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Efficacy of a probiotic supplement in patients with atopic dermatitis: a randomized, double-blind, placebo-controlled clinical trial

Background: Atopic dermatitis (AD) is a multifactorial long-standing inflammatory skin disease with a high incidence worldwide in both adults and children. According to the recognized correlation between skin and intestine–the so-called "gut–skin axis"–gut unbalances can affect skin by inducing systemic inflammation and triggering dermatological diseases such as AD. *Objectives:* To evaluate the efficacy of a food supplement containing selected strains of probiotics in ameliorating AD symptoms and skin conditions in adult volunteers. *Materials & Methods*: Eighty adult subjects showing mild-to-severe AD, skin dryness, desquamation, erythema and itching were enrolled in a randomized controlled trial to receive, for 56 days, a placebo or a mixture of lactobacilli (*L. plantarum* PBS067, *L. reuteri* PBS072 and *L. rhamnosus* LRH020). The latter was chosen according to the patients' production of post-biotic metabolites and B-group vitamins, anti-inflammatory and anti-oxidant capacity and anti-microbial activity. Clinical and instrumental dermatological evaluation was performed at T_{0d}, T_{28d} and T_{56d}, and then at T_{84d} (after a one-month wash-out). Inflammatory cytokine levels from skin tape stripping, sampled close to AD lesions at T_{0d} and T_{56d}, were also measured. *Results:* Subjects receiving the probiotic mixture showed an improvement in skin smoothness, skin moisturization, self-perception, and a decrease in SCORAD index as well as in the levels of inflammatory markers associated with AD at T_{28d}, with a positive trend up to T_{56d} which was maintained at T_{84d}. *Conclusion:* Administration of selected probiotic strains resulted in a fast and sustained improvement in AD-related symptoms and skin conditions.

Key words: adult subjects, atopic dermatitis, probiotics, randomized controlled trial, SCORAD

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Atopic dermatitis (AD) is an inflammatory, chronic, pruritic skin disease with a large scale of severity and a high rate of recurrence and infections due to scarring, which can seriously affect health-related quality of life [1]. AD-related symptoms are itching, redness, dry and scaly skin and recurrent eczematous lesions [2]. AD incidence has increased worldwide over the past several decades, and affects 60% of the population, especially children (60% in the first year and 85% before five years old); it typically clears during adolescence but may persist into adulthood [3]. The higher incidence in children derives from several factors such as the type of delivery, familiarity, Western diet with high glycaemic load and pollution [4, 5]. AD development is characterized by external stimuli (allergens), immune mechanisms (inflammatory cytokines) and alteration of gut and skin microbial community [6-8].

The gut microbiome can strongly influence the host immune system, providing protection against pathogens and triggering an immune protective response. A dysbiotic status can increase the possibility to develop an autoimmune and inflammatory response even in a distant body area such as the skin [9].

This correlation was described for the first time in 1930 with a theory addressing the inter-relationship between emotional states, intestinal microbiota and systemic skin inflammation: the well-known "gut-skin-brain axis" [10].

To date, the contribution of the gut microbiome to the adaptive immune system has been well characterized, and has been shown to involve the maintenance of homeostasis between effector T cells (Th1, Th2, and Th17) and regulatory T cells [11].

AD is known to initiate with Th2, Th22 and Th17 cell activation (acute phase), before chronicity, defined by the onset of a Th1 cell response alongside the continued activation of Th2 and Th22 cells [12]. This mechanism could be explained by the presence of a positive feedback loop made by TSLP (thymic stromal lymphopoietin), IL-4 and IL-13. TSLP, produced by keratinocytes, drives Th2 polarization and activates dendritic cells, while IL-4 and IL-13 act on keratinocytes to further increase TSLP level [13, 14]. Thymus and activation-regulated chemokine (TARC) levels also increase in the stratum corneum of skin lesions of AD patients, which is correlated with disease severity, especially with erythema, oedema/papules, and oozing/crusts.

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These two cytokines have been used as indicators of skin inflammation in AD lesions [15, 16].

To counteract AD, several treatments exist, for example, palliative care such as the daily use of emollients to reduce trans epidermal water loss, while others are prescribed to cure and prevent recurrence, such as a wide range of corticosteroid treatments [17]. For severe AD treatment, a recent antibody has been approved, Dupilumab, which is an injectable human lgG4 monoclonal antibody that inhibits IL-4 and IL-13 cytokine responses, and has provided remarkable results [18]. However, its use is still debated regarding its suitability as a routine treatment. Although anti-inflammatory drugs are considered as the first pharmacological treatment, in recent years, concerns have arisen due to the high incidence of side effects [19]. In addition, corticosteroids are not feasible for delicate parts of the body, such as the eyelids, and are discouraged in childhood [20].

In this context, the modulation of the gut microbiome, which may positively affect the skin, provides a safe and innovative approach to the disease, thus probiotics may be administered to alleviate AD symptoms and prevent recurrence [21].

Although several studies have been carried out to evaluate the efficacy of probiotics on AD, the majority have focused only on analysis of SCORAD and the gut microbiota. Furthermore, most of the clinical trials were performed over an extended period [22, 23].

The objective of this study was to evaluate the clinical effect of oral intake of a combination of three probiotic strains *(L. plantarum* PBS067, *L. reuteri* PBS072 and *L. rhamnosus* LRH020) as a treatment for AD symptoms in adult subjects based on analysis of efficacy as an emollient and a hydrating and soothing agent.

Materials and methods

Human volunteers and study design

This single-centre, randomized, double-blind, placebo-controlled, parallel group study was carried out at Complife Italia Srl facilities in compliance with the Helsinki Declaration (1964) and its amendment. The study protocol and informed consent form were approved by the "Independent Ethical Committee for Non-Pharmacological Clinical studies" (Genova, Italy). All subjects provided written informed consent before starting the study.

Eighty subjects of both sex, aged between 18 and 50 years, with mild-to-moderate AD based on the SCORAD index, were enrolled according to a list of inclusion and exclusion criteria (*see table 1*), and assigned equally to two groups (40 subjects in the active group and 40 in the placebo group), according to a previously prepared randomization list, to receive one capsule/day of food supplement or placebo for 56 days.

Composition of the food supplement containing the probiotic mix was as follows: 1×10^9 CFU *L. plantarum* PBS067, 1×10^9 CFU *L. reuteri* PBS072 and 1×10^9 CFU *L. rhamnosus* LRH020, excipients such as corn starch (26 mg) and vegetable magnesium stearate (1 mg). The placebo composition was as follows: 99 mg corn starch and 1 mg vegetable magnesium stearate. Capsules of the probiotic mixture and placebo were supplied by Roelmi HPC Srl (Italy).

Volunteers were also supplied with a moisturizing cream, to be used throughout the study period, instead of their usual body cream.

Clinical visits were scheduled as follow: initial visit (T_{0d}), intermediate (T_{28d}) and final visit (T_{56d}); moreover, a follow-up visit was planned 28 days after the last administration (T_{84d}).

During the visits, a clinical assessment of safety as well as clinical and instrumental evaluation of the selected parameters and skin tape strippings for the analysis of inflammatory cytokines were carried out. Furthermore, a self-assessment questionnaire about the perceived efficacy of the treatment was completed by the volunteers at both T_{56d} and T_{84d}.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
Good general health
Female or male subjects
Age between 18 and 50 (±2) years old
Phototype I to IV
Mild to moderate AD (SCORAD score between 15 and 25)
Subjects who have not been recently involved in any other similar study
Willingness to follow the proposed alimentary treatment over the entire study period
Willingness to use only the cream consigned at the beginning of the study for body care
Willingness to provide images before and after the study
Willingness to use the product to be tested only over the study period
Willingness to not use products likely to interfere with the product to be tested
Willingness to not vary normal daily routine (*i.e.* lifestyle, physical activity, *etc*.)
Using effective contraception (oral/not oral), not expected to be changed during the trial
Aware of the study procedures and having signed an informed consent form
Exclusion criteria
Current antibiotic administration
Known history of chronic medical condition such as congenital heart disease, liver or kidney disease, or immune deficiency
Treatment with probiotics within the six months preceding enrolment
Treatment with systemic steroids and antihistamines within the three months prior to enrolment
Topical treatment with immunomodulators (tacrolimus or pimecrolimus) within the three months prior to enrolment
Acute or chronic infectious diseases
Pre-existing hypersensitivity to components contained in the probiotic
Not meeting the inclusion criteria
Pregnant or intending to became pregnant during the study
Breastfeeding
Use of sun-beds or self-tanning products one month before the study or intention to use these during the present study
Any condition that the principal investigator deems inappropriate for participation
Adult protected by the law (under guardianship, or hospitalized in a public or private institution, for a reason other than research, or incarcerated)
Volunteer unable to communicate or cooperate with the investigator due to language problems, poor mental development, or impaired cerebral function.

Table 2. Clinical classification of skin smoothness.

Level of skin smoothness	Score
Not smooth	1
A little smooth	2
Smooth	3
Clearly smooth	4

Assessment of clinical effects

All clinical parameters were assessed by a dermatologist under controlled room conditions (T = 22 ± 2 °C and RH = 40-60%) after 15-20 minutes of acclimatization. AD severity was evaluated according to the SCORAD (SCORing AD) index (as developed by the European Task Force on Atopic Dermatitis [ETFAD]). Skin smoothness was clinically evaluated in accordance with the clinical scores presented in *table 2*. Skin moisturization was measured according to the Corneometer® method, using Corneometer® CM 825 (Courage + Khazaka, electronic GmbH). Trans epidermal water loss (TEWL) was measured indirectly using a Tewameter® TM 300 (Courage + Khazaka, electronic GmbH). Skin reactions, both physical (erythema, oedema, dryness and desquamation) and functional (tightness, itching and burning), were scored according to a clinical 5-point scale (0 = none; 1 = very mild; 2 = mild; 3 = moderate; 4 = severe) and were evaluated each time a sign (physical or functional) appeared (*i.e.* a new sign or a sign that had worsened relative to previous evaluation), and its causality with the tested products was investigated.
Subjects were asked to express their personal opinion on the treatment by answering a questionnaire about product acceptability and efficacy at the end of the treatment period and at the end of the follow-up period.

Digital macrophotography and skin tape stripping

Images of the skin areas affected by AD were acquired from each subject at T_{0d}, T_{28d}, T_{56d}, and T_{84d} using a professional digital reflex camera (NIKON D300 digital camera).
Skin tape strippings were sampled using Corneofix® (Courage + Khazaka) under temperature, relative humidity and pressure-controlled conditions: 10 consecutive strips placed close to the area selected for clinical and instrumental evaluation were collected. Skin strippings were stored at -80 °C until extraction (phosphate buffer solution + triton 1%), and cytokines, TNF-alpha, TARC and TSLP, were determined using commercially available kits for ELISA (RayBio® Human TSLP ELISA, RayBio® Human TARC [CCL17] ELISA, BosterBio Human TNF-Alpha ELISA).

Statistical analysis

Descriptive analysis

Data were summarized using frequency distributions (number and percentage) for categorical/ordinal variables. For continuous variables, the following values were calculated: mean value, minimum value, maximum value, standard error of the mean (SEM), individual variation/individual percentage variation, mean variation/mean percentage variation. All calculations were performed using a Microsoft® Excel 2013 (vers. 15.0.4885.1001; Microsoft, USA) worksheet with Microsoft® Windows 8.1 Professional (Microsoft, USA).
The results of the self-assessment questionnaire were calculated as percentage (%) of subjects who selected a given answer. For each question, the number of subjects who selected a particular answer was counted (number of subjects) and this was then divided by the total number of subjects (percentage of answers).

Table 3. Demographic and baseline characteristics.

	Probiotic Mix	Placebo
Sex		
Male	7	5
Female	33	35
Age (±SEM)	39 ± 1.8	38 ± 1.4
SCORAD (±SEM)	20.9 ± 0.5	19.7 ± 0.4

SEM: standard error mean.

Statistical methods

The instrumental data were submitted to an ANOVA test followed by a Tukey-Kramer post-test (intra-group analysis); the inter-group statistical analysis was performed on the data variations versus T_{0d} by means of the Bilateral Student's t-Test for unpaired data. The clinical data were analysed using the Mann-Whitney U / Wilcoxon Rank-Sum Test (two samples).
Statistical analysis were performed using NCSS 10 statistical software (NCSS, LLC. Kaysville, Utah, USA) running on Windows Server 2008 R2 Standard (Microsoft, USA).
A p value <0.05 was considered statistically significant for all analyses.

Results

The clinical study was carried out from November 2018 to February 2019. Treatments with food supplement and placebo were well tolerated by all subjects and no adverse events were reported during the study period; furthermore, there were no drop-outs. There were no significant differences between the probiotic and placebo group with regards to any of the baseline characteristics including age, gender and initial SCORAD score (*table 3*), confirming the unbiased randomization.
Treatment resulted in an overall improvement in SCORAD index in both groups, however, a statistically significant and progressive decrease in SCORAD index was measured for the food supplement group throughout the administration period (from 20.9 ± 0.5 at T_{0d} to 16.9 ± 0.5 at T_{28d} and to 13.7 ± 0.6 at T_{56d}), moreover, improvement in SCORAD index remained favourable after one month of discontinuation of product (14.8 ± 0.6 at T_{84d}).
A slight, but not significant, decrease in SCORAD index was also appreciated in the placebo group, but the food supplement resulted in significantly lower SCORAD index throughout the treatment period compared to the placebo group ($p < 0.001$ vs baseline and vs placebo) (*figure 1*).

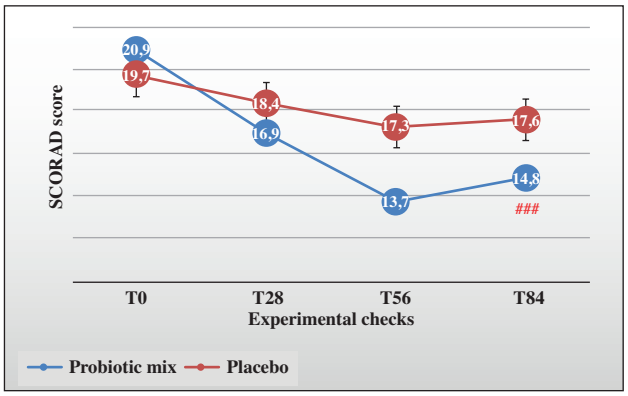

Figure 1. SCORAD score.

Table 4. Skin smoothness as percentage of improved subjects.

	T28	T56	T84
Probiotic Mix	57.5%***##	82.5%***##	77.5%***##
Placebo	15.0%	37.5% **	30.0%**

** Wilcoxon test $p<0.05$ vs T_0; ** Wilcoxon test $p<0.01$ vs T_0; *** Wilcoxon test $p<0.001$ vs T_0; ## Mann-Whitney U Test for Difference between active vs. placebo $p<0.01$*

Clinical evaluation of skin smoothness resulted in a similar profile: the food supplement group showed a progressive increment in percentage of subjects with amelioration of skin smoothness with respect to the beginning of the study (57.5% at T_{28d} and 82.5% at T_{56d}) and a sustained percentage (77.5%) at T_{84d}. Such improvement was statistically significant compared to baseline as well as to the placebo group (*table 4*).

An overall improvement in skin moisturization was also recorded, detected as a reduction in trans epidermal water loss (TEWL) and an increase in skin moisturization indexes, measured using a corneometer. TEWL showed a statistically significant reduction, compared to baseline, at T_{28d} (-9.5%) and T_{56d} (-19.3%), and this remained reduced at one month after the last intake of probiotics (-15,0%). Moreover, the decrease in TEWL observed in the food supplement group, from T_{28d} to T_{84d}, was statistically significant also with respect to the placebo group (*table 5*).

A similar trend was observed for skin moisturization: the probiotic group exhibited a progressive and significant increase in this parameter throughout the administration of the food supplement, an effect that was also evident one month later (22.9% at T_{28d}, 33.7% at T_{56d}, and 28.3% at T_{84d}); a statistically significant difference ($p < 0.001$) with respect to the placebo group was appreciated at all time points during the study (*table 6*).

Digital macrophotography acquired throughout the study provided objective confirmation of clinical improvement of the skin affected by AD (*figure 2A-C*).

A non-invasive technique, such as skin tape stripping, was used to measure cytokine expression in areas of skin close to areas with AD lesions. With respect to the placebo group, TNF alpha, a marker of global inflammatory status, showed a progressive and statistically significant decrease at T_{28d} and T_{56d} (*table 7*), and levels of TARC and TSLP, specific inflammatory markers of AD lesions, were considerably lower at T_{28d} and T_{56d}. (*table 8, 9*).

With regards to AD symptoms recorded throughout the study, no differences between the two treatments were recorded for erythema and oedema, perhaps due to the emollient effect of moisturization cream used throughout the trial. However, a constant improvement in dryness, desquamation as well as tightness, itching and burning was observed in the probiotic group, whereas such symptoms were stable in the placebo group.

Moreover, there was almost a 90% positive response for tightness, skin desquamation and itch intensity at the end of the treatment (T_{56d}) and after one month of follow-up. Indeed, the self-assessment questionnaire revealed that 75% of subjects in the probiotic group judged their skin to be from "quite hydrated" to "well hydrated "after 56 days of treatment, and 60% of them reported the same answers 28 days after the last administration. In the placebo group, such figures were respectively, 35% and 28%.

This persisting positive effect was also perceived for itching. In the probiotic group, 70% of subjects judged the decrease in itching to be from "good" to "very good", while the remaining 30% perceived this effect as "sufficient" at T_{56d}. When interviewed after the follow-up period, 85% of subjects declared that this effect was maintained, from

Table 5. Transepidermal water loss (TEWL).

	T_0	T_{28}	T_{56}	T_{84}
Probiotic mix	14.6 ± 1.3	13.2 ± 1.1 (-9.5%) *##	11.6 ± 1.0 (-19.3%) ***###	12.0 ± 1.0 (-15.0%) ***###
Placebo	12.8 ± 1.1	12.2 ± 0.9 (-3.1%)	11.7 ± 0.9 (-6.0%) **	12.0 ± 0.9 (-2.8%) ***

The % variation vs. T0 (i.e. $[T_x\text{-}T_0]/T_0$) is presented in brackets.
** Tukey-Kramer's $p<0.05$ vs T_0; ** Tukey-Kramer's $p<0.01$ vs T_0; *** Tukey-Kramer's $p<0.001$ vs T_0. bilateral Student's t-test for unpaired data (based on data variation versus T_0) active vs placebo: $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$*

Table 6. Skin moisturization.

	T_0	T_{28}	T_{56}	T_{84}
Probiotic mix	23.1 ± 0.9	28.5 ± 1.3 (22.9%) ***###	30.8 ± 1.3 (33.7%) ***###	29.4 ± 1.2 (28.3%) ***###
Placebo	24.7 ± 1.1	26.0 ± 1.2 (5.5%)	27.5 ± 1.3 (11.5%) ***	27.0 ± 1.3 (9.9%) ***

The % variation vs. T_0 (i.e. $[T_x\text{-}T_0]/T_0$) is presented in brackets.
**** Tukey-Kramer's $p<0.001$ vs T_0; $^{\#\#\#}$ bilateral Student's t-test for unpaired data (based on data variation versus T_0); active vs placebo $p<0.001$*

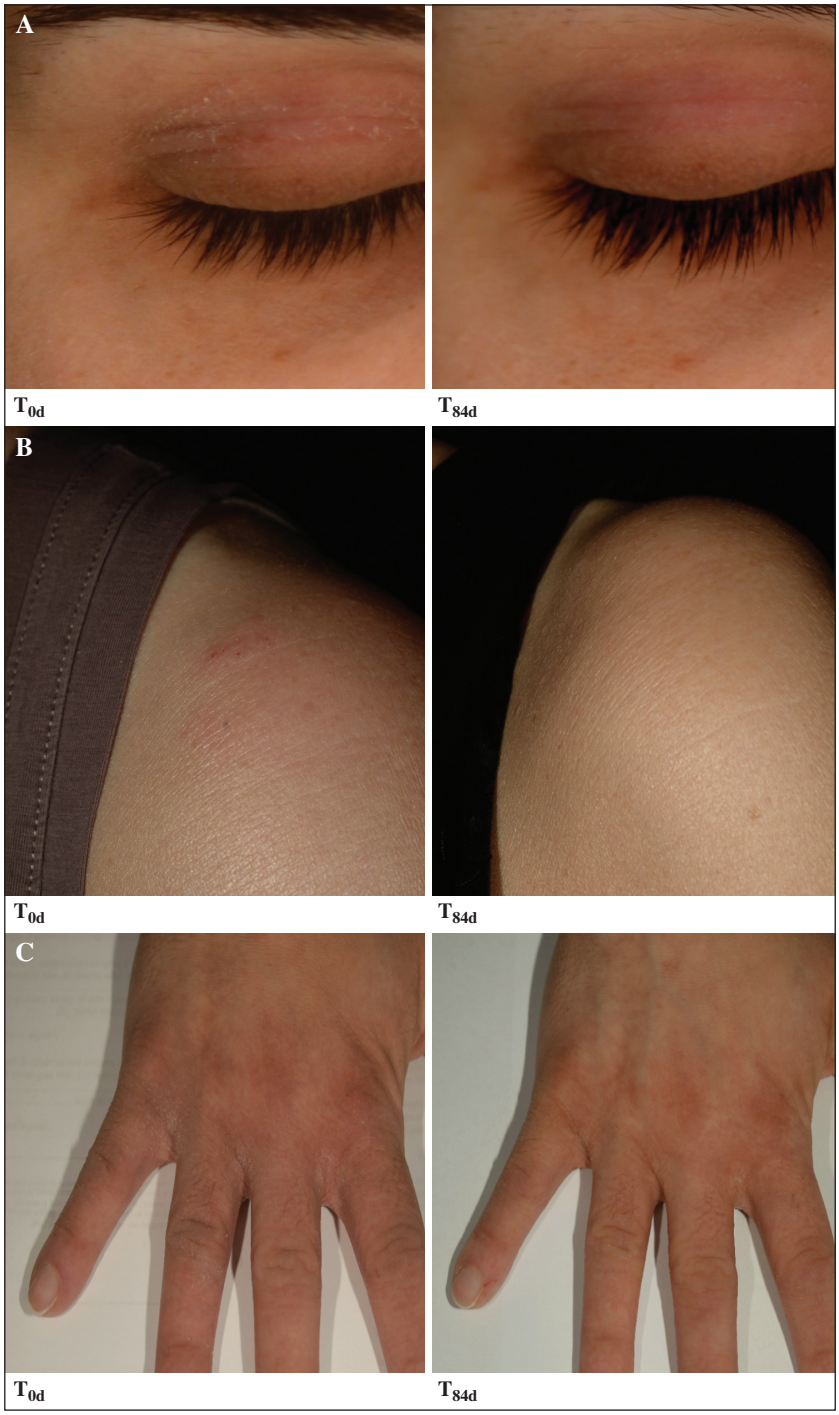

Figure 2. (**A**: T_{0d}-T_{84d}; **B**: T_{0d}-T_{84d}; **C**: T_{0d}-T_{84d}). Representative macrophotographs showing the efficacy of the probiotic mixture in ameliorating AD symptoms on three different subjects (**A-C**).

Table 7. Inflammatory cytokines: TNF-alpha (pg/ml).

	T_0	T_{28}	T_{56}
Probiotic mix	108.2 ± 9.3	82.5 ± 8.3 (-23.6%) #	72.7 ± 6.1 (-32.0%) ###
Placebo	111.1 ± 4.0	105.9 ± 4.1 (-3.0%)	105.3 ± 3.6 (-3.5%)

The % variation vs. T_0 (i.e. $[Tx-T_0]/T_0$) is presented in brackets.
bilateral Student's t-test for unpaired data (based on data variation versus T_0) active vs placebo: # $p<0,05$; ### $p<0,001$

Table 8. Inflammatory cytokines: TARC (pg/ml).

	T_0	T_{28}	T_{56}
Probiotic mix	23.8 ± 0.2	22.5 ± 0.2 (-5.6%) ##	22.3 ± 0.1 (-6.2%) ###
Placebo	23.1 ± 0.1	22.5 ± 0.2 (-2.5%)	22.7 ± 0.1 (-1.6%)

The % variation vs. T_0 (i.e. $[T_x\text{-}T_0]/T_0$) is presented in brackets.
bilateral Student's t-test for unpaired data (based on data variation versus T_0) active vs placebo: ## $p<0.01$; ### $p<0.001$

Table 9. Inflammatory cytokines: TSLP (pg/ml).

	T_0	T_{28}	T_{56}
Probiotic mix	28.9 ± 0.5	25.6 ± 0.4 (-11.1%) ###	25.9 ± 0.4 (-10.1%) ###
Placebo	27.9 ± 0.5	27.0 ± 0.5 (-3.4%)	27.4 ± 0.5 (-1.8%)

The % variation vs. T_0 (i.e. $[T_x\text{-}T_0]/T_0$) is presented in brackets.
bilateral Student's t-test for unpaired data (based on data variation versus T_0) active vs placebo: ### $p<0,001$

"yes enough" to "yes a lot", while the remaining 15% declared "a little".
In the placebo group, a decrease in itching sensation was perceived as "good" to "very good" by 38% and as "sufficient" by 45% of subjects after 56 days of treatment, and in terms of maintenance of the effect, this was judged as "yes enough" and as "a little" by 25% and 40% of subjects, respectively.

Discussion

The correlation between skin and gut microbiota is a common topic in the scientific community [24-26]. In recent decades, scientific studies evidenced that bacterial dysbiosis in the gut is often associated with the pathogenesis of many extra-intestinal disorders [27].
A large number of studies have explored the potential efficacy of probiotics in the prevention and treatment of AD [28-38], yet the picture remains unclear and conflicting; several clinical studies show improvement in the severity of AD after taking probiotics for a minimum of eight weeks, but strong evidence to support their effectiveness remains elusive [39, 40].
Given that the gut bacterial microbiota is very different in subjects with AD, dysbiosis seems to be an essential step in the occurrence of dermatitis. In fact, recent studies have shown the importance of the gut-skin axis which links the gut dysbiosis to skin inflammation. Essentially, gut dysbiosis can influence gut absorption, allowing toxins or inflammatory agents to enter the blood stream, and finally reach the skin. In 2001, the probiotic strain *Lactobacillus rhamnosus* GG was reported to reduce the incidence of AD in at-risk infants up to the age of seven years [29]. Another randomized, double-blind, placebo-controlled study investigated the effects of the use *of L. plantarum* CJLP133 strain in the prevention of AD symptoms. The study was performed for a time period of 12 weeks among children who were from one to 12 years old. An improvement in AD scores (SCORAD) was found, with a concomitant decrease in IFN-γ, eosinophils, and interleukin-4 [41].
The present study was carried out to demonstrate *in vivo* efficacy of a multi-strain probiotic complex to alleviate symptoms correlating with AD. The complex was composed of *L. plantarum* PBS067, *L. reuteri* PBS072 and *L. rhamnosus* LRH020. These proprietary strains were selected, based on *in vitro* screening, for their remarkable performance in modulating inflammatory status and improvement in cellular antioxidant potential; they were also shown to be the most effective at inhibiting the growth of AD-related skin pathogens, such as *S. aureus* and *S. epidermidis* (data not shown). Moreover, these strains can themselves produce B-group vitamins (B7, B9, B12), which are recognized to contribute to the maintenance of normal skin [42].
Results obtained in the present clinical study confirm that the *in vitro* activities exhibited by such selected strains could have an important role in ameliorating AD, due to their ability to produce active compounds that can reach distal sites through the circulation and carry out beneficial activity. In the same way, probiotics can migrate and reach other body areas where they exert a positive global effect through the production of antimicrobial compounds and enhancement of anti-inflammatory cytokines and antioxidant enzymes [43].
Administration of the multi-strain probiotic complex to subjects affected by mild-to-moderate AD resulted in a statistically significant decrease in SCORAD index. This decrease was already observed after the first four weeks of probiotic administration, was maintained throughout the period of intake, and lasted for more than a month during follow-up. This decrease in SCORAD is in agreement with other studies using probiotics to counteract AD symptoms [25, 44-47].
Generally, the overall decline of cutaneous moisturization in AD is related to impairment of skin barrier integrity [26]. Skin moisturization improved during the whole treatment, and volunteers in the active group presented with clear evidence of skin restoration, showing an enhancement of all the tested parameters: TEWL, which was significantly reduced in the active group compared to the placebo group, and the level of skin moisturization, which was increased starting from the first month of treatment, maintaining a positive trend up to T_{84d} (one month of follow-up). This outcome is evidence of the beneficial effect of the probiotic complex on the maintenance of skin moisturization, contributing to a reinforced skin barrier [48].

An improvement in skin smoothness is strongly linked to skin evenness. In this context, such improvement is essential in the general aesthetic evaluation of skin. The remarkable results obtained with the probiotic treatment are evident based on, not only clinical analysis, but also through comparison of digital photography taken at the beginning and the end of the treatment, and after the follow-up period.

AD is considered a primarily T cell-driven disease with a skewed response that leads to activation of T helper 2 (Th2) cells and an alteration of the innate immune system. In this regard, analysis performed on the skin of volunteers showed a reduction in pro-inflammatory cytokines TNF-alpha, TARC and TSLP, involved in the allergic immune response, during the eight-week period of probiotic treatment.

The selected strains provided a consistent positive trend regarding AD symptoms over time, while symptoms remained relatively stable in the placebo group. Alongside the dermatologist's evaluation, the self-assessment questionnaire revealed an overall amelioration of AD-related symptoms, with even better results during the follow-up period, especially regarding itching and skin hydration. A possible explanation for this long-term effect can be linked to the proven gastro-intestinal colonization of the selected strains and their persistence after one month from the end of intake, as reported by Mezzasalma *et al*. [49].

In summary, administration of the probiotic mixture of *L. plantarum* PBS067, *L. reuteri* PBS072 and *L. rhamnosus* LRH020 resulted in an amelioration of AD-related symptoms in an adult population. Even though adults represent a small percentage of AD patients overall, relative to the paediatric population, this result remains meaningful.

The probiotic treatment resulted in a statistically significant improvement throughout the whole period, and highest levels of improvement were detected soon after four weeks of food supplement intake. The sustained clinical improvement recorded at the end of probiotic intake and even during the follow-up period suggests that different treatment protocols (prolonged period, cyclic treatment, *etc*.) could achieve even better results, and considerably alleviate AD symptoms and prevent recurrence of the acute phase. To the best of our knowledge, this is the first clinical study assessing probiotic efficacy against AD symptoms with fast recovery and long-term performance, using a broad set of clinical and instrumental parameters beyond the traditional SCORAD index.

Probiotic intake in AD is an interesting intervention, since it is directed not to the symptoms, but rather to the cause of the disease (intestinal dysbiosis). Thus, overall more effective and long-lasting improvement can be achieved, as indicated by this clinical trial. To date, in the context of the gut-skin axis theory, there are now new options for clinicians with attractive, novel, well-tolerated treatments, thus overcoming the historical division between dermatology and gastroenterology [43]. Modulation of the intestinal microbiota by strain-specific probiotics could thus be a basis to manage skin diseases. Treatment focused on gut dysbiosis could help relieve distress related to skin disorders and may be complementary to standard dermatological therapy [50].

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