN-γ has antiviral, immunoregulatory and anti-tumor properties (Schröder *et al.*, 2004), and it is additionally well established that IL-10 is an anti-inflammatory cytokine being capable of inhibiting synthesis of pro-inflammatory cytokines (Kluth and Rees, 1996). Therefore the observed tendency towards a decrease in these two parameters in the present study might suggest a beneficial effect of the intervention. Additionally the two probiotic strains NCFM and the Bi-07 which were selected for this study have recently shown to be effective in reducing fever, rhinorrhea, and cough incidence and duration and incidence of antibiotic prescription, as well as the number of missed school days attributable to illness, in children from 3 to 5 years of age (Leyer *et al.*, 2009). Probiotics were consumed as either NCFM alone or NCFM in combination with Bi-07 and both groups resulted in the beneficial effects when compared to placebo. The combination of NCFM and Bi-07 did show a more pronounced reduction of fever, coughing and rhinorrhea incidence compared to NCFM consumption alone (Leyer *et al.*, 2009). This additional beneficial effect is believed to be an effect of Bi-07. Thus, there are some data suggesting some beneficial effects of the strains in children. However, a significant effect could not be confirmed by this study with regards to treating AD in children less than two years of age.

Our results are in agreement with recent published reviews which have not been able to finally establish enough evidence to support the use of probiotics for treatment of AD in children (Boyle *et al.*, 2008; van der Aa *et al.*, 2009). There is evidence that probiotic treatment can lower the increased intestinal permeability associated with eczema (Rosenfeldt *et al.*, 2004). However, as probiotics are ineffective in treating AD the decrease in intestinal permeability associated with their use seems to be insufficient. Thus it is concluded that probiotic treatment does not reduce the need for other eczema treatment such as topical corticosteroids (Boyle *et al.*, 2008).

There could be several reasons explaining why we did not find an effect of the probiotic intervention of the present study. First, the power of the study was low. With the included number of fifty children we were only able to detect a difference of 1 standard deviation. Secondly the children included were having a mild to moderate degree of AD, and were generally well-treated. Additionally, only a small number of children with increased IgE levels were included in the trial. Other studies of children with AD have found a more pronounced beneficial effect of probiotics among children with a positive skin prick test and increased IgE levels (Rosenfeldt *et al.*, 2003). Furthermore previous data have indicated that probiotic treatment is more effective on food sensitized children suffering from AD (Sistek *et al.*, 2006). The intervention period of 8 weeks and the experimental dose of 10$^{10}$ CFU of the probiotics per day are believed to be appropriate, as earlier studies have showed that probiotic given to children with moderate-severe eczema for 6 weeks, lowered the level of ECP and improved the IgE associated form of eczema (Rosenfeldt *et al.*, 2003). Furthermore, the compliance was high in this study. The general low total IgE concentrations seen in the present study is believed to be due to the mild to moderate degree of AD, but may also be a result of the age of the recruited children. If the children had been younger, their immune system would have been more immature, and more sensitive to environmental and food allergens and possibly also to the effect of probiotics. Also the children had passed the “introduction period” to different foods, where the oral tolerance is more unstable. The higher fraction of boys in the NCFM group is not believed to influence the results. In general the literature reports a higher fraction of boys developing allergy related disorders (Smidesang *et al.*, 2009) and a earlier peak in prevalence of AD among boys (Halkjaer *et al.*, 2006), however there is to our knowledge not reported differences in AD severity or clinical signs related to the gender of young children.

The significant correlation between the cytotoxic agent ECP secreted by activated eosinophils during allergic and inflammatory processes and the SCORAD index at baseline suggest the use of ECP as a measure of the degree of inflammation and for monitoring the degree of AD in children. This possible correlation has previously been investigated in the literature, and it is concluded that the ECP have a role in the pathogenesis of AD (Murat-Susic *et al.*, 2006). Studies have investigated the use of ECP as a possible clinical marker of disease activity and immunological status in children having AD by comparing with those of nonatopic children; however these previous studies have not been able to detect a correlation (Murat-Susic *et al.*, 2006; Schulte-Herbruggen *et al.*, 2007). The clearly significant correlation between ECP and SCORAD index observed in this study should be further confirmed by clinical studies, but is indicative of ECP being a clinical marker of the disease.

Recent literature have found increased levels of faecal calprotectin among children having bowel inflammation disease, and have therefore suggested the use of this measure as a simple and non-invasive marker of intestinal inflammation (Paduchova and Durackova, 2009). The use of faecal calprotectin as a measure of intestinal inflammation has, to our knowledge, not previously been investigated in children below two years of age having AD. The
present study did not confirm any significant change between the groups after intervention, and could indicate the difficulties in using faecal calprotectin as a measure for inflammation among AD children in this age group. Secondly it could be speculated that the anti-inflammatory effect of the probiotic intervention was to low to be measured by faecal calprotectin. More studies are needed to establish the use of faecal calprotectin as a measure of intestinal inflammation among children having AD.

In conclusion there was no overall clinical or immunological effect of probiotic supplementation of the two probiotics *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* Bi-07 in young children with AD. However, a post hoc analysis showed a significant reduction in severity of AD in the Bi-07 group and possible beneficial effects of this probiotic strain could be of interest. Further and larger probiotic AD treatment studies would be helpful in clarifying whether specific probiotic strains, such as Bi-07 or other strains could have a role in treating AD.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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