Pharmacological Research 163 (2021) 105284

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Rice flour fermented with *Lactobacillus paracasei* CBA L74 in the treatment of atopic dermatitis in infants: A randomized, double- blind, placebo-controlled trial

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ARTICLE INFO

Keywords:
Atopic dermatitis
SCORAD
Microbiota
Postbiotics
Topical corticosteroids
Sparing-effect

ABSTRACT

To assess the effect of a fermented rice-flour obtained from *Lactobacillus paracasei* CBA L74 in managing infants with moderate to severe atopic dermatitis. Infants with moderate to severe atopic dermatitis, aged 6–36 months, were randomly assigned to receive once-daily consumption of rice flour containing heat-killed probiotic *Lactobacillus paracasei* CBA L74 or placebo for 12 weeks as supplementary approach to topical treatment. Primary outcome was SCORAD index change from baseline to 12 weeks; secondary outcomes were gut microbiota composition, as evaluated by the analysis of fecal samples, and serum cytokines at baseline and at the end of the intervention period in both groups, and steroid usage over the treatment period and one month after stopping it. V3–V4 region of the 16S ribosomal RNA gene was sequenced to evaluate changes in the gut microbiota. SCORAD index decreased over the treatment period in both groups. The difference in the SCORAD change was -2.1 (-5.5 to 1.3; p = 0.223) for the experimental vs. the placebo group, not reaching the minimal clinical difference of 8.7 units. The use of topical steroids, measured as finger tips units, decreased from 4 to 16 weeks, in both groups; the reduction was significantly higher in experimental than in placebo group (p value from Wilcoxon rank sum test = 0.031). No significant differences were observed for cytokines levels between groups. The composition of gut microbiota at the phylum and class taxonomic levels resulted very similar, at baseline and after intervention, in both groups. Similarly, no significant differences were observed in the relative abundance of bacterial genera between groups. In conclusion, though the heat-killed *Lactobacillus paracasei*was not proved to be effective in reducing the severity of atopic dermatitis, it showed a steroid sparing effect the value of which needs to be further investigated.

1. Introduction

Atopic Dermatitis (AD), an itchy eczema with a chronic relapsing course, is a common clinical manifestation of atopy in the first years of life and the most common chronic inflammatory skin disease, with a prevalence of 20 % in children [1]. AD, as a multidimensional disease, has a great impact on patients and their families' quality of life, comparable with epilepsy or type 1 diabetes. It also deeply affects social activities and patient well-being [2,3].

Based on the available knowledge, AD immunopathogenesis is characterized by an inflammatory reaction, characterized by a complex interplay between skin barrier disruption and immune inflammation [4,5].

Nowadays, it is recognized that type 2 cytokines play a common pathogenetic role in atopic diseases [6], leading to epidermal barrier dysfunction and skin inflammation [7].

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https://doi.org/10.1016/j.phrs.2020.105284
Received 20 September 2020; Received in revised form 27 October 2020; Accepted 27 October 2020
Available online 4 November 2020

Imbalances in gut microbial composition and function, termed dysbiosis, have been linked to the pathophysiology of many disorders, including allergic diseases and AD, especially during early childhood [8,9].

Accordingly, lists of taxa specifically under- or over-represented in AD have been produced by a number of recent papers, with conflicting results. Reddel et al. (2019) reported a dysbiotic status of the gut microbiota, characterized by the reduction of *Bifidobacterium*, *Blautia*, *Coprococcus*, *Eubacterium*, *Propionibacterium* and an increase in *Faecalibacterium*, *Oscillospira*, *Bacteroides*, *Parabacteroides in children with AD* [1]. Zheng et al. (2016), in a case-control study of infants with eczema and controls, identified five genera significantly enriched in the former (*Escherichia/Shigella*, *Veillonella*, *Faecalibacterium*, *Lachnospiraceae incertae sedis* and *Clostridium XIVa*) and four in the latter (*Bifidobacterium*, *Megasphaera*, *Haemophilus* and *Streptococcus*) [10].

The conventional management of pediatric AD targets the restoration of the skin barrier using moisturizers and the prevention of flare-ups via topical corticosteroids (TCS). Calcineurin inhibitors are used as second-line effective agents [11]. Moderate-to-severe AD affected patients need to be treated with long-term applications of topical corticosteroids; long-term TCS therapy has been associated with adverse local effects, including skin atrophy and flares rebounds [12,13]. The AD skin barrier impairment can also increase TCS percutaneous absorption that leads to rare but severe adverse systemic effects, such as the hypothalamic pituitary-adrenal (HPA) axis suppression,poor growth, hypertension, hyperglycemia [14]. These safety concerns are increased in pediatric patients, whose greater body surface area-to-weight ratio is thought to cause increased percutaneous absorption.

Furthermore, parents' TCS-phobia often causes poor adherence to an appropriate treatment which may have a dramatic effect on diseases outcomes [15]. In addition, long-term safety data of mid- to high-potency TCS and calcineurin inhibitors are lacking for pediatric patients [16,17]. These considerations, joint to the observed gut dysbiosis, raised the interest for complementary treatment strategies, directed at immunomodulation, possibly targeting gut microbiota via dietary supplements [18–20].

Available data regarding the effects of probiotics and other functional food (prebiotics, e.g., non-digestible oligosaccharides or dietary substrates, stimulating the growth or activities of specific taxa within the gut microflora) are not conclusive yet and conflicting, as evidenced from recent meta-analyses [21,22].

Thus, one different possibility could be exploiting the probiotic-like effects of inactivated bacteria, with all their metabolic products (e.g., SCFA), of microbial fractions or isolated bacterial components, known as postbiotics [23].

The present randomized, double-blind, placebo- controlled study aimed to evaluate the effects of a fermented rice flour obtained from *Lactobacillus paracasei* CBA L74 in infants with moderate to severe atopic dermatitis.

2. Methods

2.1. Study design and study population

A prospective, randomized, double-blind, 2-arm placebo-controlled trial was conducted at Department of Pediatrics, Vittore Buzzi Children's Hospital in Milan (Italy). The study protocol was approved by the local ethics committee and was conducted in accordance with the principles of the amended Declaration of Helsinki and Ethical Guideline for Epidemiological Research. Written informed consent was obtained from parents or legal representative prior to enrollment in the study. Trial was registered in the Registry Clinical Trial (www.clinicaltrial.govI.D NCT 03157284).

Inclusion criteria were as follows: infants and young children age 6 months-36months old at the enrolment, with a diagnosis of AD according to Hanifin and Rajka criteria and moderate to severe atopic dermatitis according to the SCORAD (index)system [24]

SCORAD (SCORing Atopic Dermatitis) is a reliable and validated tool to assess the extent and severity of eczema in clinical trials [25]. It was developed by the European Task Force on Atopic Dermatitis in 1993 [24]. This index is a composite score, which combines objective criteria, such as extent and intensity of lesions and subjective ones, namely daily pruritus and sleeplessness. It ranges between 0 (lowest possible score) and 103 (highest possible score) and it is interpreted as follows: < 25: mild AD; 25–50 moderate AD; > 60: severe AD.

The major exclusion criteria were: mild dermatitis (SCORAD < 25), children with comorbid chronic diseases and/or dermatologic conditions other than AD, children using antihistamines, oral corticosteroids, pre and/or probiotics during the 4 weeks before enrollment, treatments with topical calcineurin inhibitors in the previous three months and recognized allergy or intolerance to probiotics or excipients of the tested product.

Records and diaries were kept of concomitant medications and adverse effects. Topical use of corticosteroids was allowed and all subjects were asked not to change the kind of topical therapeutics, as far as possible, through the intervention period.

Fifty-eight infants and young children (age 6–36 months), fulfilling the inclusion criteria, were enrolled in the study (Fig. 1). They were randomly assigned to receive rice fermented with *Lactobacillus paracasei* CBA L74 or placebo. All enrolled subjects were assessed on day 0 (baseline) and underwent medical examination at week 4, 8 and 12. At each visit, the Medical Investigators examined the number of days and the finger tips units (FTU) of TCS usages, the consumption of allotted study diets (experimental product and placebo) and the occurrence of adverse events based on the individual's study diary.

A flare was defined as a worsening of the disease leading to use of topical corticosteroids for at least 3 consecutive days.

2.2. Recruitment and randomization of participants

Children aged 6 months to 3 years were recruited over two years, from January 2017 to March 2017 and from October 2018 to February 2019, among patients admitted to the Allergy Unit or Dermatology Unit for AD evaluation. Two dermatologists with expertise in pediatric dermatology evaluated their AD and SCORAD index.

Participants were then randomized, using computer-generated 4-block design lists, drawn up by a statistician. Both the investigators and the subjects were blinded throughout the study regarding the assignment of the experimental product vs placebo.

2.3. Sample size

The minimum clinically important difference (MCID) for SCORAD is 8.7 units [24]. Using an analysis of covariance for repeated measures (RM-ANCOVA) [26], we calculated that a sample size of 25 subjects per group (Experimental group, EXP *vs.* Placebo group, PLA) ensures 80 % power to detect a difference of 8.7 as statistically significant at an alpha level of 0.05 assuming a mean difference (standard deviation) of -41.6 (17.0) between the first and the last measurement for EXP [27], three repeated measurements beyond baseline [27], a correlation of 0.5 between repeated measures [27], and the same standard deviation of the difference (17.0) for the EXP and PLA groups. Assuming an estimated dropout rate of 15 %, we decided to enroll 29 subjects per group.

2.4. Interventions

Subjects were treated for a period of 12 weeks. During the study period all patients received treatment or placebo, moisturizers, and topical corticosteroids in case of disease flares, according to the guidelines for the management of AD [13]. Patients in the experimental group received a daily 8 g of a rice-dried powder containing heat-killed *Lactobacillus paracasei* CBA L74; placebo rice-powder was matched for

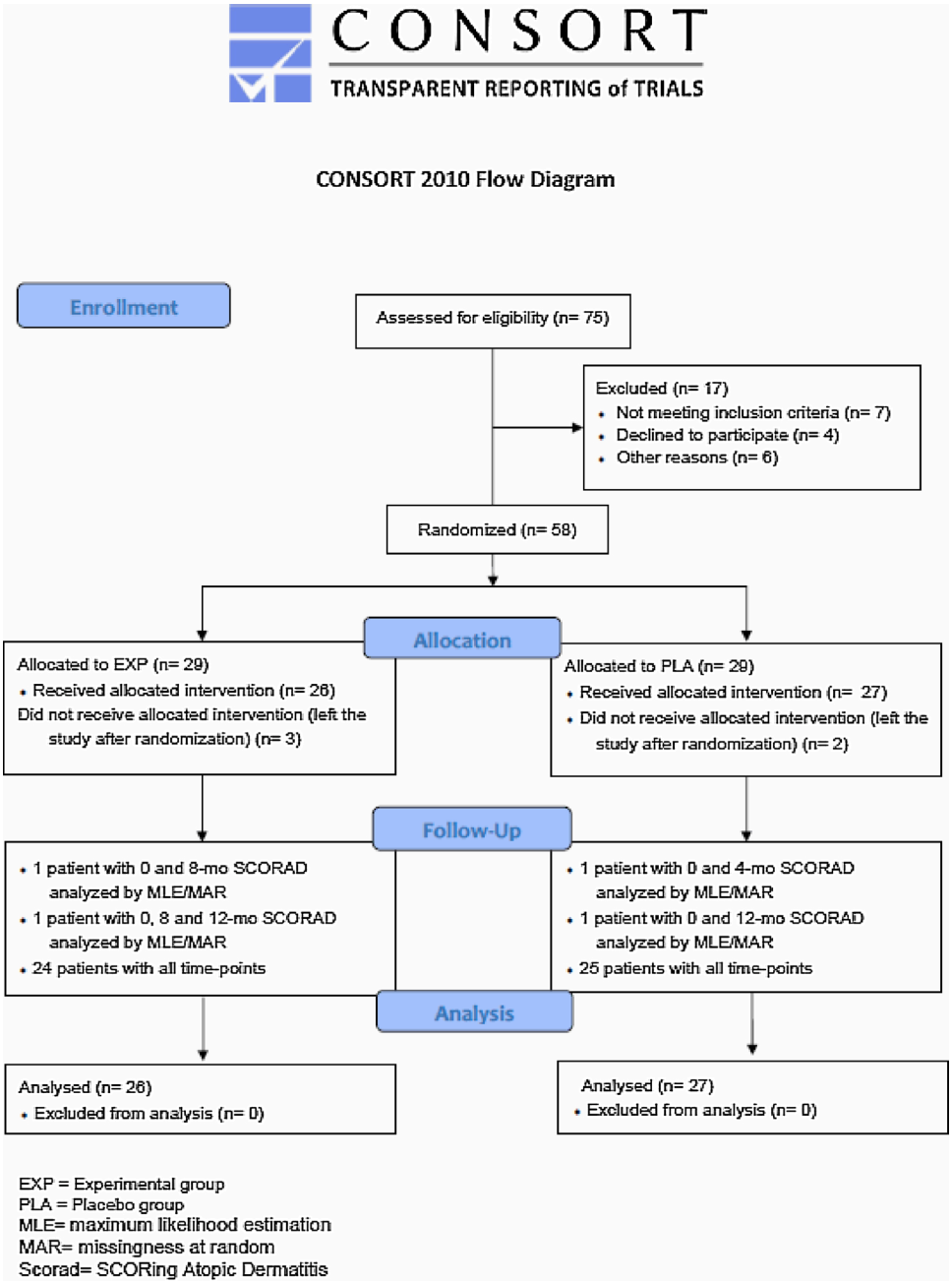

Fig. 1. The flow diagram of the study.

size, shape, and volume of contents. Both the fermented matrix and the placebo were dispensed by the protocol dedicated staff. Once daily each subject was fed with 8 g of *Lactobacillus paracasei* CBA L74 powder or placebo diluted in beverage or liquid food.

2.5. Outcomes evaluation

The primary outcome was the change in the severity of AD after 12 weeks of intervention, compared to baseline. The standardized SCORAD scoring system was used to evaluate AD severity [24]. Assessments and SCORAD Index were made at baseline and after 4, 8 and 12 weeks of treatment.

Secondary outcomes were the evaluation of the steroid-usage in both group at baseline, at 4, 8, 12 weeks after the start of treatment, and one month after stopping it; taxonomic composition and diversity indices of the gut microbiota, as evaluated by the analysis of fecal samples and serum cytokines levels at baseline and after 12 weeks intervention period.

The overall consumption of topical corticosteroids, measured as number of finger tips units (FTU) over the overall study period, from 4 to 16 weeks, was recorded by the parents and reported to the investigators.

Taxonomic composition and diversity indices were available for 21 PLA children out of 27 and 22 EXP children out of 26. Reasons were the following: one of the fecal samples at T0 or T12 (generally the latter) was not conferred in eight patients; two patients dropped-out from the study.

2.6. Serum cytokine assessment

Serum samples of PLA and EXP patients were collected both before (T0) and after 12 weeks (T12) from the treatment start, and then frozen at -80 °C. On the day of assessment, samples were allowed to gradually

thaw at +4 °C, spun at 10,000xg for 10 min and supernatants were then used for the evaluation of G-CSF, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17A, MCP-1, MIP-1β and TNF-α levels by a magnetic microsphere-based Bio-Plex Pro Human Cytokine 17-plex immunoassay on a Bio-Plex 200 system (both from Bio-Rad), following manufacturer's instructions. Briefly, 50 µl of 4-fold diluted serum samples were sequentially mixed with immunomagnetic beads, detection antibodies and with PE-labeled streptavidin, with appropriate washing between each step. Finally, hybridized beads were loaded on a Bio-Plex 200 reader (Bio-Rad) and fluorescence results were analyzed using the in-house Bio-Plex Manager software, v.6.0 (Bio-Rad).

2.7. Production of 16S rRNA amplicons (V3-V4 regions) and sequencing

Amplicon metagenomic analysis was performed in each subject enrolled in the study, at the initial (T0) and final (T12 timepoints). DNA was extracted from 200 mg of each faecal sample by the QIAamp DNA Stool Mini kit (Qiagen; Hilden, DE), following the manufacturer's protocol to assure an unbiased representation of the various bacterial populations. The DNA concentration of each sample was assessed fluorometrically. For amplicon production, the V3-V4 hypervariable regions of the prokariotic 16S rRNA gene were targeted [28]. PCR was set up in a 50-µl volume with template DNA, 1x HiFi Hot Start Ready Mix (Kapa Biosystems, Wilmington, MA), 0.5µM of each primer. The amplification was carried out on a Bio-Rad T100 thermal cycler (Bio-Rad, Hercules, CA) and included: initial denaturation (95 °C for 3 min); 30 cycles at 94 °C for 30 s (s), 55 °C for 30 s, 72 °C for 30 s; final extension (72 °C for 5 min). Clean-up of amplicons was performed using Agencourt AMPure XP SPRI magnetic beads (Thermo Fisher Scientific). Illumina sequencing libraries were finally prepared through the link of indexes (Nextera XT Index Kit, Illumina, San Diego, CA), quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA), normalized and pooled. Libraries were subjected to paired-end sequencing (2 × 300 bp format) on an Illumina MiSeq platform at BMR Genomics (Padova, Italy). Two amplicons were produced and sequenced for each subject enrolled in the study at the two timepoints.

2.8. Bioinformatics and community analyses

The bioinformatics analysis of sequencing data was based on the Mothur pipeline [29]. Briefly, raw FASTQ files were quality-filtered using Trimmomatic [30] and high-quality reads analysed following the SOP Mothur procedure. Chimeric sequences were identified and removed using UCHIME [31]. The remaining sequences were clustered into OTUs at the 97 % homology level using VSEARCH [32]. OTUs were finally annotated, and taxonomy assigned against the reference database SILVA [33]. The main ecological indexes of within-sample, alfa diversity (Shannon, Chao, inverse Simpson, Observed Richness) were computed using Mothur. Diversity in composition among samples (□-diversity) was evaluated at all taxonomic ranks (from phyla to genera) byplotting the relative heatmap using the function heatmap.2 of the Gplots R library [34], and the relative Principal Component Analysis (PCA) using the R library Ade4 [35].

2.9. Statistical analysis

Continuous variables are reported as median (50^{th} percentile) and interquartile range (IQR, 25^{th} and 75^{th} percentiles). Discrete variables are reported as the number and proportion of subjects with the characteristic of interest. Besides analyzing the change in SCORAD using the originally planned RM-ANCOVA [26], we used random effects linear regression (RER) to handle missing data using maximum likelihood estimation (MLE) [36]. Theoretically, multiple imputation (MI) could be applied to RM-ANCOVA to handle missing data, but we preferred MLE over MI because it is simpler to implement and more efficient [36]. MLE assume missingness at random (MAR), which was highly plausible on the basis of the missingness pattern (reported under Results). The response variable of the RER model was SCORAD; the predictors were baseline SCORAD, treatment (discrete, 0 = Placebo group; 1 = Experimental group), time (discrete, 0 = baseline; 1 = other times) and a treatment*time interaction (discrete); and the random effect was the child. It should be noted that the final time was coded as originally implied by the RM-ANCOVA design, i.e. as a weighted mean of SCORAD at 4, 8 and 12 weeks. RER was also used to analyze the other outcomes. All analyses were performed applying the intention to treat principal. Missing data were handled using MLE under the MAR assumption [36]. RER models similar to the above were used for the study of the other continuous outcomes. The only exception is that we did not include the baseline value of the outcome among the RER predictors. Changes in topical FTU of topical steroids from week 4 to week 16 were compared between groups using the Wilcoxon rank sum test.

Statistical analysis was performed using Stata 16.0 (Stata Corporation, College Station, TX, USA).

3. Results

75 children were assessed for eligibility and 17 were excluded for the following reasons: 7 not satisfying the inclusion criteria, 4 declining to participate, and 6 for other reasons.

58 children were randomized into the EXP ($n = 29$) and PLA ($n = 29$) groups.

3 children in the EXP and 2 in the PLA group decided not to proceed with the study shortly after randomization, leaving 26 children in the EXP and 27 children in the PLA group. 24 of the EXP children had all the planned SCORAD data points (0, 4, 8 and 12 months) available, one had just the baseline and 8-mo data points, and one had just the baseline, 8-mo and 12-mo data points.25 of the EXP children had all the planned data points available, one had just the baseline and 4-mo data points, and 1 had just the baseline and 12-mo data points.

3.1. Baseline characteristics of the study groups

No differences between groups at baseline (Table 1).

Even if sex distribution is likely due to chance, we took it into account by performing the RER analysis of the main outcome using sex as further covariable of the regression model.

3.2. Main outcome

The main outcome was the change of SCORAD in the EXP *vs.* the PLA group. As estimated by RER, the mean (95 %CI) SCORAD was 41.5 (37.5–45.5) at baseline and 21.8 (19.2–24.4) at the end of the treatment period in the PLA group, with corresponding values of 42.5 (38.4–46.6) and 18.7 (16.0–21.3) in the EXP group. The difference in the SCORAD change was -2.1 (-5.5 to 1.3) for the EXP *vs.* the PLA group ($p = 0.223$) (Fig. 2).

When sex was added as covariable to the RER model, the SCORAD

Table 1
The baseline characteristics of patients in both groups.

	PLA N = 29	EXP N = 29
Sex (female)	14 (48 %)	20 (69 %)
Age at enrollment (months)	12 (8; 23)	12 (6; 16)
Birth weight (g)	3250 (3000; 3600)	3230 (3040; 3645)
Delivery		
vaginal	22 (76 %)	21 (72 %)
cesarean	7 (24 %)	8 (28 %)
Formula milk (any)	6 (21 %)	3 (10 %)
Family - Atopy	23 (79 %)	18 (62 %)
Family - smoke	13 (45 %)	10 (34 %)
Family - pets	7 (24 %)	7 (24 %)
SCORAD	41 (32; 48)	44 (35; 46)

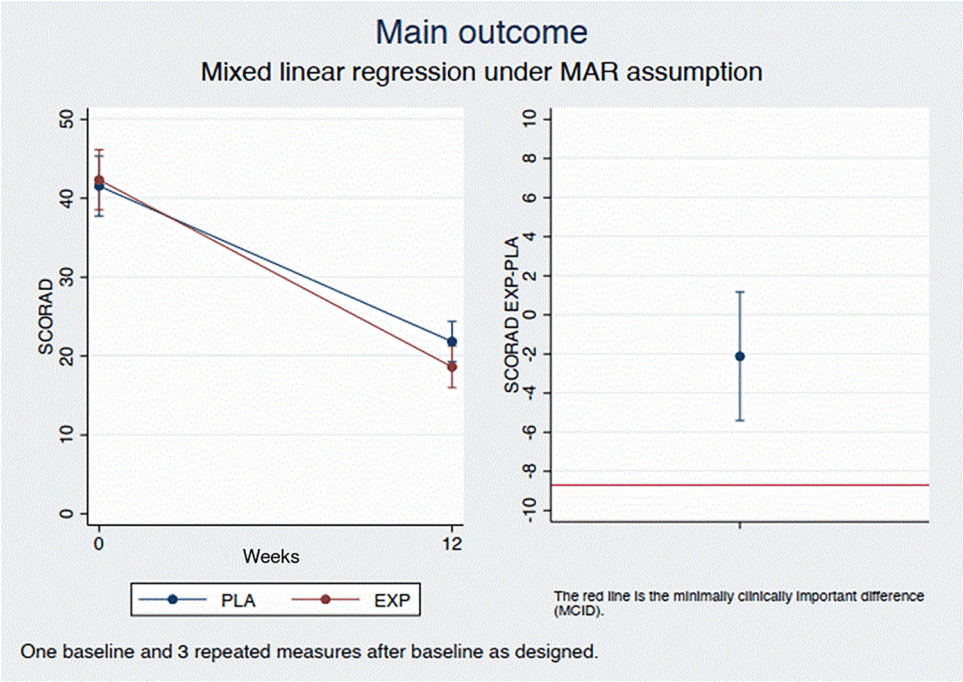

Fig. 2. SCORAD change from baseline to 12 weeks in Experimental (Exp) vs Placebo (PLA) group.

change was -2.7 (-6.1 to 0.7) in both boys and girls ($p = 0.126$ for the comparison). Thus, the different sex distribution of the EXP and PLA groups (Table 1) did not appear to have impacted the SCORAD change even if, of course, the 95 %CI of the within-sex difference are wide because of the reduced effective sample size.

It should be noted that the change in SCORAD calculated by the originally planned RM-ANCOVA was virtually identical that that obtained by MLR, i.e. -2.2 (95 %CI -5.6 to 1.3, p = 0.221). As explained under Statistical analysis, the reason why we preferred RER over RM-ANCOVA is its ability to take into account missing data using (MLE) [37].

3.3. Secondary outcomes

Evaluation of taxonomic composition and diversity indices of the gut microbiota as evaluated by the analysis of fecal samples and cytokines pattern:

The change in the relative abundance of nine preselected fecal bacterial genera for the EXP vs. the PLA groups (Akkermansia, Bacteroides, Bifidobacterium, Clostridium sensu stricto, Faecalibacterium, Lachnospira, Lactococcus, Rothia and Veillonella) was assessed. These 9 genera were selected on the basis of the available literature pointing to their possible association, either positive and negative with atopy. Instead of Lachnospira, the genus annotated as "Lachnospiraceae_unidentified" was considered, in agreement with the output of the analytical pipeline.

To this aim, two 16S rRNA amplicons covering the V3-V4 regions were obtained, sequenced and analysed at T0 and T12 timepoints for PLA and EXP groups. After quality filtering, a total of 40,495,212 high-quality reads were obtained (20,247,606 for the second repeat) and clustered into 12096 operational taxonomic units (OTUs) at 97 % similarity level. The average number of OTUs was 356.86 per sample (standard deviation: 151.94; min: 89; max: 826). These OTUs were classified into 19 phyla, 40 classes, 74 orders, 140 families and 330 genera.

The composition at the phylum and class taxonomic levels resulted very similar for PLA and EXP, at both timepoints, despite minor differences in relative rankings. Some differences were observed between the two groups for the Bacteroidetes and Firmicutes phyla. These differences, however, were limited to the T0 timepoint, and then tended to disappear at 12 weeks (T1) (Fig. 3). The same results were observed for classes distribution between PLA vs EXP (Fig. 4a-b)

As for classes, *Bacteroidia* show the behaviour described above for the corresponding phylum (*Bacteroidetes*); *Clostridia* (phylum: *Firmicutes*) display minor differences in the PLA and EXP groups that remain constant at both timepoints.

In most if not all subjects, the majority of preselected genera had low relative abundances, below the 0.05, which we considered the limit to give reliable results. On the basis of this criterion, we did not perform a formal analysis for the following genera: *Akkermansia*, *Clostridium*, *Faecalibacterium*, *Lactococcus*, *Rothia* and *Veilonella*. As determined by RER, the changes in prevalence for the 3 remaining genera in the EXP *vs.* PLA group were: 0.00 (95 %CI -0.06 to 0.05, $p = 0.877$) for *Bacteroides*, -0.02 (-0.04 to 0.01, $p = 0.186$) for *Lachnospira* and 0.00 (-0.02 to 0.02, $p = 0.903$) for *Veilonella*.

As shown in Fig. 4c, *Bacteroides* spp and "*Lachnospiraceae*_unidentified were the most abundant. The other 7 ones, together, made up <10 % of all genera in all cases and < 5 % in 6 out of 7 cases. *Rothia* and *Veillonella* resulted virtually indetectable. The very small baseline values possibly make it difficult to appreciate any change of these genera between EXP and PLA groups (Fig. 5). *The* change in the main ecological indexes of within-sample (a) diversity (Chao1, Shannon, Observed Richness, Inverse Simpson) were evaluated using RER. The treatment*time interaction was not significant for all cases (data not shown). As for between-sample (b) diversity, the principal component analysis on the Bray-Curtis dissimilarity matrix confirmed that the bacterial consortia had similar structures in PLA and EXP at both time points, taking into account the taxonomic level of family. In detail, children did not seem cluster on the basis of the postbiotic (not shown). As well as for gut microbiota composition, no significant differences were observed for the cytokines pattern between groups at baseline and after 12 weeks (data not shown).

We also evaluated if any treatment-dependent change occurred in the serum cytokine profile between the EXP and PLA. We thus assessed the levels of several relevant immunological factors – including the Type 2-related cytokines IL-4, IL-5 and IL-13 – in the serum of EXP and PLA patients, before and after treatment. Similar to the gut microbiota

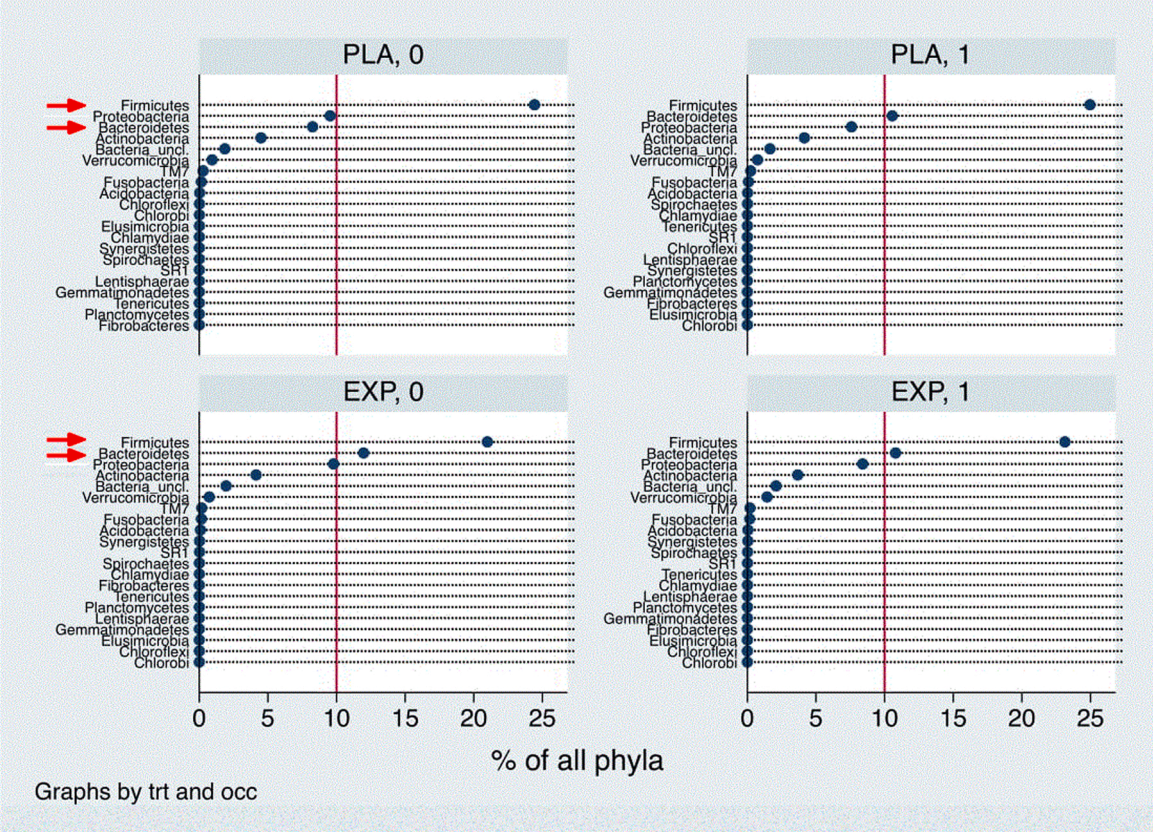

Fig. 3. Phyla distribution Experimental (EXP) vs Placebo (PLA) group at T0 and T1.

composition, no relevant treatment-associated changes in cytokine levels were noted between the two groups of patients after 12 weeks of intervention.

Steroid-sparing effect. Eight patients in the experimental group and 6 in the placebo group did not use any topical corticosteroids during the overall study period. Among patients reporting some corticosteroids use, FTU of topical steroids decreased from 4 to 12 weeks in both groups: EXP (median decrease: -10; interquartile range, I.Q.R -20; 0), PLA (median =-3.5; I.Q.R -10, 3; p value from Wilcoxon rank sum test = 0.197; at 16 weeks a significantly higher decrease was observed in experimental (median decrease: -14, I.Q.R: -25, -8) vs placebo group (median decrease: -4, I.Q.R: -10, 0); p value from Wilcoxon rank sum test = 0.031 (Fig. 6).

4. Discussion

Beside the well-known pre- and probiotics, the –biotic family includes postbiotics, bioactive compounds produced by food-grade microorganisms during a fermentation process. Postbiotics include microbial cells, cell's constituents and metabolites [23]. Probiotics have been deeply investigated as gut microbiota modulators; nevertheless, their therapeutic effect on AD is still controversial [38,21,39,40]. Reports on postbiotics products have been emerging for less than a decade and, more specifically, *in vivo* studies are a still relatively unexplored field in AD affected patients. *Lactobacillus paracasei* has been diffusely investigated and showed beneficial effects *in vitro* and *in vivo* as pro-inflammatory cytokines inhibitor ad T-regulatory cell-like response inductor [41].

Preclinical data show that *Lactobacilli*-originated postbiotics may be effective in reducing contact hypersensitivity reaction and development of atopic skin lesion [42]. Their biological responses have also been observed in human trials [43–45]. Postbiotics use could be an advantage in terms of safety profile, longer shelf life, and resistance to mammalian enzymes [46,47]. Furthermore, fermented matrix no need for refrigerated storage,they are stable to digestive system conditions and there is the chance to mix them in warm to hot liquids (47). To our knowdledge, *in vivo* studies using postbiotics in AD are limited to three, all of which using heat-killed *Lactobacillus paracasei,* and two performed in pediatric patients. The former, by Moroi et al., evaluated the effect of *Lactobacillus paracasei* K71 in a randomized, double-blind, placebo-controlled study on adult AD patients, who were treated with conventional topical corticosteroid and tacrolimus [48]. The second study, performed on pediatric subjects, considered only 12 children with moderate to severe AD in a repeated-measure cohort design [27]. The most recent one, by Yan et al., compared the effects of consumption of heat-killed *Lactobacillus paracasei* GM-080 to placebo, as a supplementary approach in AD affected infants, aged 4–30 months [49].

The present RTC primarily tested the effect of a fermented rice flour, containing heat killed *Lactobacillus paracasei* CBA L74 (Heinz Italia SpA, Latina, Italy) on AD severity as complementary approach to the conventional, topical treatment. To our best knowledge, this is the first study evaluating the effect of a postbiotic on clinical outcome, e.g AD severity, and gut microbiota composition. Over the period treatment, as determined by three repeated measures, SCORAD decreased of -3.1 (-5.5 to 2.3) in the EXP *vs.* the PLA group, the observed difference was below the minimal clinical difference of 8.7 units. Different sex distribution in the EXP and PLA groups had no impact on SCORAD change. Yan et al. obtained the same results while administering daily heat-treated probiotic *Lactobacillus paracasei* GM-080 for 16 weeks, as supplementary approach, in AD infants aged 4–30 months. Moroi et al. found a scant benefit in adult with AD who received a supplementary diet containing *Lactobacillus paracasei* K71 vs placebo. Even if Δ values of scores deviated more from baseline in the intervention group at weeks 8 and 12 (i.e., the differences resulted greater than for the placebo group), none of the differences reached a significant level. It should be pointed out that in this study skin severity scores were measured using the criteria of the

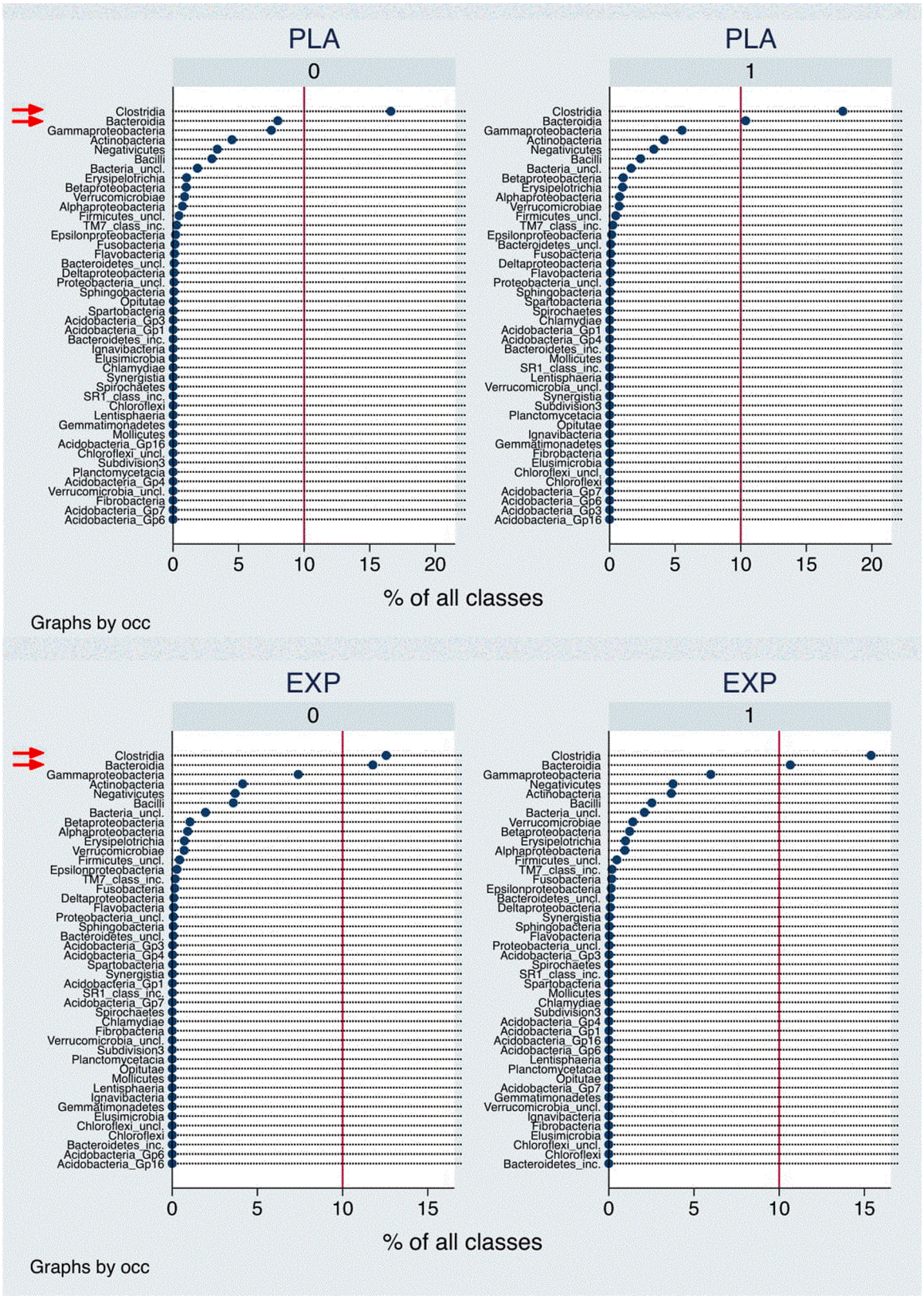

Fig. 4. Class distribution Experimental (EXP) vs Placebo (PLA) group at T0 and T1.

Japanese Dermatological Association, which is not considered among the instruments valid to measure the AD severity, according to the International Guidelines [50]. Promising results in Beretta et al.'s pilot study, might have been affected by the upcoming summer season, which could have led to AD remission, and were limited by the lack of a placebo control group.

So far, heat-inactivated *Lactobacillus paracasei* CBA L74 was not proved to be effective in reducing AD severity in infants with moderate

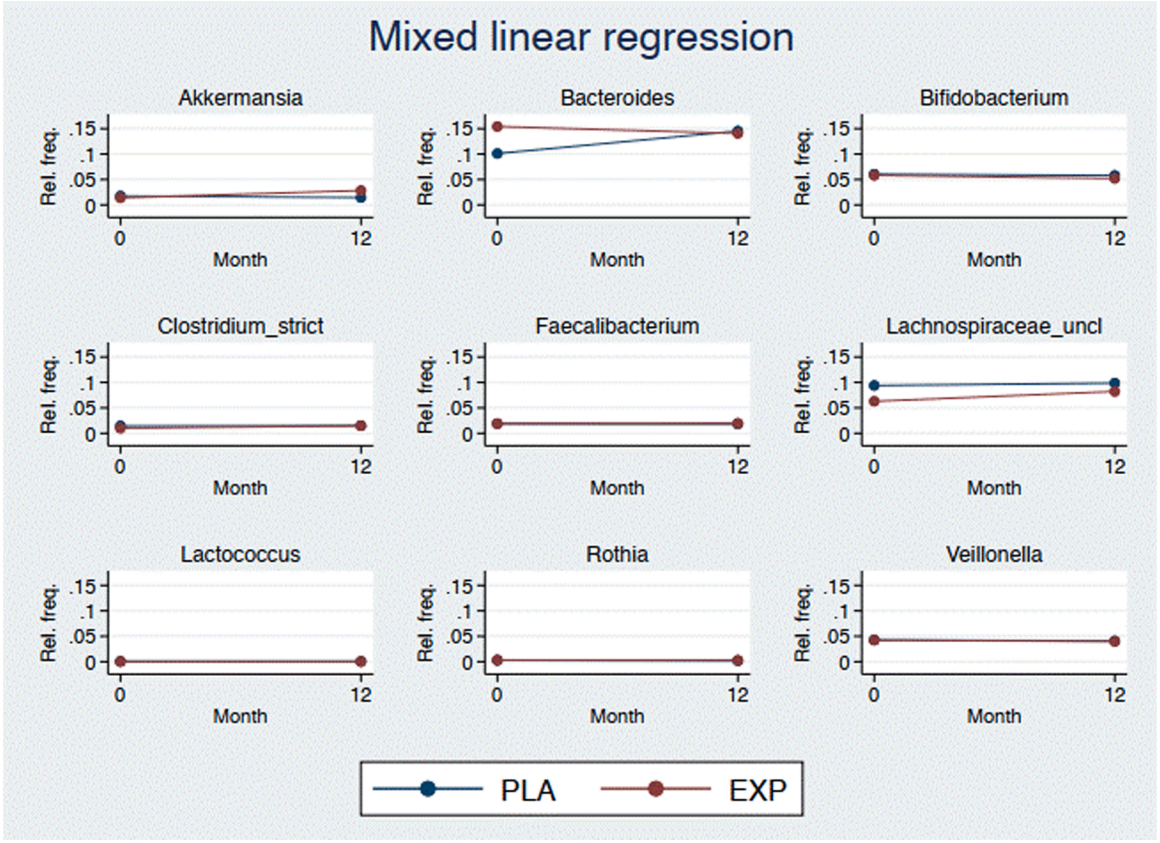

Fig. 5. Genera distribution Experimental (EXP) vs Placebo (PLA) group at T0 and T1.

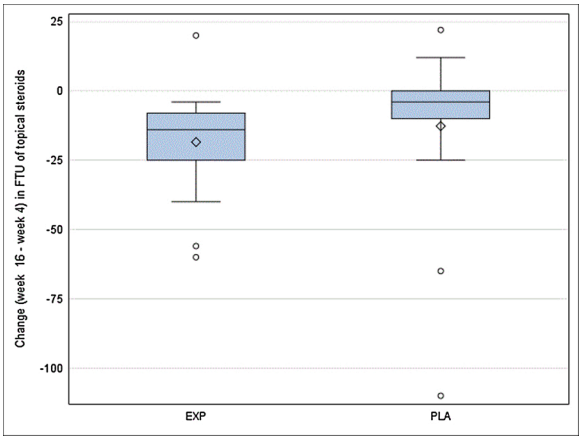

Fig. 6. Box plot for the change (week 16 – week 4) in FTU of topical steroids in Exp vs PLA groups.

to severe atopic dermatitis. Although the consumption of corticosteroids decreased at the end of the intervention period in both groups, the decrease was higher in experimental group, reaching a significant difference one month after stopping treatment. The steroid sparing effect observed in the group treated with heat-inactivated *Lactobacillus paracasei* CBA L74 might be explained by its anti-inflammatory properties, as proven by preclinical data [42] and in human trials [43–45]. In our setting, no relevant treatment-related change in the level of serum cytokines – including IL-4, IL-5 and IL-13 – was observed, suggesting that such anti-inflammatory effect might be potentially mediated by cell and/or metabolites, other than cytokines.

Regarding the analysis of the 9 pre-specified bacteria genera, there are differences between the results here reported and literature data; these may be due to demographic differences in the examined cohorts, different geographical locations, environment and diets, methodological and experimental differences.

In our hands, and for our population, slightly differential trends among EXP and PLA limited to *Akkermansia* (trend to increase at T1 in exp, in agreement with the results reported by Fujimura et al., that described the depletion in the gut microbiota associated with atopy and asthma). However, the caveats linked to the scarce representation of these genera remain [51].

Data for *Bacteroides* differ to those reported in Reddel et al. (2019). They reported an increase of the genus in atopic subjects while in our cohort we see it constant in the EXP group, considering the timepoints T0 and T12. On the contrary, an increase is appreciated in T1 for PLA. One important point is the different age of the enrolled subjects: 1–6 years for Reddel; 6–36 months for us. Another issue is that data on *Bacteroides* are not congruent in the various papers.

In our cohort, *Bacteroides* presents a behaviour comparable to what described above for its phylum, *Bacteroidetes,* and class, *Bacteroidia*. The same applies, to a minor extent, to *Lachnospiraceae_unclassified* compared to *Firmicutes* (phylum) and *Clostridia* (class). In summary, with regard to gut microbiota no significant differences were observed between the groups.

In conclusion, the present study did not prove the efficacy of a fermented rice flour obtained from heat treated *Lactobacillus paracasei* CBA L74 as a complementary approach in significantly reducing AD severity. However, heated killed *Lactobacillus paracasei* CBA L74 showed a corticosteroid sparing effect beyond the treatment period. This issue deserves further and more specific investigations in the light of the growing interest for steroid-sparing strategies. An effective adjunctive treatment in AD management could be able to minimize corticosteroid adverse effects and improve the patients and parents' adherence to the

therapy.

Funding

None to declare for all the Authors

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The study was supported by a grant from Heinz Italia SpA, Latina, Italy to the Department of Pediatrics of Vittore Buzzi Children's Hospital, University of Milan. The sponsor had no influence on the study design; the collection, analysis, and interpretation of data; the writing of the manuscript; or the decision to submit the manuscript for publication.

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