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Effect of an oral probiotic formula on scalp and facial skin condition, glucose, and lipid metabolism

Peng Yu[1], Xiaoyue Teng[2], Tingzhu Liu[3], Yuhan Li[3], Jiayi Ni[4*], Siliang Xue[2*], Juan Wang[3*]

[1]School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, China; [2]Department of Dermatology, West China Hospital of Sichuan University, Sichuan, China; [3]Memoo (Beijing) Technology Co. Ltd., Beijing, China; [4]Sprim (China) Consulting Co. Ltd., Shanghai, China† Peng Yu and Xiaoyue Teng contribute equally to this work.

***Corresponding Authors:** Siliang Xue, Department of Dermatology, West China Hospital of Sichuan University, Chengdu, Sichuan, China; Juan Wang, Memoo (Beijing) Technology Co. Ltd., Beijing, China

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ABSTRACT

Objective: We conducted this study to explore the effect of oral probiotic supplementation on hair density as a primary outcome in subjects with hair loss and at high risk of metabolic syndrome. The secondary objectives were to assess probiotic effects on skin barrier function,metabolic health and stress responses.

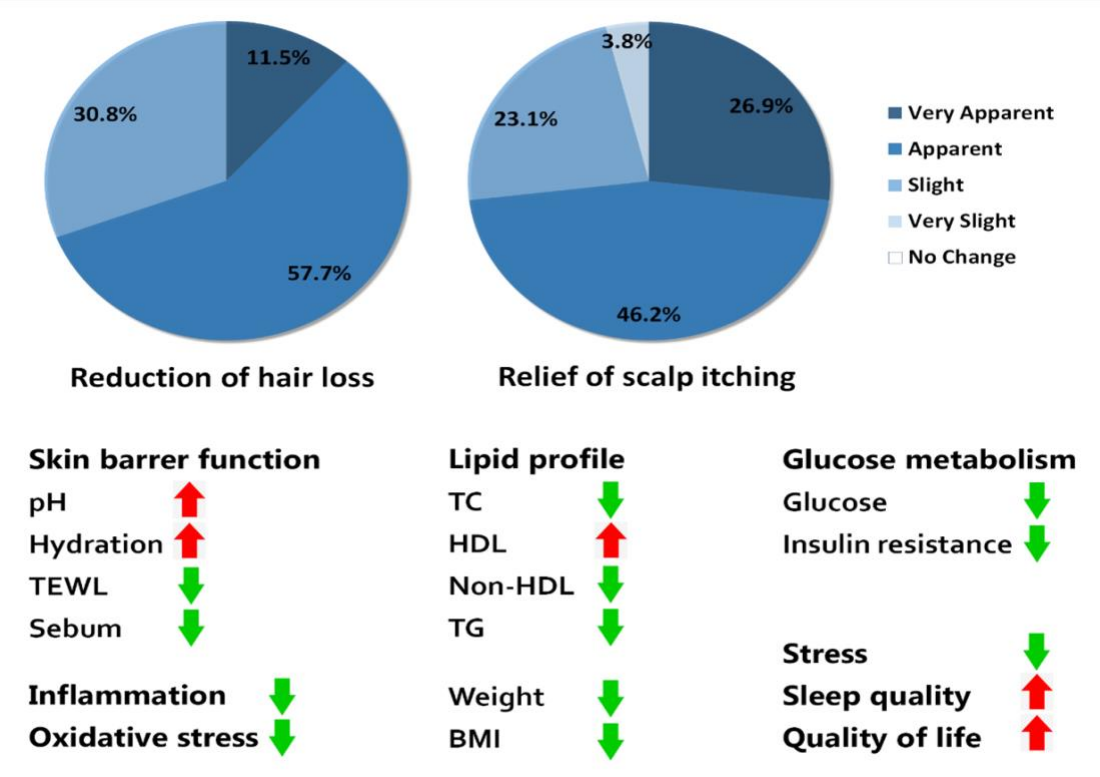
Methods: We supplemented the diets of Chinese adults presenting with hair loss and high risk of metabolic syndrome (n = 26, male gender 38.5%, age = 33.6 ± 4.5 years) with a multi-strain probiotic formula at a dosage of 18.1 billion colony forming units (CFU) twice daily for 12 weeks. We compared the hair density, hair loss, anthropometrics measures, blood biochemistry markers, skin biophysical characteristics and stress-associated responses between baseline and the end of the trial.

Results: After 12 weeks of probiotic supplementation, 96.2% of the study participants had improvement in hair density (median density level increased: 1; interquartile range: 1-2). Participants reported reduced hair loss both quantitatively and qualitatively. The majority (73.1%) of the participants reported apparent relief of scalp itching. Stratum corneum hydration and pH increased, while transepidermal water loss and sebum decreased on both scalp and facial skin. Body weight and body mass index decreased following probiotic consumption. Most components of glucose metabolism and the lipid profile were significantly better, with increases in high-density lipoprotein cholesterol and reductions in glucose, homeostasis model assessment-estimated insulin resistance, total cholesterol, non-high-density lipoprotein cholesterol

and triglycerides. Inflammation and oxidative stress markers improved with increases in interferon-γ and superoxide dismutase, and reductions in high-sensitivity C-reactive protein, interleukin-6, interleukin-31 and malondialdehyde. No changes were observed in glycated hemoglobin, insulin, immunoglobulin E and interleukin-10 levels. Besides, perceived stress relieved in participants accompanied with improved sleep quality as well as better overall perception of life quality and health.

Conclusion: Twice-daily supplementation with the test probiotic formula over a 12-weeks period may exert profound beneficial effects on hair growth, skin condition, glucose and lipid metabolism, and stress-associated psychological and physiological responses in participants presenting with hair loss and high risk of metabolic syndrome.
This study has been registered at Chinese Clinical Trial Registry (ChiCTR2100050498).

Keywords: hair density, hair loss, metabolic syndrome, oral probiotic supplementation



INTRODUCTION

Not only are probiotics considered beneficial to digestive health, but increasing evidence suggests a continually functioning interaction between gut microbiome, the brain, and the skin [1–4]. There is substantial evidence linking various gut microbiota and local immunity networks with systematic effects on the immune system [5–7]. The gut-brain-skin axis has been used to explain the relationship between gut microbiota, emotional states, and local and systemic inflammation. According to this theory, a person's emotional states, such as stress or anxiety, can cause gut dysbiosis which subsequently resulting in a generalised inflammation [8]. Excessive

inflammation plays critical roles in the relationship between stress and stress-related diseases, such as insulin resistance, diabetes, hypertension and depression. Besides, stress-induced inflammation in the skin may premature induction of hair follicle and inhibit hair growth [9]. One of the remedies could be using probiotics to restore a balanced gut environment. By such means, excessive inflammation and the onset of diseases could be prevented through reducing the sensitivity to infections, maintaining immune tolerance and protecting tissue barrier function [10,11].

To date, the intervention strategies using probiotics have been largely studied. Increasing evidence supporting the claims of beneficial effects has been attributed to probiotics. Some of the most studied effects include improving gastrointestinal health [12–14], enhancing immune reaction [15–17], and improving metabolic health [18–20]. More recently, great interest has been raised by the possible use of ingested probiotics to modulate the immune system of the skin [21–23]. While the potential skin benefit of such probiotic strategies is generally acknowledged, its actual efficacy in clinical practice and the underlying mechanisms of these effects require further exploration.The probiotic formula investigated in the present study is a combination of Bifidobacterium (B) lactis Bi-07, Lactobacillus (L) acidophilus NCFM, B. lactis HN019, L. rhamnosus HN001 and Lacticaseibacillus (L) paracasei Lpc-37. Each of the strains used has been studied alone or in combination with some other strains and have well established health benefits. The first four strains have been shown to provide immunity enhancing in different age groups [15,24–26]. L. paracasei Lpc-37 has proven effect in preventing chronic stress-associated behaviors [27]. L. rhamnosus HN001 delivered positive effect on postnatal depression [28]. Previous studies have demonstrated that L. acidophilus NCFM and B. lactis HN019 improved metabolic health in patients with diabetes and metabolic syndrome [29,30]. Besides, intake of B. lactis Bi-07 alone could reduce the severity of atopic dermatitis [31]; when combined with L. acidophilus NCFM, the mixture helped relieving bloating symptoms [12]. Nevertheless, studies demonstrating their potential applicability in supporting hair and skin health are very limited. In addition, it has not been fully examined whether the combination of these strains has additive or synergistic effects.

In light of published prior evidence from the literature, we now wish to undertake a study to investigate the effect of this multi-strain probiotics formula on hair density as a primary endpoint, as well as on scalp and facial skin barrier functions, metabolic health and stress-associated responses.

MATERIALS AND METHODS

Study Design

Thirty subjects were enrolled to ensure at least 25 complete the study, accounting for a 15% potential attrition rate. All patients were administered a probiotic formula over the period of 12 weeks. The research practices of this study were in accordance with the Declaration of Helsinki. The Institutional Review Board of Shanghai Nutrition Society reviewed and approved this study. All subjects provided written informed consent prior to enrollment.

Study Product

The study product is a combination of B. lactis Bi-07 (1.2 billion colony forming units, CFU), B. lactis HN019 (4.2 billion CFU), L. acidophilus NCFM (1.2 billion CFU), L. rhamnosus HN001 (3.0 billion CFU) and L. paracasei Lpc-37 (8.5 billion CFU) at a total dose of 18.1 billion CFU. The study product was supplied in sachets (1.8 g per sachet) and were taken orally twice per day, one in the morning and one in the evening. At consumption, subjects took the contents of the sachet directly or with warm water half an hour after meal. All products were stored

refrigerated at the study site until time of use and were refrigerated by the subjects throughout the supplementation period.

Study Subjects

Eligible subjects included males and females aged 25-40 years, self-reported of hair loss and oily scalp, had impaired fasting blood glucose (6.1-6.9 mmol/L) [32] and borderline total cholesterol (5.2-6.1 mmol/L) [33], and experienced moderate or higher stress (score≥29) evaluated by the Perceived Stress Scale (PSS) [34]. Exclusion criteria were history of treatment for hair loss; current treatment for diabetes or gastrointestinal symptoms; present of active diarrhea; current use of pain relievers such as aspirin or paracetamol; use of laxatives or other supplements to improve digestive gastrointestinal function within two weeks before study entry; ongoing or recent use of antibiotics within three months prior to study entry; vaccination for upper respiratory tract infection within six months or other vaccination within 15 days prior to study entry; history of daily consumption of probiotics or prebiotic, fermented milk or yogurt; ongoing use of antihistamines medication, cough medication or high-dose vitamin C; alcohol or drug addiction; scalp organic lesions; and pregnant or breast-feeding women. Subjects were required to avoid the consumption of other fermented milk, yogurt and probiotic or prebiotic products, and to maintain their usual daily physical exercise habits.

Efficacy Evaluation

All efficacy outcomes were measured at baseline and after 12 weeks of probiotic consumption. The primary outcome was a visual scale for of hair density evaluated by expert assessors. The integer scale ranges from 0 – "no hair" to 7 – "Very high density with scalp almost invisible".

Self-reported number of lost hairs was used to evaluate product effect on hair loss. Subjects were instructed to pass their fingers through a small strand of dry hairs (approximately 100 hairs) right after combing and record the number of hairs that fell and left in their hands. Subjects also performed a cognitive assessment of the change in hair loss and scalp itching at the end of the study.

The skin condition was quantitatively evaluated by non-invasive bioengineering equipment, including those assessing stratum corneum hydration (SCH), transepidermal water loss (TEWL), skin surface pH and sebum. These parameters are widely used to evaluate skin barrier function [35,36]. All measurements were performed at two sites (one on the left and the other on the right) on both the scalp and the face using respective probes by the same investigator. SCH was measured using DermaLab USB (Cortex Technology, Hadsund, Denmark) on the scalp and Corneometer CM825 (Courage+Khazaka electronic GmbH, Koln, Germany) on the face. At each measurement, three readings were obtained, and the average of these readings was used for analyses. For both the scalp and the face, TEWL was measured by AquaFlux AF200 (Biox Systems Ltd, London, England), pH level was measured by Skin-pH-Meter PH905, and sebum was measured by Sebumeter SM815 (Courage+Khazaka electronic GmbH, Koln, Germany).

Body weight, height and body mass index (BMI) were measured using standard anthropometric techniques. Blood samples were drawn between 8 to 10 a.m. following an overnight fast of at least 12 hours to quantify glycemic status, lipid concentrations,

biomarkers of inflammation and oxidative stress. The biomarkers measured include glucose, hemoglobin A1c (HbA1c), insulin, high-density lipoprotein (HDL) cholesterol, total cholesterol (TC), triglyceride (TG), high-sensitivity C-reactive protein (hsCRP), malondialdehyde (MDA), superoxide dismutase (SOD), immunoglobulin E (IgE), interferon-γ (IFN-γ), interleukin-6, -10 and -31 (IL-6, IL-10 and IL-31), cortisol, and biotin. Glucose and insulin concentrations were used to estimate insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR) [37].

Participants completed the PSS questionnaire, the Pittsburgh Sleep Quality Index (PSQI) questionnaire, and the World Health Organization Quality of Life-BREF (WHOQOL-BREF) questionnaire to investigate the effects of the study formula on self-reported symptoms and perception of stress, sleep quality and quality of life. The Chinese language versions were used in all cases.

The PSS questionnaire consists 14 items intended to measure the degree to which individuals perceived their life circumstances as stressful within the last month [34]. Individuals rate items on a 5-point Likert scale, ranging from 0 – "Never" to 4 – "Very often". The total score was calculated by summing the scores of the 14 items.

The PSQI questionnaire assesses sleep quality over a one-month time interval. The measure contains 19 self-rated items generate seven component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction [38]. Each component score ranges from 0 to 3 points. The sum of scores for these seven components yields one global score with a range of 0 to 21 points, where 0 indicating no difficulty and 21 indicating severe difficulties in all areas.

The WHOQOL-BREF instrument comprises 26 items, which measures the broad domains including physical health, psychological health, social relationships, and environment [39]. The raw item scores in the questionnaire range from 1 to 5 and were summarized to areas scores of each domain, as well as two independent items "overall perception of quality of life" and "overall perception of health". The area scores were then transferred to 100 points scales.

Participants were instructed to record their daily food and beverage intake during the 3 days before the baseline and the last 3 days before the end of the study according to the food models and scales provided. The portion sizes were converted to grams and summarized by food categories. Physical activity in the past week of the baseline and in the last week of the study period were assessed using a continuous measure for metabolic equivalent of task (MET-minutes) derived using the algorithms provided by the Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) [40]. These data were collected to assess if there was a change in diet and exercise frequency.

Statistical Analysis

Demographic and baseline characteristics were summarized. Continuous outcome variables are reported as means ± SD or median (quartiles), and categorical outcomes are reported as n (%). Prior to testing, distributional assumptions for the outcomes were assessed and transformations or nonparametric versions of the tests were used if deemed necessary. The

differences between baseline and post-intervention outcomes was evaluated using paired t-test for normal distributed continuous outcomes and Wilcoxon signed ranks test for non-normal or ordinal outcomes. A mixed model was used to assess skin biophysical characteristics, accounted for multiple measurement sites. The number and percent of adverse events (AEs) and serious adverse events (SAEs) were summarized. All analyses were conducted for enrolled participants who consumed at least 1 dose of the study product. The significance level for statistical tests was set at 0.05. Statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Among the 30 participants who enrolled, four withdrew early before consumption of the test product for personal reasons. Thus, 26 participants completed all study procedures and were included in the analyses. Of these participants, mean age was 33.6 years (standard deviation, SD: 4.5 years) and 38.5% were men. All participants had moderate or higher stress level, impaired fasting glucose and borderline total cholesterol (Table 1). Baseline characteristics are similar between all enrolled participants and the participants who completed the study (Table S1). During the study, all participants complied with dietary restrictions and maintained similar diet (Table S2) and physical activity levels (Table S3) at baseline and end of study.

Table 1. Baseline characteristics

	Participants (n=26)
Male, n (%)	10 (38.5)
Age, year	33.6±4.5
Body weight, kg	73.5±17.2
Height, cm	165.5±8.7
Body mess index (BMI), kg/m^2	26.7±5.0
Body temperature, °C	36.4±0.3
Systolic blood pressure, mmHg	128.7±16.1
Diastolic blood pressure, mmHg	84.6±11.9
Pulse, time/min	78.3±9.6
Perceived Stress Scale score	43.4±6.4
Fasting blood glucose, mmol/L	6.6±0.2
Total cholesterol, mmol/L	5.5±0.2

Data are presents as mean ± standard deviation or frequency (%).

Visual Scale of Hair Density

More than half of the participants had fairly low hair density, 9 (34.6%) had moderate density, and 3 (11.5%) had low hair density with scalp easily visible at baseline. At the end of the study, 96.2% of the participants showed evidence of increase in hair density assess by the visual assessment (median density level increased: 1; interquartile range, IQR: 1, 2). The proportion of participants with fairly low or low hair density was markedly reduced. Instead, 61.5% of the participants showed fairly high-density hair with very little visiblescalp though hair according to the visual assessment (Table 2).

Table 2. Visual scale of hair density

	Baseline	Week 12
Hair density, n (%)		
0 No hair	0 (0.0)	0 (0.0)
1 Very low density with scalp clearly visible	0 (0.0)	0 (0.0)
2 Low density with scalp easily visible	3 (11.5)	0 (0.0)
3 Fairly low density with scalp visible	18 (53.9)	1 (3.9)
4 Moderate density with a little scalp visible	9 (34.6)	8 (30.8)
5 Fairly high density with very little scalp visible	0 (0.0)	20 (61.5)
6 High density with scalp indistinctly visible	0 (0.0)	1 (3.9)
7 Very high density with scalp almost invisible	0 (0.0)	0 (0.0)
Improved hair density, n (%)	25 (96.2)	
Difference of week 12 vs. baseline, median (Q1, Q3)	1 (1, 2), p<0.001	

Q1, the first quartile; Q3, the third quartile.
Data are presented as frequency (%). The differences of post-intervention versus baseline scales were presented as median change (Q1, Q3) of the scales and evaluated using Wilcoxon signed ranks test.

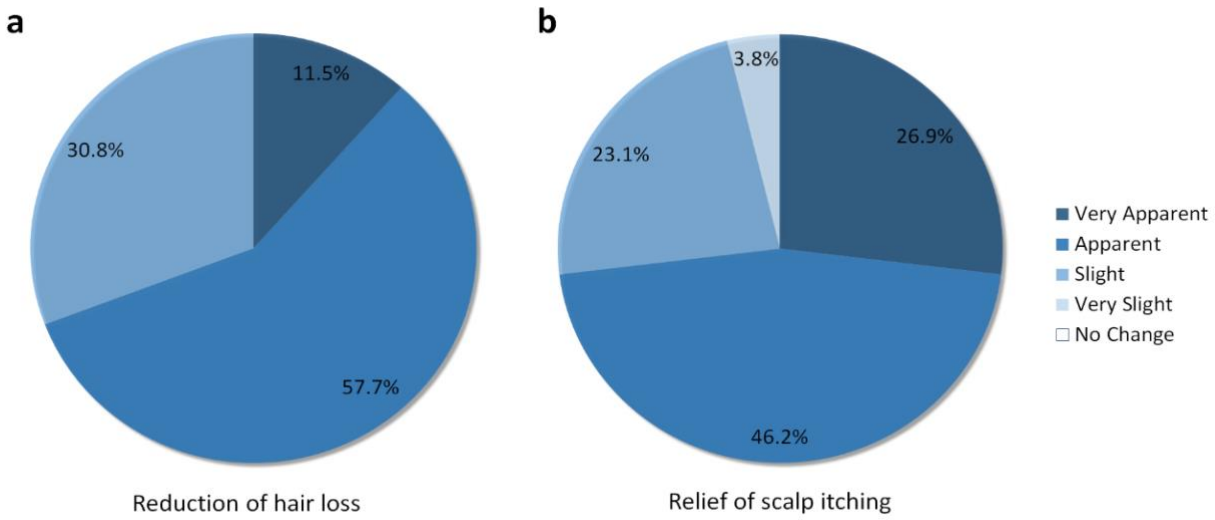


Figure 1. Self-reported reduction of hair loss and relief of scalp itching. a. Reduction of hair loss (%). b. Relief of scalp itching (%).

Skin Biophysical Characteristics

There were significant increases in the scalp pH (mean change: +0.4; 95% confidence interval, CI: 0.3, 0.5) and hydration level (+3.2 µS; 0.3, 6.1), as well as significant decreases in the scalp TEWL (-5.6 g/m^2·h; -9.1, -2.1) and sebum (-36.0 µg/cm^2; -42.9, -29.1) after product intervention (Figure 2). There were similar changes from baseline in the facial skin biophysical characteristics, with mean changes of +0.3 (95% CI: 0.2, 0.4) in pH, +7.2 µS (4.1, 10.3) in hydration, -6.1 g/m^2·h (-9.5, -2.8) in TEWL, and -53.4 ug/cm^2 (-64.6, -42.3) in sebum (Figure 3).

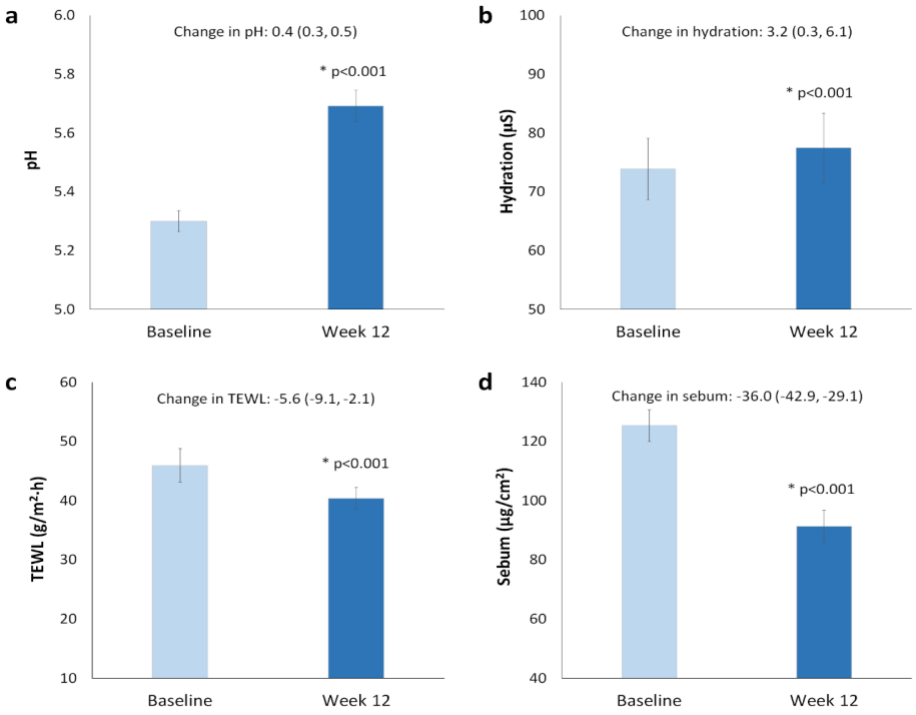


Figure 2. Biophysical characteristics of the scalp. A. pH (Mean ± SE). b. Hydration (Mean ± SE). c. Transepidermal water loss (Mean ± SE). d. Sebum (Mean ± SE). Changes are presented as mean (95% confidence interval). *: significant different compared to baseline measurements by mixed model. Abbreviations: TEWL, transepidermal water loss; µS, microSiemens.

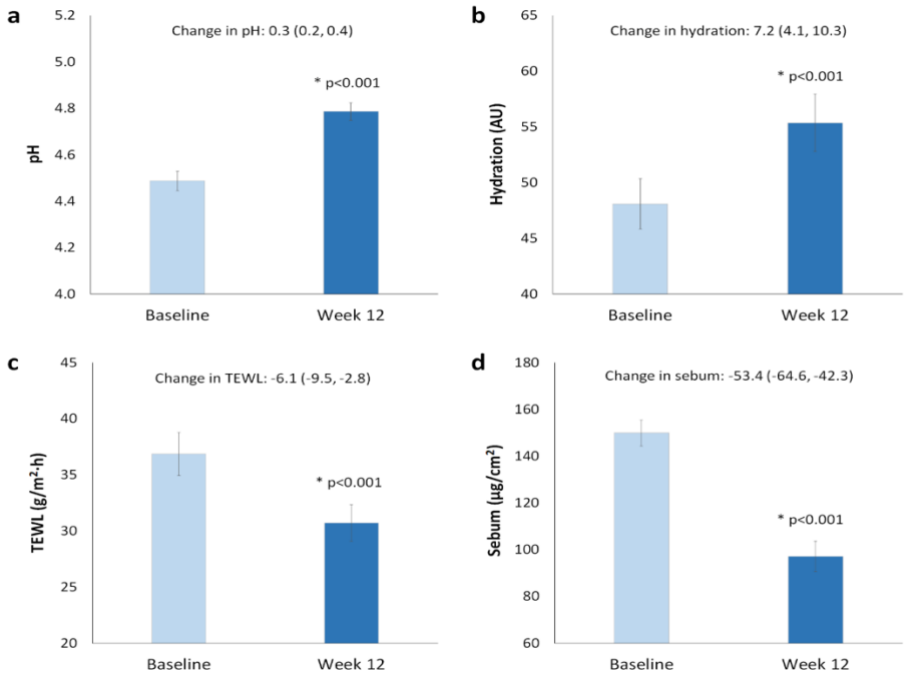


Figure 3. Biophysical characteristics of the facial skin. A. pH (Mean ± SE). b. Stratum corneum hydration (Mean ± SE). c. Transepidermal water loss (Mean ± SE). d. Sebum (Mean ± SE). Changes are presented as mean (95% confidence interval). *: significant different compared to baseline measurements by mixed model. Abbreviations: TEWL, transepidermal water loss; µS, microSiemens.

Anthropometric and Blood Biomarkers

Both body weight (mean change: -0.87 kg; 95% CI: -1.55, -0.18) and BMI (-0.31 kg/m^2; -0.58, -0.05) significantly decreased in response to probiotic consumption. There were significant reductions in serum level of cortisol (-42.1 nmol/L; -80.0, -4.2), glucose (-0.6 mmol/L; -0.8, -0.4), HOMA-estimated insulin resistance (-0.7; -1.1, -0.1),TC (-0.6 mmol/L; -0.8, -0.3), non-HDL-cholesterol (-0.7 mmol/L; -0.9, -0.5), TG (-0.2 mmol/L; -0.3, -0.1), hsCRP (-0.2 mg/L; -0.4, -0.1), IL-6 (-0.3 pg/ml; -0.5, -0.1), IL-31 (-0.5 pg/ml; -1.0, -0.04) and MDA (-0.9 nmol/ml; -1.2, -0.5). Besides, probiotic consumption resulted in significant increases in serum biotin (+0.3 ng/ml; 0.2, 0.5), HDL (+0.1 mmol/L; 0.02, 0.2), IFN-γ (+22.2 pg/ml; 12.9, 31.6) and SOD (+17.0 nmol/ml; 10.5, 23.5). There was no significant change in HbA1c, insulin, IgE and IL-10 levels (Table 3).

Table 3. Anthropometric and blood biomarkers

Outcomes	Baseline	Week 12	Week 12 vs. Baseline	
			Difference	p-value
Body weight, kg	73.5±17.2	72.7±16.8	-0.87 (-1.55, -0.18)	0.015
BMI, kg/m^2	26.7±5.0	26.4±4.9	-0.31 (-0.58, -0.05)	0.023
Cortisol, nmol/L	370.1±144.0	328.1±88.1	-42.1 (-80.0, -4.2)	0.031
Biotin, ng/ml	0.4±0.1	0.7±0.2	0.3 (0.2, 0.5)	<0.001
Glucose metabolism markers				
Glucose, mmol/L	6.6±0.2	6.0±0.5	-0.6 (-0.8, -0.4)	<0.001
Glycated hemoglobin [a], n (%)	6.2 (6.0, 6.3)	6.1 (5.9, 6.2)	0.01 (-0.2, 0.1)	0.175
Insulin [a], µU/ml	15.9 (11.0, 28.5)	17.2 (11.4, 26.7)	-0.9 (-2.1, -0.15)	0.099
Insulin resistance, HOMA-IR	4.8 (3.2, 8.7)	4.3 (2.9, 7.3)	-0.7 (-1.1, -0.1)	<0.001
Lipids				
Total cholesterol, mmol/L	5.5±0.2	4.9±0.5	-0.6 (-0.8, -0.3)	<0.001
HDL-cholesterol, mmol/L	1.1±0.2	1.3±0.2	0.1 (0.02, 0.2)	0.014
Non-HDL-cholesterol mmol/L	4.3±0.2	3.7±0.5	-0.7 (-0.9, -0.5)	<0.001
Triglyceride mmol/L	2.0±0.3	1.7±0.2	-0.2 (-0.3, -0.1)	<0.001
Inflammation markers				
High-sensitivity CRP [a], mg/L	1.4 (0.8, 2.9)	1.1 (0.5, 1.9)	-0.2 (-0.4, -0.1)	0.001
Immunoglobulin, E IU/ml	19.5±2.9	19.4±3.4	-0.1 (-1.4, 1.3)	0.940
Interferon-γ, pg/ml	106.1±55.6	128.4±58.7	22.2 (12.9, 31.6)	<0.001
Interleukin-6, pg/ml	3.8±0.4	3.4±0.5	-0.3 (-0.5, -0.1)	0.003
Interleukin-10, pg/ml	22.8±1.0	22.5±1.0	-0.3 (-0.7, 0.2)	0.292
Interleukin-31, pg/ml	7.9±0.9	7.4±0.8	-0.5 (-1.0, -0.04)	0.036
Oxidative stress markers				
Malondialdehyde, nmol/ml	6.7±1.5	5.8±1.2	-0.9 (-1.2, -0.5)	<0.001
Superoxide dismutase, U/ml	127.9±13.7	144.9±20.7	17.0 (10.5, 23.5)	<0.001

BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; Q1, the first quartile; Q3, the third quartile.

Unless otherwise stated, data are presented as mean ± standard deviation. Differences between post-intervention and baseline measurements are presented as mean (95% confidence interval) and evaluated by paired t-test.

[a]·Data are presented as median (Q1, Q3). Differences between post-intervention and baseline measurements are presented as median (Q1, Q3) and evaluated by Wilcoxon signed rank test.

Self-reported Outcomes

PPS scores significantly decreased (-9.1 points; 95% CI: -12.6, -5.6) from baseline to end of study indicating a significant effect of the probiotic formula in reducing perceived stress. Participants reported a significant lower global PSQI score (-4.2 points; -5.1, -3.3) at end of study compared to baseline, indicating an improvement in the overall sleep quality. Probiotic consumption tended to improve subjective sleep quality and sleep duration, reduce sleep latency, sleep disturbances and daytime dysfunction, but have no effect on habitual sleep efficiency. In addition, participants reported a significant improvement in the overall perception of quality of life (+0.4 points; 0.1, 0.7) and perception of health (+0.5 points; 0.2, 0.8), as well as in the area of physical health (+10.3 points; 3.6, 17), psychological health (+9.8 points; 3.3, 16.3) and social relationships (+7.4 points; 2.0, 12.8). There was no significant change in the environment area score (Table 4).

Table 4. Self-reported outcomes

	Baseline	Week 12	Week 12 vs. Baseline	
			Difference	p-value
Perceived stress	43.4±6.4	34.3±6.8	-9.1 (-12.6, -5.6)	<0.001
Pittsburgh sleep quality index (PSQI)	11.9±2.8	7.7±3.2	-4.2 (-5.1, -3.3)	<0.001
Subjective sleep quality [a]	2 (2, 3)	1 (1, 2)	-1 (-1, 0)	<0.001
Sleep latency [a]	2 (1, 3)	1.5 (0, 2)	0 (-1, 0)	0.008
Sleep duration [a]	1.5 (1, 2)	1 (0, 1)	-0.5 (-1, 0)	0.001
Habitual sleep efficiency [a]	1 (0, 1)	0 (0, 1)	0 (-1, 0)	0.186
Sleep disturbances [a]	3 (2, 3)	1 (1, 2)	-1 (-1, -2)	<0.001
Use of sleeping medication [a]	0 (0, 0)	0 (0, 0)	0 (0, 0))	1.000
Daytime dysfunction [a]	2 (2, 3)	1 (1, 2)	-1 (-2, 0)	<0.001
Quality of life				
Overall perception of quality of life	3.2±0.5	3.6±0.6	0.4 (0.1, 0.7)	0.022
Overall perception of health	3.0±0.8	3.5±0.5	0.5 (0.2, 0.8)	0.001
Physical health (PHYS)	60.9±18.2	71.2±14.2	10.3 (3.6, 17.0)	0.004
Psychological (PSYCH)	58.0±13.4	67.8±13.5	9.8 (3.3, 16.3)	0.005
Social relationships (SOCIL)	59.6±13.5	67.0±14.0	7.4 (2.0, 12.8)	0.010
Environment (ENVIR)	58.4±11.88	61.1±13.4	2.6 (-2.3, 7.6)	0.279

Q1, the first quartile; Q3, the third quartile.

Unless otherwise stated, data are presented as mean ± standard deviation; the difference of post-intervention versus baseline total score was presented as mean (95% confidence interval) and evaluated using paired t-test. [a]Data are presented as median (Q1, Q3); the differences of post-intervention versus baseline item scores were presented as median (Q1, Q3) and evaluated using Wilcoxon signed ranks test.

Adverse Events

There were four adverse events during the study. The adverse events reported included dry skin (2), torticollis (1) and light injury (1). None of the adverse events were judged by the investigators to be related to the study product.

DISCUSSION

The growing awareness of intestinal microflora as a mediator in both emotional state and systemic low-grade inflammation has raised the interesting possibility that the addition of probiotic supplements might serve as an intervention to restore the composition of the gut microbiota, and thus to improve skin and hair conditions, strengthen barrier function, to support metabolic health, and to reduce stress responses. Because of the health benefits that probiotics offer, they are considered as a functional food by many. The Functional Food Center (FFC) defines functional foods as "Natural or processed foods that contain biologically-active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms'' [41]. The FFC goes through a systematic evaluation when identifying functional foods. For probiotics to be safely consumed as functional food products, it is important to understand the various mechanisms through which probiotics exert their action.

In the present study, all enrolled participants were administered an oral probiotic formula over a period of 12 weeks. Overall, 96% of the participants demonstrated beneficial effects in terms of the hair density assessed by the visual scale. All participants reported reduction of hair loss both quantitatively and qualitatively by self assessment. The mechanism of probiotics on hair growth is likely multifactorial. A recent clinical trial investigated the effect of probiotics from fermented food on alopecia and showed a 93% improvement in terms of thickness and hair count following probiotics consumption [42]. The authors proposed a possible mechanism that, intake of probiotics could lead to a beneficial effect on peripheral vascular blood flow and hence promote hair growth. This is because metabolic diseases such as hypercholesterolemia negatively affect microvascular function, which can be reversed by lipid-lowing therapy [43]. The participants of our study were at high risk of metabolic syndrome at baseline. Probiotics supplementation lowered serum glucose, insulin resistance, and improved most lipid markers. These actions might have improved microvascular function, which contributed to hair growth. However, such potential mechanism needs to be confirmed with a double-blind, placebo-controlled study including more participants.

A common link among hair growth and the components of metabolic syndrome may be related to inflammation, since these participants generally present in a pro-inflammatory state [44]. Our study found a significant increase of serum IFN-γ, which act as a master regulator of immune responses and inflammation [45,46]. We also detected significant reductions in the inflammation makers including hsCRP, IL-6 and IL-31, adding to existing evidence that probiotic bacteria can stimulate the anti-inflammatory component of the immune system to release cytokines and hormones that disrupt the damaging inflammatory cycle [47,48]. By supporting the body to reduce inflammation, probiotics could enable the hair follicles to sustain growth [47].

Another potential reason for the beneficial effects of probiotics on hair density might be the reduction of

oxidative stress. Oxidative stress is associate with many skin conditions. On the scalp, the hair is impacted prior to emergence and oxidative stress appears to play an important role in premature hair loss [49]. In the present study, probiotic supplementation resulted in a significant decrease of serum MDA and a significant increase of serum SOD. MDA is the end product of lipid peroxidation and act as a marker of elevated oxidative stress, while SOD could help eliminate harmful substances generated during metabolic processes [50,51]. Their changes indicated that probiotic consumption could effectively reduce oxidative stress in the body and hence might reduce hair loss.

Moreover, we observed a significant increase of serum biotin concentration following probiotic consumption. This might be because that probiotic bacteria are able to synthesize most of the water-soluble B vitamins, including biotin in humans [52]. Although biotin has been shown to improve hair growth in young children with biotin deficiency, it's association with hair growth in healthy individuals remains unclear [53].

Participants of our study self-reported having oily scalp at baseline. Skin biophysical measurements showed a decrease in sebum and TEWL paralleled with an increase in pH and SCH in both the scalp and the facial skin after 12 weeks, suggesting an association of probiotics consumption with the improvement of oily skin condition and the skin barrier function [36,54]. The changes observed in skin barrier parameters in response to probiotics are in line with the results of previous randomized trials [55–57]. Furthermore, while all participants noticed relief of scalp itching, 73% felt apparent or very apparent itching relief. Reduction of serum concentration of IL-31, a cytokine that is known to be implicated in itching and skin barrier dysregulation, also suggested a beneficial effect of probiotics in skin barrier improvement [58].

Participants in the present study reported significantly lower PSS scores following intervention with the study probiotic formula. Serum IL-6 level significantly decreased from baseline to end of study indicating a reduction of stress-related inflammation [59]. Probiotic consumption significantly reduced the levels of cortisol, which is correlated with stress-related disorders [60]. Previous studies have found an impact of probiotics on the cortisol response in stressed participants [61–63]. Apart from being regarded as a stress hormone, cortisol counteracts insulin and stimulates the synthesis of glucose [64]. The decrease of cortisol levels might also mediate the improvement in glucose metabolism, especially insulin resistance.

The gut microbiome has been linked to sleep disturbances [65], and some studies support the role of probiotics in improving sleep quality in humans [66,67]. Participants of the present study had significantly better global sleep quality and perception of quality of life and health following probiotic consumption. Sleep and the immune system are bidirectionally related [68]. While probiotics might improve sleep quality through reducing the inflammatory cytokines of the immune system, a better sleep could in turn regulate immune response and enhance metabolic and overall health.

Based on our knowledge, this study is the first one to investigate this multi-strain probiotic supplementation in individuals with hair loss and high risk of metabolic syndrome. In addition to assessing the effect of this formula on hair density, we used both psychological and physiological makers to evaluate other potential effects which may be related to hair condition. Despite all the positive outcomes found in the present study, some limitations must be acknowledged. First, this was not a double-blind, placebo-controlled study. Second, although the study design was intended to minimize the effect of individual variability and the small number of

participants, individual changes can occur over time. Moreover, due to the small sample size, we did not perform subgroup analysis. However, age group and gender could be underlying factors influencing probiotic effect. Finally, although the strains used in this study have known health benefits which have been demonstrated in previous studies, because of the complex interactions existing among strains and between probiotics and the host, the complete mechanisms of action for the study formula remain unknown. Therefore, monitoring changes in the human gut microbiome after probiotic intaking can provide a better understanding of the mechanisms underlying its health benefits. We would like to address these limitations in randomized-control trials with larger sample size in the future.

CONCLUSION

In conclusion, our findings suggest that twice-daily consumption of probiotics preserves the diversity of the gut microbiota and may exert profound beneficial effects on hair growth, skin barrier function, glucose and lipid metabolism, and stress-associated psychological and physiological responses in participants presenting with hair loss and risk of metabolic syndrome. Probiotic execute actions via the gut-brain-skin axis, which involve many bidirectional interactions with the immune system. These complex mechanisms require exploration in future researches.

List of Abbreviations: AE: adverse event, BMI: body mass index, CFU: colony forming units, CI: confidence interval, FFC: Functional Food Center, HbA1C: hemoglobin A1c, HDL: high-density lipoprotein, HOMA-IR: homeostasis model assessment of insulin resistance, hsCRP: high-sensitivity C-reactive protein, IFN- γ: interferon-γ, IgE: immunoglobulin E, IL: interleukin, IPAQ: International Physical Activity Questionnaire, IQR: interquartile range, MDA: malondialdehyde, MET: metabolic equivalent of task, PSQI: Pittsburgh Sleep Quality Index, PSS: Perceived Stress Scale, Q1: the first quartile, Q3: the third quartile, SAE: serious adverse event, SCH: stratum corneum hydration, SD: standard deviation, SE: standard error, SOD: superoxide dismutase, TC: total cholesterol, TEWL: transepidermal water loss, TG: triglyceride, WHOQOL-BREF: World Health Organization Quality of Life-BREF.

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