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Dual intervention on the gut and skin microbiota attenuates facial cutaneous aging†

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The gut and skin microbiota are microbial barriers, resisting harmful foreign microorganisms and maintaining internal homeostasis. Dysbiosis of the gut and skin microbiota is involved in aging progression. However, interventions targeting facial skin wellness taking into account the gut–skin axis are scarce. In this study, the impact of an eight-week intervention with oral (O), topical (T), and both oral and topical (OT) xylo-oligosaccharides (XOS) by regulating gut and skin microbiota on facial cutaneous aging was investigated in a double-blind placebo-controlled trial in females. An increase in the proportion of participants with skin rejuvenation was observed, along with a significant reduction in facial pores after OT intervention. The reduction of cutaneous *Cutibacterium* by OT intervention was greater than that in the O and T groups. These interventions can change the skin microbial structure. Intestinal *Bifidobacterium* was enriched only by dual treatment with oral and topical XOS. Function prediction analysis revealed a decrease in K02770 encoding fructose-1-phosphate kinase involved in *de novo* lipid synthesis from fructose with dual intervention, suggesting that inhibition of lipophilic *Cutibacterium* may contribute to reducing facial pores. Overall, the dual XOS intervention approach is most effective for improving both gut and skin microbiota, as well as facial skin aging.

1. Introduction

As the largest organ of the human body, the skin serves as the outermost protective barrier, maintaining the internal homeostasis and defending against harmful foreign substances.[1] Facial skin aging is a significant concern from both psychological and social perspectives, especially in women. Facial skin aging encompasses various age-related changes such as UV spots, wrinkles, pores, roughness, loss of elasticity, *etc*.

Millions of bacteria, fungi and viruses have been identified residing in representative cutaneous regions, covering both the skin surface and subcutaneous tissues.[2] Dysbiosis or substantial compositional alterations in the skin microbiota are associated with numerous cutaneous disorders. Therefore, comprehending changes in skin microbiota niches and special species on healthy skin is imperative for developing therapeutics aimed at restoring a healthy skin microbial community. In the pilosebaceous unit, *Cutibacterium acnes* (*C. acnes*) is the most prevalent and abundant species accounting for approximately 90% of skin bacteria.[3,4] In particular, it predominates in invaginations of oily skin sites in the face which are anoxic and sebum-rich, such as pores or sebaceous follicle units.[5,6] An enlarged facial pore is considered a genuine clinical entity that generates aesthetic concerns and is perceived as one of the markers of aging skin[7] and a potential target for anti-aging treatment.[8] The proliferation of pathogenic *C. acnes* strains in pores can lead to the development of acne and impair skin health. An increased anaerobic growth environment for lipophilic microorganisms such as *C. acnes* is provided by the expansion in pore size and number, along with the sebum production. Sebum levels on facial cheeks have been shown to be positively correlated with the abundance of *Cutibacterium* spp.[9] Therefore, the involvement of *C. acnes* in pore-related skin problems may result in skin aging.

The concept of the gut–skin axis proposes that the gut microbial barrier can impact cutaneous pathology and health.

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Both the gut and skin are colonized by numerous microbes and serve as crucial organs for communication with the environment. Recently, dysbiosis of the gut microbiota has been observed in common skin diseases such as acne,[10] atopic dermatitis (AD),[11] and psoriasis.[12] The correlation between gut microbiota and aging has been extensively investigated, whereas limited research has been conducted regarding the correlation between gut microbiota and skin aging. Bidirectional crosstalk between the gut and skin has been extensively studied, linking gastrointestinal health with skin homeostasis. One of the latest progress of research on the relationship between the gut and the skin is focusing on the skin microbiota involved in the gut–skin axis, along with its potential to ameliorate various skin and/or gut diseases.[13] Considering the bidirectional influence, impaired skin barriers can also affect the health of the gastrointestinal tract micro-ecosystem. For instance, allergens penetrating through the skin can induce the sensitization of the intestinal mucosa.[14] However, we consider that the gut–skin axis represents not merely an interaction between the gut and the skin but rather jointly establishes a microbial barrier to resist the invasion of foreign microbes and safeguard the host between the two microbial barriers.

Besides the regulation of the gut microbiota, growing evidence of prebiotic strategies has been reported in the fields of cosmetics and dermatology in recent years.[15] Comprehensive insights into innovative approaches focused on the maintenance and enhancement of skin health, as well as management of skin disease, through targeted-regulation of the microbiome.[16] Prebiotics, such as xylose-oligosaccharides, galactose-oligosaccharides, fructose-oligosaccharides, and inulin, have exhibited the ability to enhance the proliferation of beneficial bacteria, thereby maintaining or repairing skin barriers.[17] For example, 5% gluco-oligosaccharides could inhibit an increase in *S. aureus* cells.[18] Galacto-oligosaccharides were reported to inhibit the growth of harmful skin microbiota while promoting beneficial bacteria.[19] Clinical trials have demonstrated that supplementation with non-digestible oligosaccharides can prevent eczema by prompting gut microbiota closer to that of breastfed infants.[20] However, there is currently a lack of research investigating their regulation on both terminals of the gut–skin axis. This study aimed to explore the effects of prebiotics administered orally and/or topically on skin condition, the gut and skin microbiota as well as indicators associated with skin aging.

2 Experimental

2.1 Ethical statement

This study protocol was approved by the Ethics Committee at the Affiliated Hospital of Jiangnan University (ID: 2019122701). All human subject procedures were performed in accordance with the Guidelines of the Declaration of Helsinki. The trial was registered with the Chinese Clinical Trial Registry (ChiCTR2000031913). All participants provided written informed consent. A total of 79 healthy female participants were recruited for this study. The study design and participant flow are shown in Fig. 1. The inclusion and exclusion criteria for the recruitment of participants are presented below.

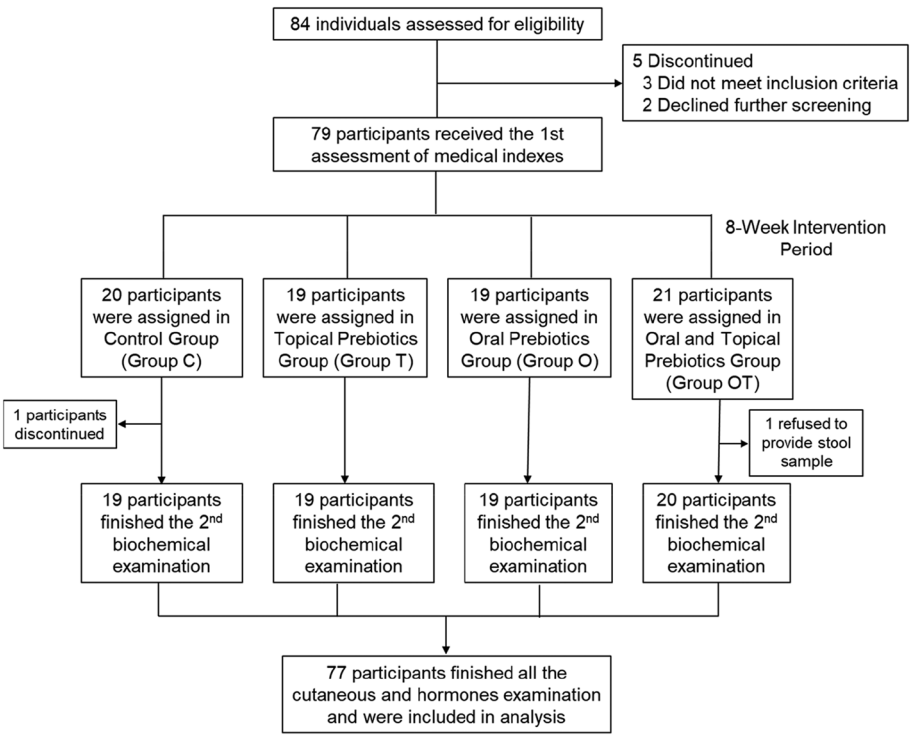

Fig. 1 Flow chart of the experiment.

2.2 Inclusion and exclusion criteria for recruitment of participants

2.2.1 Inclusion criteria. Healthy female aged 30–45 years; healthy skin without dermatitis, eczema or acne; informed of the purpose and the protocol of the study and signed a written informed-consent form; co-operative and available during the study period.

2.2.2 Exclusion criteria. Use of oral antibiotics or oral steroids in the 6 months prior to the initiation of the study; current participation in another clinical test within the 3 months prior to the initiation of the study; skincare therapy using lasers or peeling within the 3 months prior to the initiation of the study; skincare product contains probiotics or prebiotics; women who had undergone, or planned to undergo, pregnancy or breastfeeding; any condition judged by the investigator to be unsuitable for participation in the study.

2.3 Protocol

2.3.1 Sample size calculation. In our preliminary study, 20 participants were initially recruited and randomly divided into 4 groups, and their skin age was assessed using VISIA. The finding showed that combined treatment with oral and topical XOS exhibited the highest proportion of individuals with skin age younger than their chronological age following the intervention. In detail, the average drop-out values of skin age in each group were as follows: C group, 0; T group, 0.4; O group, −1.2; OT group, 3.2. Therefore, to further investigate how dual intervention modulates age-related indicators and mechanisms associated with youthful skin, we subsequently recruited more participants for in-depth research. Based on the result of the preliminary study, the total sample of 76 subjects (19 per group) achieves 90% power to detect the difference in skin age, with a significance level of 0.05, as calculated using PASS software. Finally, a sample size of 84 participants was needed considering a drop-out rate of 10%.

Participants completed the same testing protocol pre- and post-intervention with XOS. In this clinical trial, we investigated the effect of an eight-week intervention with XOS in a double-blind placebo-controlled study in females aged 30 to 45. Stool samples and scraped skin surface samples were collected pre- and post-intervention. While maintaining their regular skincare routine, participants were explicitly instructed not to alter their usual habits.

2.4 Materials

2.4.1 Questionnaire. Prior to the commencement of the intervention trial, each participant was required to provide informed consent and complete a questionnaire pertaining to the study. The questionnaire encompassed inquiries regarding basic demographic information such as age, frequency of going to bed late, frequency of skin care, *etc*. Those who stayed awake past 11 PM were considered as going to bed late. For the assessment of skin properties, we implemented a comprehensive evaluation approach that integrates survey questionnaires with expert assessments from professional dermatologists.[21,22]

2.4.2 XOS/placebo intervention. The cosmetic serum formulation[19] served as the basis for our study, with modifications made to incorporate XOS. The preparation of XOS (provided by Henan Heagreen Biotechnology Co., Ltd) included PEG-60 hydrogenated castor oil (0.29%), cetyl ethylhexanoate (0.52%), arginine (0.15%), hydroxyethyl cellulose (0.10%), carbomer #941 (0.08%), carbomer #940 (0.10%), XOS (7.0%), and deionized water to reach 100%. A control serum without XOS was also used in the study. Participants took orally a daily dose of 3.0 g of the XOS or placebo (maltodextrin) for an eight-week period, with both supplements provided in unlabeled packaging and similar in color and taste. Xylo-oligosaccharides are commonly utilized in food formulas, and due to their potential prebiotic benefits, they have been incorporated into cosmetics as one of the active constituents in recent years. XOS serve as "oral cosmetic agents" when taken orally, while as a component of "cosmetic serum formulation" when applied topically.

2.4.3 Sample collection and processing. The skin microbiota sampling swabs were premoistened in sterile PBS and utilized to scrape the skin on the forehead and both cheeks of each participant for 1 min, applying consistent pressure and frequency under the supervision of a professional doctor. Subsequently, swabs were promptly transferred into sterile centrifuge tubes and stored at −80 °C until further analysis. A unique sampling box was provided for participants for stool collection, while fasting venous blood samples were collected from each participant. All blood samples were centrifuged at 3000*g* for 20 min to obtain the serum, placed in inert separating glue coagulant tubes, and stored at −80 °C for later use. All samples were collected at the baseline and at the end of the intervention.

2.4.4 Clinical indexes detection. Crucial facial skin features and physiological characteristics were detected by the VISIA complexion analysis system (Canfield Imaging System, Fairfield, New Jersey, United States), which performed at the baseline and post-intervention, with three digital images taken of each participant's face (left- and right-side and forehead). The eight fundamental skin indicators, spot, wrinkle, texture, pore, UV spot, brown spot, red area, and porphyrin, examined by VISIA are crucial for evaluating the condition of facial skin. Participants were strictly instructed to refrain from washing their face and using any cosmetic formulations for a duration of 12 hours prior to VISIA detection, whether at the baseline or after intervention. The levels of plasma hormone were measured using an automatic chemiluminescence immunoassay analyzer (UniCel Dxl 800, Beckman Coulter, California, USA).

2.4.5 DNA extraction, database construction and sequencing of skin and gut microbiota. Sequencing was performed at Shanghai Honsun Biological Technology Co., Ltd (Shanghai, China). Briefly, genomic DNA was extracted from samples scraped from skin surface and feces, followed by detection using 1% agarose gel electrophoresis. PCR for DNA was performed to amplify the hypervariable V3–V4 region of the bacterial 16S rRNA gene using universal primers. Fluorescence

quantification was the preliminary quantitative result of electrophoresis, and the PCR products were detected and quantified using the QuantiFluor™-ST blue fluorescence quantification system. Sequencing processing and taxonomic classification were conducted using both forward and reverse reads when paired-end data were available. The collected fluorescence signals from each round were tallied to obtain the sequence information for template DNA fragments.

2.5 Analysis

2.5.1 Bioinformatics analysis. The Illumina Miseq sequencing system (Illumina, San Diego, CA, USA) was used for 16S high-throughput sequencing analysis of the samples. Trimmomatic and FLASH were utilized to demultiplex and quality-filter the raw pyrosequencing reads obtained from the sequencer. UCHIME was used to detect and remove chimera sequences arising from PCR amplification, while high-quality sequences were assigned to the respective samples based on barcodes and clustered into operational taxonomic units (OTUs) using the USEARCH platform with a 97% sequence similarity threshold. The QIIME bioinformatics software was utilized to analyze and process the data of each group. Richness estimators (ACE and Chao1) and diversity indices (Shannon and Simpson) were calculated as α-diversity based on the level of OTUs. β-Diversity was evaluated by calculating the Bray–Curtis distance and visualized by principal coordinate analysis (PCoA). The linear discriminant analysis effect size (LEfSe) method was used to perform linear discriminant analysis (LDA) on samples based on the taxonomic composition and different grouping criteria to identify communities or species that significantly differentially contribute to sample delineation. Spearman analysis was used to examine the correlation between clinical data and dominant microbiomes.

2.5.2 Statistics. Various statistical strategies were employed to determine the statistical significance based on different types of data. A two-way repeated-measures analysis of variance (ANOVA) with a mixed effects model was used for intra- and intergroup comparisons by using the GraphPad Prism 9.0 software. Pearson chi-square tests (two tailed) were applied to analyze the proportion of participants in intergroup comparisons. All the graphs are presented as mean values with standard error of the mean (SEM). The LDA score and PERMANOVA analyses were performed using R software for statistical analysis. $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Baseline characteristics

The baseline characteristics and the basic information did not exhibit any significant differences among the four groups (Table 1). The use of questionnaire scores is one of the ways to assess skin properties. For the assessment of skin properties, we implemented a comprehensive evaluation approach that integrates survey questionnaires with expert assessments from professional dermatologists. This method of skin property evaluation is more suitable for our study as it enables a comprehensive assessment of the subjects' skin properties. Additionally, in the study design, our initial focus was on investigating anti-aging effects; however, given the multifactorial nature of skin aging, we subsequently discovered a corre-

Table 1 Baseline characteristics of the subjects

	Randomization group				
Characteristic	All (N = 79)	Group C (N = 20)	Group T (N = 19)	Group O (N = 19)	Group OT (N = 21)
Age, (mean ± SEM)	36.74 ± 0.47	35.79 ± 1.05	37.00 ± 1.03	36.16 ± 0.86	37.95 ± 0.76
Skin property, *N* (%)					
Oily	33 (41.77)	9 (45.00)	6 (31.58)	10 (52.63)	8 (38.10)
Dry	37 (46.84)	9 (45.00)	10 (52.63)	6 (31.58)	12 (57.14)
Slight oily	6 (7.59)	1 (5.00)	2 (10.53)	3 (15.79)	0 (0.00)
Slight dry	3 (3.80)	1 (5.00)	1 (5.26)	0 (0.00)	1 (4.76)
Frequency of skin care, *N* (%)					
Once a day	29 (36.71)	10 (50.00)	7 (36.84)	6 (31.58)	6 (28.57)
Twice a day	50 (63.29)	10 (50.00)	12 (63.16)	13 (68.42)	15 (71.43)
Type of sunblock used, *N* (%)					
Physical	15 (18.99)	5 (25.00)	2 (10.53)	5 (26.32)	3 (14.29)
Chemical	11 (13.92)	1 (5.00)	1 (5.26)	3 (15.79)	6 (28.57)
Combined use	30 (37.97)	5 (25.00)	10 (52.63)	6 (31.58)	9 (42.86)
Nonuse	23 (29.11)	9 (45.00)	6 (31.58)	5 (26.32)	3 (14.29)
Frequency of going to bed late, *N* (%)					
Hardly	11 (13.92)	3 (15.00)	3 (15.79)	2 (10.53)	3 (14.29)
Once or twice a week	46 (58.23)	11 (55.00)	9 (47.37)	15 (78.95)	11 (52.38)
Three to five times a week	14 (17.72)	4 (20.00)	4 (21.05)	1 (5.26)	5 (23.81)
Almost everyday	8 (10.13)	2 (10.00)	3 (15.79)	2 (10.53)	1 (4.76)
Hormone levels, (mean ± SEM)					
Estradiol (ng L^{-1})	39.97 ± 3.18	46.92 ± 10.17	38.00 ± 5.04	37.38 ± 4.47	37.46 ± 3.57
Testosterone (ng mL^{-1})	0.47 ± 0.02	0.49 ± 0.05	0.45 ± 0.05	0.47 ± 0.03	0.48 ± 0.04

N = number; demographic characteristics of the final sample (N = 77).

lation with the sebum level. Nevertheless, our original design did not anticipate the association between improved skin condition and sebum production, and thus sebum levels were not measured at the baseline.

3.2 Changes in clinical indexes

Two participants dropped out, leaving a final sample size of 77 who completed all aspects of the experiment. The sample size for each group was as follows: control group (C, n = 19), oral XOS group (O, n = 19), topical XOS group (T, n = 19), and oral combined topical XOS group (OT, n = 20).

The intensity and speed of the aging process differ markedly between individuals. Some women look younger than their chronological age, while others look much older.[23] Therefore, skin age (SA) is also one of the main analysis indexes of the VISIA system. Comparison of the SA pre- and post-intervention on the left-side and right-side facial skin in each group is shown in Fig. 2. For the left-side facial skin, the SA in the OT group tended to be lower than that in the C group pre- and post-intervention (Fig. 2G, p = 0.08). However, the drop-out value of SA pre- and post-intervention was significantly greater in the OT group than in the O and T groups (Fig. 2G, p < 0.05). The drop-out value of SA of the right-side face in the OT group was greater than that in the T group (Fig. 2H, p = 0.05). In conclusion, the drop-out value of skin age in the OT group was the maximum among the four intervention groups. This suggests that oral combined topical XOS may rejuvenate middle-aged women by mainly improving pores.

Furthermore, only the combination of oral and topical XOS could promote the proportion of participants whose SA decreased (Fig. 2, p < 0.05), indicating that dual intervention may tend to alleviate skin aging in women.

Although the combination of topical and oral treatment demonstrated the most effective results in improving pores and alleviating skin aging, it is also imperative to meticulously observe and analyze the efficacious phenotype of single treatment. Why did a lower number of participants derive benefits from single treatment? We considered that this result could be attributed to the placebo and nocebo effects. Michael S. Roberts *et al.* comprehensively reviewed consumer perception of topical products, encompassing factors such as consumer perception, cultural differences among consumers, sensory attributes of the product, and perception of products on the placebo and nocebo effects.[24] In summary, these topical products may exert either positive (placebo) and/or negative (nocebo) perceptions independent of the presence or absence of any active ingredient.[24] This has largely enhanced our perception of these phenomena. In this context, the nocebo effect refers to the adverse reaction caused by patients' doubts or negative expectations regarding the treatment modality. The broader category of nocebo encompasses the psychological phenomenon where patients interfere with their treatment due to suspicions that a placebo has been administered. A portion of the subjects may be convinced that they were assigned to the control group, perceiving that the preparation used had no positive impact on the skin, thus triggering the occurrence of a nocebo effect. In our study, when comparing the difference in the SA < CA proportion pre- and post-intervention in each group, we observed a decrease in SA < CA proportion in the T_Post group compared to the T_Pre group, which could be attributed to a potential nocebo effect. Additionally, a nocebo effect was observed with topical admin-

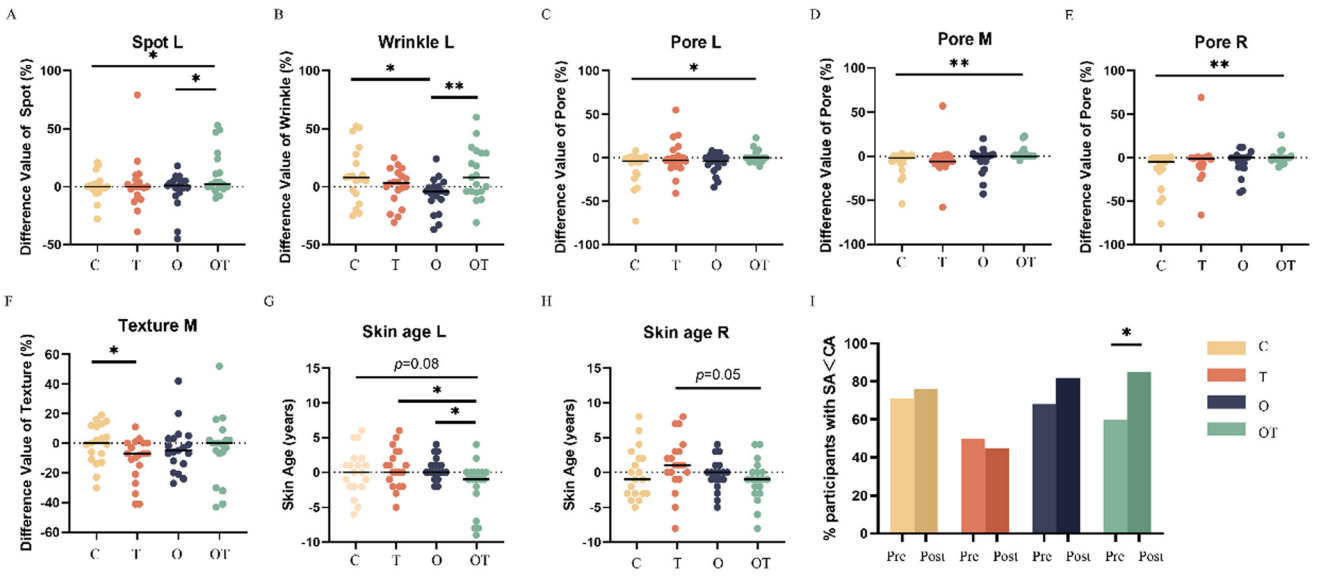

Fig. 2 Changes of VISIA indexes: (A) spot, (B) wrinkle, (C) pore on the left-side facial skin, (D) pore, (F) texture on the forehead facial skin, (E) pore on the right-side facial skin, (G) left-side facial skin age, and (H) right-side facial skin age in each group pre- and post-intervention. (I) Changes in the percentage of participants with improved skin age pre- and post-intervention in each group. *p < 0.05, **p < 0.01, and ***p < 0.001 using two-way repeated-measures analysis of variance (ANOVA) for inter-group comparisons.

istration, whereas no such effect was evident with oral administration. The psychological and physiological mechanisms underlying nocebo effects remain an enigmatic challenge requiring further investigation.

The score of pores on three parts of the subject's facial skin was significantly increased after OT intervention compared with the control group (Fig. 2C–E), which showed that combining oral and topical XOS can improve the pores in female facial skin. Furthermore, left-side facial spots and wrinkles were significantly improved in the OT group compared with those of the O group (Fig. 2A and F). Among various skin aging parameters, wrinkles, pores, hyperpigmented spots, and skin roughness are considered as particularly representative manifestations of an older appearance in female skin.[23]

The skin is the largest non-reproductive organ influenced by androgen action, but little is known about the impact of androgens on the skin microbiota. Androgens can directly stimulate sebaceous gland cells and increase sebum secretion in females, leading to acne, hirsutism, and androgenic alopecia.[25] In the current study, no significant changes were observed in estradiol and testosterone levels before and after the intervention in each group (Fig. S1†), indicating that no matter oral or external use of XOS could not affect sex hormone secretion in a state of health. Therefore, the regulation of hormone levels is not the mechanism by which XOS affects facial pores.

3.3 Effect of XOS on female skin microbiota and gut microbiota

The microbiota sequencing results of skin and stool samples under different intervention methods were analyzed.

3.3.1 Summary of the basic information of sequencing data. A total of 77 feces and 231 skin surface swab samples (77 left- and right-side cheeks and forehead, respectively) were collected, followed by DNA extraction and sequencing of the V3–V4 region using 16S rRNA gene primers. OTU clustering analysis was performed, and taxonomic classification was conducted on representative sequences. Statistical analysis revealed a total of 48 298 599 original data sequences with a final read count of 46 549 481.

3.3.2 α-Diversity analysis of skin and gut microbiota

3.3.2.1 α-Diversity analysis of skin microbiota. The α-diversity was utilized to assess the microbial richness and diversity. For the forehead skin microbiota, the Ace and Chao1 indices in group T were significantly reduced post-intervention (Fig. S2-A and B,† $p < 0.05$), indicating a significant decrease in the number of species of skin microbiota. The Simpson index in the X group was significantly decreased post-intervention compared to pre-intervention (Fig. S2-D†). The Shannon index in the OT group significantly increased post-intervention compared with pre-intervention in the forehead and right-side facial skin (Fig. S2-C,† $p < 0.05$), indicating a significant increase in community diversity of skin microbiota. The Simpson index decreased significantly post-intervention in group OT (Fig. S2-D,† $p < 0.05$), indicating an increase in the community diversity of forehead skin microbiota after oral and topical XOS intervention. No significant changes were observed in the Shannon index in the other three groups post-intervention. In summary, these results showed that OT intervention significantly increased both species richness and community diversity of skin microbiota among women.

3.3.2.2 α-Diversity analysis of gut microbiota. There were no changes in the diversity and richness of gut microbiota after the intervention of prebiotics (Fig. S3†), indicating that XOS was not sufficient to change the types and richness of gut microbiota in healthy women, but the enrichment and influence of XOS on a specific bacterium in gut microbiota need further study.

3.3.3 β-Diversity analysis of skin and gut microbiota

3.3.3.1 β-Diversity analysis of skin microbiota. β-Diversity analysis focused on the structural differences between different groups of samples. In this study, the skin microbiota in the left-side forehead and right-side facial skin of each group pre- and post-intervention were compared. There was no significant difference in the skin microbiota on the L-, M-, and R-side in the C_Post group compared with that in the C_Pre group (Fig. 3A–C). The skin microbiota of L-, M-, and R-side facial skin in the T_Post group was different from that in the T_Pre group, and the skin microbiota of M- and R-side facial skin had significant differences pre- and post-intervention (PERMANOVA, $p < 0.05$), among which the difference was the largest on the R-side pre- and post-intervention (PERMANOVA, $p = 0.001$). The left-side facial skin microbiota in the O_Post group was significantly different from that in the O_Pre group (PERMANOVA, $p = 0.005$). For M- and R-side facial skin, a significant difference was observed in the OT_Post group compared with the OT_Pre group (PERMANOVA, $p < 0.05$).

3.3.3.2 β-Diversity analysis of gut microbiota. PCoA was used to analyze the β-diversity of the gut microbiota structure and showed that there was no significant difference in each group pre- and post-intervention (Fig. S4†). These results indicated that neither oral nor topical XOS intervention has significantly changed the overall structure of gut microbiota in healthy women.

3.3.4 Effect of XOS on key bacteria genera screened by LEfSe

3.3.4.1 Exploring intervention effects on skin microbiota in oral and/or topical XOS treatment. The LEfSe analysis was used to screen out the key differential bacterial genera in each group comparison before and after the intervention, as well as between-group comparisons after intervention on the skin, with a primary focus on the following genera. At the end of the OT intervention, the abundance of *Cutibacterium* was significantly reduced compared to OT_Pre, which was consistent with the result in left-side facial skin after intervention in the same group (Fig. 4). In the bacterial community of the right-side facial skin, the abundance of *Cutibacterium* significantly reduced in the T, O, and OT groups post-intervention compared with pre-intervention. This genus is a kind of cutaneous symbiotic bacteria, belonging to lipophilic microorganisms, which decreased significantly after XOS intervention, indicating that XOS may reduce the abundance of lipophilic microor-

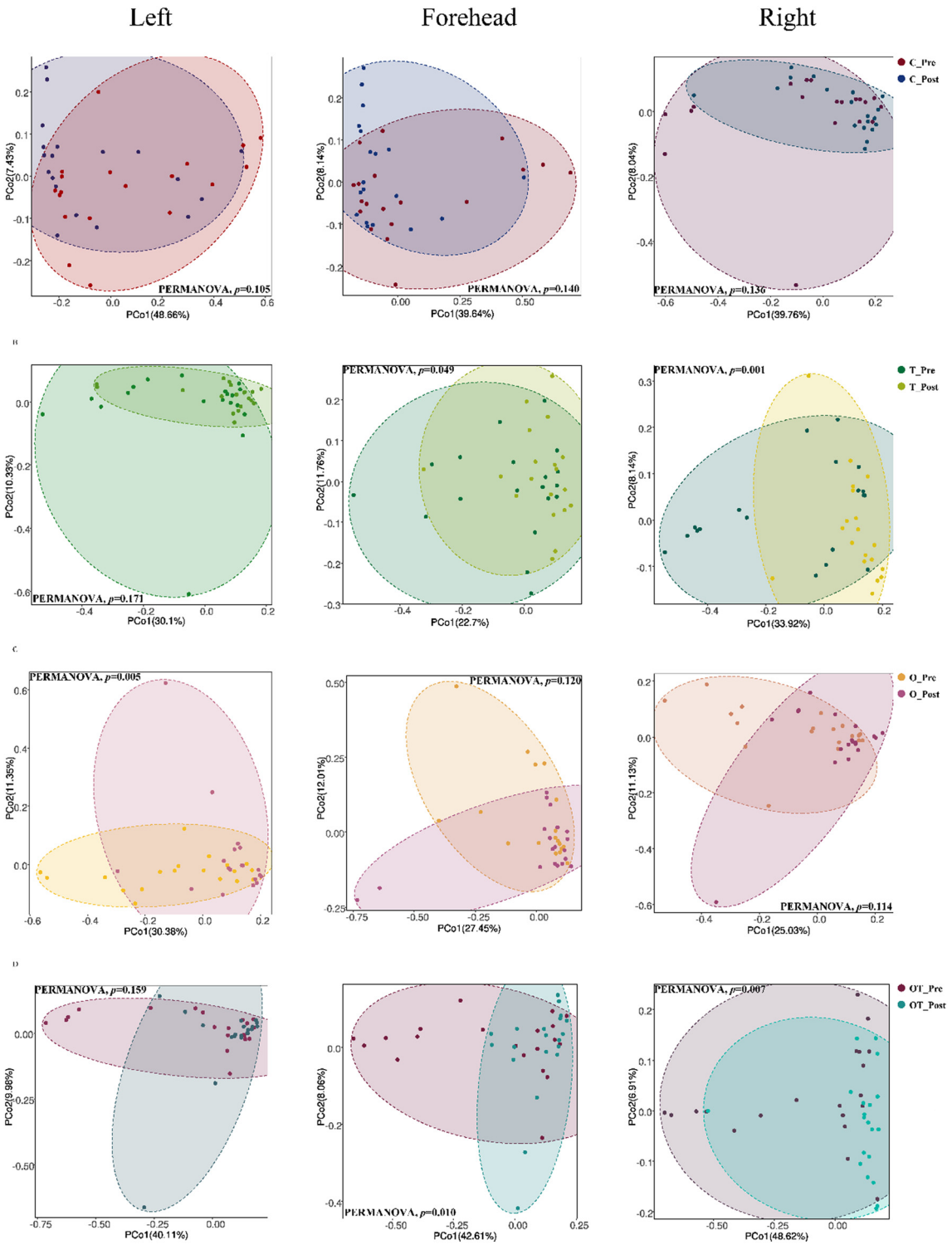

Fig. 3 PCoA of the skin microbiota in (A) C, (B) T, (C) O, and (D) OT groups at left-side, forehead, and right-side facial skin sites pre- and post-intervention based on the Bray–Curtis distance of 16S rRNA V3–V4 sequencing data and PERMANOVA statistical analysis, T_Pre_M *vs.* T_Post_M, $p < 0.05$; T_Pre_R *vs.* T_Post_R, $p < 0.01$; O_Pre_L *vs.* O_Post_L, $p < 0.01$; OT_Pre_M *vs.* OT_Post_M, $p < 0.05$; and OT_Pre_R *vs.* OT_Post_R, $p < 0.01$.

ganisms by inhibiting the secretion of skin lipids. Additionally, abnormal and excessive proliferation of *Cutibacterium* is one of the factors involved in the pathophysiological mechanism of adult acne.[26] The imbalance of skin microbiota leads to the proliferation of *C. acnes*, which is an important process causing acne. The activation of innate immunity by *C. acnes* involves the upregulation of PARs, TNF-α, TLRs, INF-γ production, and the secretion of interleukins

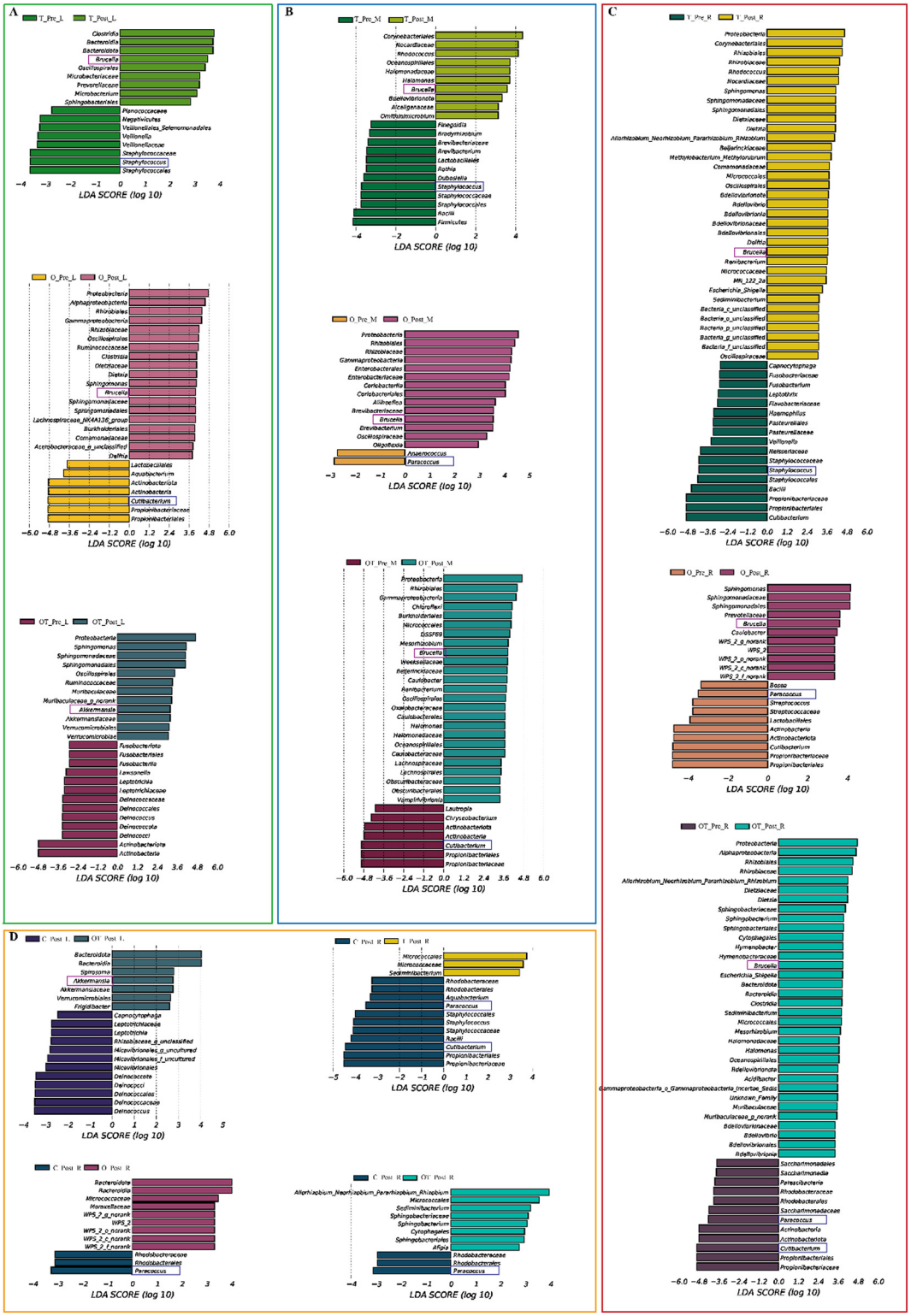

Fig. 4 Key cutaneous genera screened by the LEfSe analysis. Key genera of (A) left-side facial skin; (B) forehead; and (C) right-side facial skin in each group post-intervention compared with pre-intervention. (D) Key genera of each post-intervention group compared with the C_Post group. The linear discriminant analysis (LDA) effect size measurements (LEfSe) method was used for selecting key cutaneous microbiota.

(IL-8, IL-12, IL-1), TNF, and MMPs by keratinocytes, ultimately leading to hyperkeratinization of the pilosebaceous unit.[27] These conclusions suggest that the excessive proliferation of *C. acnes* may be reduced by inhibiting excessive keratosis of sebaceous units of hair follicles and reducing the occurrence and incidence of acne by XOS. Moreover, the abundance of

Staphylococcus significantly decreased after T intervention at the forehead and L- and R-side facial skin sites, respectively. *Staphylococcus aureus*, which belongs to the genus *Staphylococcus*, is an opportunistic pathogen that can exacerbate skin damage in AD,[28] produce enzymes that disrupt the epidermal barrier, secrete molecules that induce immune responses, and stimulate mast cells to damage the skin,[29] adversely affecting the skin.

Among the comparative analysis results of genera in the OT intervention group, the key genus, *Akkermansia*, was mainly significantly enriched compared to the OT_Pre group, as well as compared to the C_Post group (Fig. 4). The only strain in the genus *Akkermansia* is *Akkermansia muciniphila* (*A. muciniphila*). *Akkermansia* is recognized as a potentially beneficial bacterium. Oral administration of *Akkermansia* can enhance the integrity of the intestinal barrier and intestinal cell proliferation and regeneration, improve cognitive function, and reduce weakness and muscle atrophy.[30] *A. muciniphila* and its derived acetic acid can improve natural aging-related systemic disorders, suggesting that intervention with potent stimulative capacity on *A. muciniphila* growth was an alternative and effective way to maintain healthy aging.[31]

The abundance of *Paracoccus* significantly decreased in the T_Post_R, O_Post_R, and OT_Post_R groups compared with the C_Post_R group (Fig. 4D). *Paracoccus yeeii* is isolated from patients with wound or inflammation, such as ankle wound, otitis media, and neck incision drainage.[32] This species can be enriched in skin wound and inflammatory interface, representing a previously undescribed *Paracoccus* species associated with human infection.[32] Whether oral, topical, or oral combined topical XOS, the intervention resulted in a significant reduction of bacterial species which positively associated with skin infections. This result suggested that XOS may attenuate skin infections by inhibiting the abundance of *Paracoccus*, thereby exerting a protective effect on the skin.

In addition to the above conclusions, *Brucella* was significantly enriched in the T_Post_L, T_Post_M, T_Post_R, O_Post_L, O_Post_M, O_Post_R, OT_Post_M, and OT_Post_R groups compared with each group pre-intervention, respectively (Fig. 4). *Brucella* has long been known as a pathogenic bacterium, found in livestock such as cattle and sheep.[33] In addition, none of the subjects were infected with *Brucella* disease at the end of the intervention, implying that enriched *Brucella* on the skin may be non-pathogenic strains of *Brucella*.

3.2.4.2 Effect of XOS on key gut bacterial genera. Key genera in the gut were screened by LEfSe analysis in each group pre- and post-intervention (Fig. 5). At the genus level, *Peptostreptococcales_Tissierellales*, which can produce butyric acid,[34] were significantly reduced after the topical intervention. In addition, oral intervention resulted in an enrichment of *Prevotella*, which is known for its glycolysis and is sensitive to bile salt.[35] Studies have demonstrated that administration of XOS increased the abundance of *Prevotella* spp. in mouse intestines, indicating an alleviating effect of this intestinal colonizer in allergic sensitization.[36] Importantly, *Bifidobacterium* was significantly enriched at the end of the OT intervention compared with that in the OT_Pre and C_Post groups (Fig. 6). Supplementation with XOS significantly increased the abundance of *Bifidobacterium* spp. throughout the intestines, which is beneficial for recovery of intestinal diseases.[37] Moreover, XOS exhibited an anti-inflammatory effect by alleviating skin inflammation manifestation in dermatitis mice.[36] Furthermore, *Bifidobacterium longum* upregulated tryptophan metabolism and activated AHR-mediated immune response, thereby alleviating AD symptoms, based on the interactions within the gut–skin axis.[38] Increased intestinal probiotics, such as *Bifidobacterium*, may generate tryptophan-derived end metabolites which regulate sebum synthesis.[39] Interestingly, this bacterium only enriched after the intervention in group OT. Both the gut and skin possess extensive innervation and vascularization, as they are essential for immune and neuroendocrine functions.[10] Notably, preliminary experiments indicated that oral probiotics improved the clinical symptoms in patients with psoriasis, perhaps correlated with the gut microbiome-mediated crosstalk between the immune system and the nervous system by secreting neurotransmitters in psoriasis.[40] What is noteworthy is that the results of alterations in the gut microbiome can extend beyond the intestines. For example, gastrointestinal diseases are often accompanied by skin manifestations.[41] Additionally, the gut microbiota may affect cutaneous physiology, pathology, and the immune response more directly through the metastasis and metabolites of the gut microbiota to the skin.[40] DNA of the gut microbiota has been successfully isolated from the plasma of psoriatic patients. These findings provide evidence of more direct connections between the gut microbiota and skin.[10] The communication along the gut–brain–skin axis needs to be further studied and discussed.

A robust association exists between a well-balanced gut microbiome and the process of healthy aging. Changes in the gut microbiota can affect microbial function by modulating the intestinal permeability, thereby influencing nutrient absorption, food metabolism, and immune system regulation. Changes in the gut microbiota can profoundly influence the pathogenesis of various diseases, encompassing those associated with aging. A study revealed that an altered gut microbiome composition has been linked to the process of aging and age-related inflammation, exemplified by a decrease in the presence of *Bifidobacteria* spp., an anti-inflammatory bacterial species, in aged mice.[42] These findings suggested that age-related alterations in the gut microbiota may exacerbate systemic aging, including cutaneous aging. Gao *et al.* demonstrated the interaction of *Bifidobacterium longgum* 68S with the gut–skin axis, thereby exhibiting an anti-aging effect.[43] In our study, we observed a significant enrichment of the intestinal *Bifidobacterium* in the OT group, leading us to hypothesize that this may represent one of the mechanisms by which gut microbiota contributes to the attenuation of skin aging.

Currently, the majority of studies primarily focus on investigating the impact of gut microbiota on skin health and improving skin diseases.[38–40] However, there is a scarcity of research exploring the influence of gut microbiota on skin

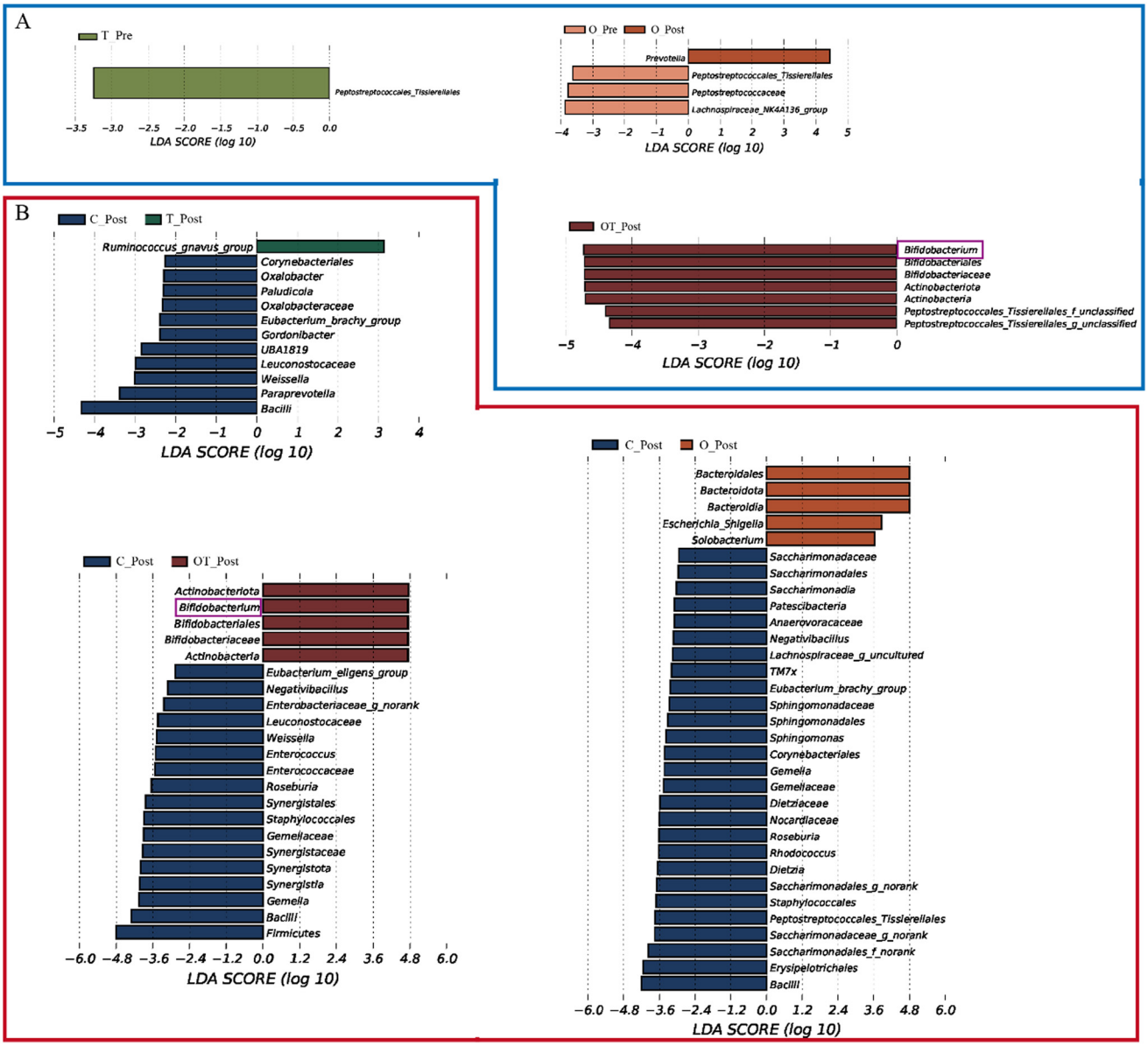

Fig. 5 Key genera screened by the LEfSe analysis in the gut. Key genera in the (A) T_Post, O_Post, and OT_Post groups compared with the pre-intervention group of each, respectively. (B) Key genera of each post-intervention group compared with the C_Post group. The linear discriminant analysis (LDA) effect size measurements (LEfSe) method was used for selecting key intestinal microbiota.

microbiota, which may provide a novel perspective to comprehend the gut–skin axis.

3.2.4.3 Changes of key bacterial genera of gut microbiota and skin microbiota pre- and post-intervention. In this study, oral and topical XOS was most effective in reducing the abundance of *Cutibacterium* (Fig. 6A). This approach may lay a foundation for preventing and reducing acne in future studies. Additionally, the abundance of *Bifidobacterium* significantly increased in the gut in the OT group compared with that in the C group (Fig. 6B, $p < 0.05$). The abundance of *Paracoccus* significantly decreased in the OT, O, and T groups compared with the C group (Fig. 6C, $p < 0.05$). Moreover, a combination of oral and topical XOS also effectively reduced the cutaneous *Cutibacterium* and *Paracoccus*, while increasing the intestinal *Bifidobacterium*. These findings indicated that oral and topical application of XOS can improve both gut and skin microbiota in women, promoting overall health. However, further research is needed to fully understand the mechanism of the gut–skin axis.

3.3.5 Changes of key pathways for prediction of skin and gut microbiota function by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt)

3.2.4.1 Skin microbial function prediction. According to the difference functional analysis of pre- and post-intervention in the OT group, the screening of key pathways and KEGG orthology (KO) was performed to predict the function of microbiota

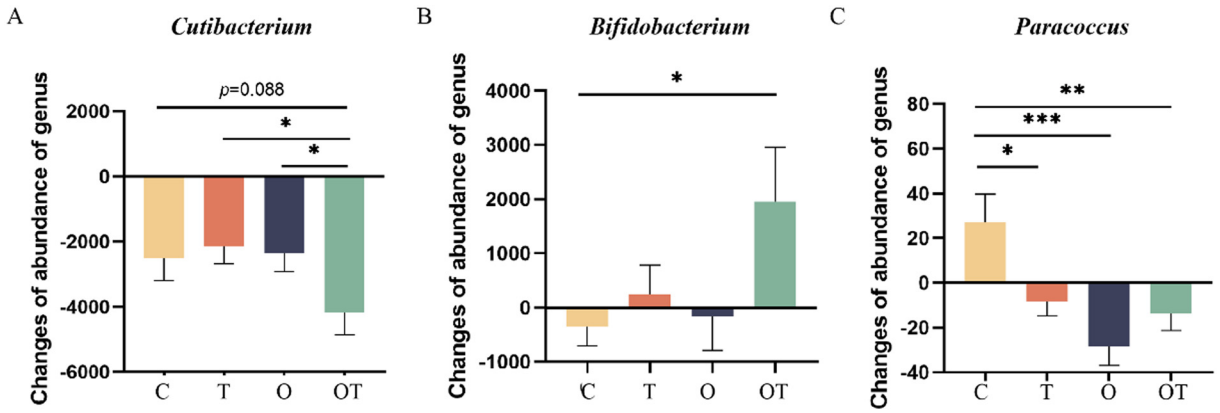

Fig. 6 Changes of the key genera of gut microbiota and skin microbiota in each group pre- and post-intervention. Changes of abundance of (A) cutaneous *Cutibacterium*; (B) intestinal *Bifidobacterium*; and (C) cutaneous *Paracoccus*. Data are presented as the means ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

on facial skin based on PICRUSt. After intervention, the OT group showed a significant decrease in K02770 (encoding fructose PTS system EIIBC or EIIC component), K00849 (encoding galactokinase), and K00016 (encoding L-lactate dehydrogenase) (Fig. 7). The metabolic pathways participated by these three key KOs were ko00051 (fructose and mannose metabolism), ko02060 (phosphotransferase system), ko00010 (glycolysis/gluconeogenesis), and ko00052 (galactose metabolism) (Fig. S5†). Among them, the EIIBC or EIIC component of the fructose PTS system participated in two reactions of the fructose and mannose metabolism pathway as well as the phosphotransferase system pathway, converting fructose to fructose-1-phosphate (F1P). L-Lactate dehydrogenase was involved in the glycolysis/gluconogenesis pathway, with its metabolites being L-lactic acid or pyruvate. The product of galactokinase involved in galactose metabolism was α-D-galactose-1-phosphate (Gal-1P).

Excessive fructose intake can increase or accelerate liver fat synthesis and ultimately lead to fatty liver, as well as increase the secretion and release of circulating triglycerides and VLDL.[44] Fructose is rapidly phosphorylated to F1P upon entering the liver cells, generating malonyl-CoA that participated in *de novo* fat synthesis and subsequent triglyceride production.[45] *C. acnes* is a lipophilic microorganism growing in an anaerobic lipid-rich environment. The levels of sebum on facial cheeks have been shown to be positively correlated with the abundance of *Cutibacterium* spp.[9] In addition, *C. acnes* secretes a lipolytic enzyme that decomposes triglycerides to form free fatty acids that are used for *C. acnes* growth.[46,47] With reduced triglyceride production, *C. acne* has no substrate to produce free fatty acids and is unable to produce more free fatty acids for its own use, resulting in a decrease in its abundance. In addition, malonyl CoA serves as both an inhibitor of fatty acid oxidation and a stimulator of fatty acid synthesis. Consequently, fatty acid oxidation and fatty acid synthesis fluctuate reciprocally with changes in malonyl CoA levels.[48] The decrease of fatty acids synthesized from the malonyl CoA pathway may also diminish the available substrates for *C. acnes* utilization. Therefore, we speculated that oral and topical XOS may inhibit cutaneous lipid synthesis and triglyceride production by reducing fructose phosphorylation, thereby decreasing the abundance of lipophilic *C. acnes*.

Lactic acid is commonly used as a therapeutic agent for acne, significantly decreasing the area and number of pores.[49] The findings of this study indicated that the lactic acid metabolism was reduced, which may play a role in attenuating facial pores. The changes were consistent with the indexes detected by the VISIA system of pores pre- and post-intervention.

Gal-1P, as a metabolite of D-gal, exhibits cytotoxic potential and can induce dermal fibroblast senescence *in vitro*. Gal-1P significantly increases the levels of NO and inducible nitric oxide (iNOS) in fibroblast medium.[50] Fibroblasts are responsible for synthesizing and secreting collagen and elastin, which directly determine skin elasticity. Reduced elasticity surrounding pores leads to increased facial pore size.[51] Therefore, XOS may alleviate fibroblast impairment by inhibiting the metabolic pathway of D-gal to Gal-1P, potentially resulting in a reduction in both volume and number of facial pores.

Additionally, XOS was found to significantly decrease glycerolipid metabolism in the T_Post_R, O_Post_L, and OT_Post_L groups (Fig. 7), indicating a reduction in lipid metabolism related genera. Furthermore, K01046 (encoding triacylglycerol lipase) was also reduced in the O_Post_L and OT_Post_L groups. Triacylglycerol lipase is involved in the production of fatty acids within the glycerolipid metabolism pathway. *C. acnes* is known to break down triglycerides into fatty acids,[46,52] which were found to decrease after the XOS intervention in our study. These results demonstrated that XOS reduced the abundance of cutaneous *Cutibacterium*, resulting in a decline in the glycerolipid metabolic pathway and subsequent reduction in lipase and metabolite production of fatty acids. However, it is imperative to validate this predic-

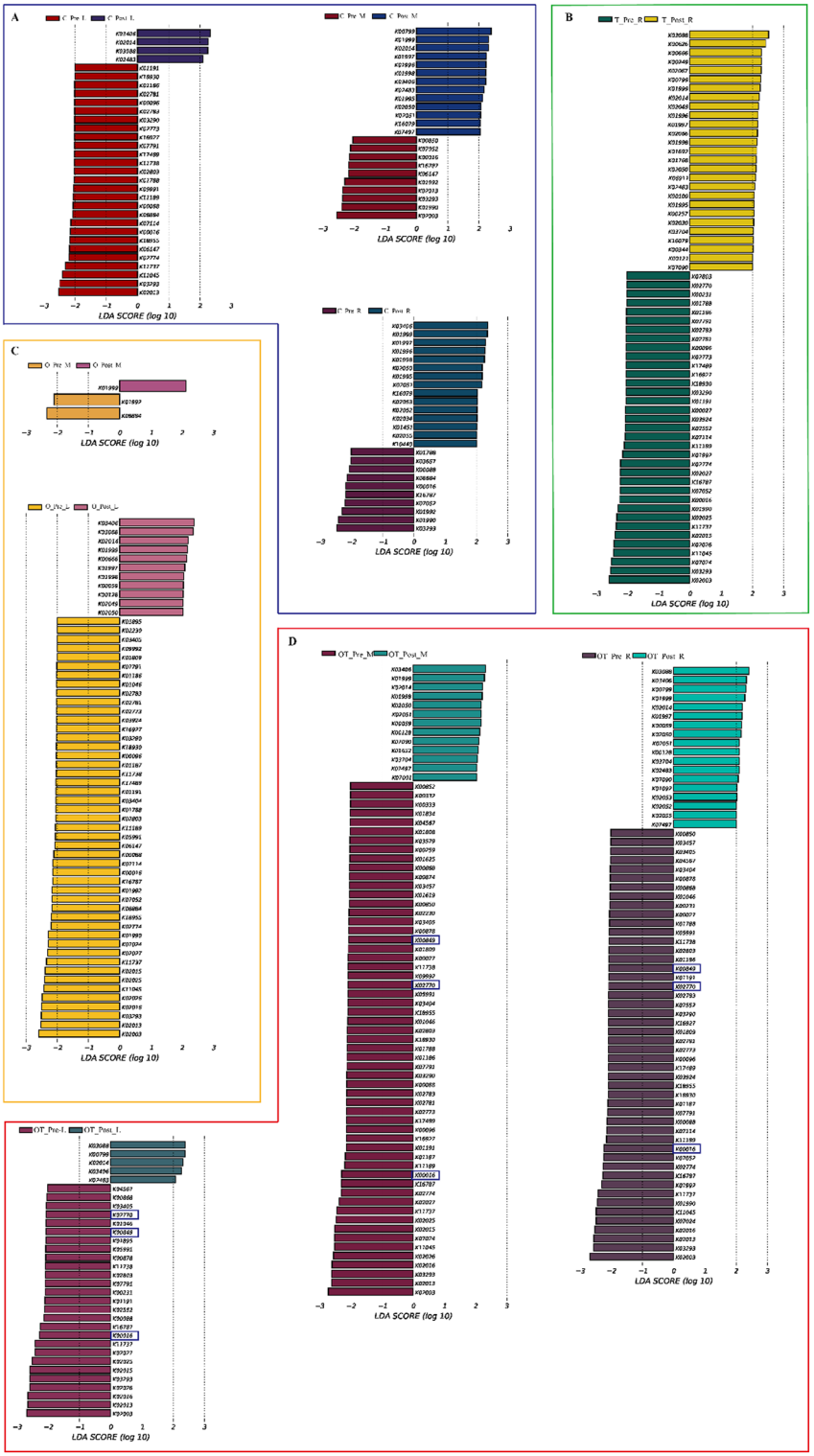

Fig. 7 Differential KO of the skin microbiota in the (A) C group, (B) T group, (C) O group, and (D) OT group at the left-, forehead, and right-side facial skin sites pre- and post-intervention, respectively.

tion with the help of other detection methods such as metabolomics and protein expression analysis. Furthermore, the identification of significantly enriched or reduced metabolic pathways warrants further investigation.

3.2.4.2 Gut microbial function predicted by PICRUSt. Key pathways were predicted based on the functional analysis of gut microbiota function and KO analysis. The pathways significantly enriched in group OT after intervention included

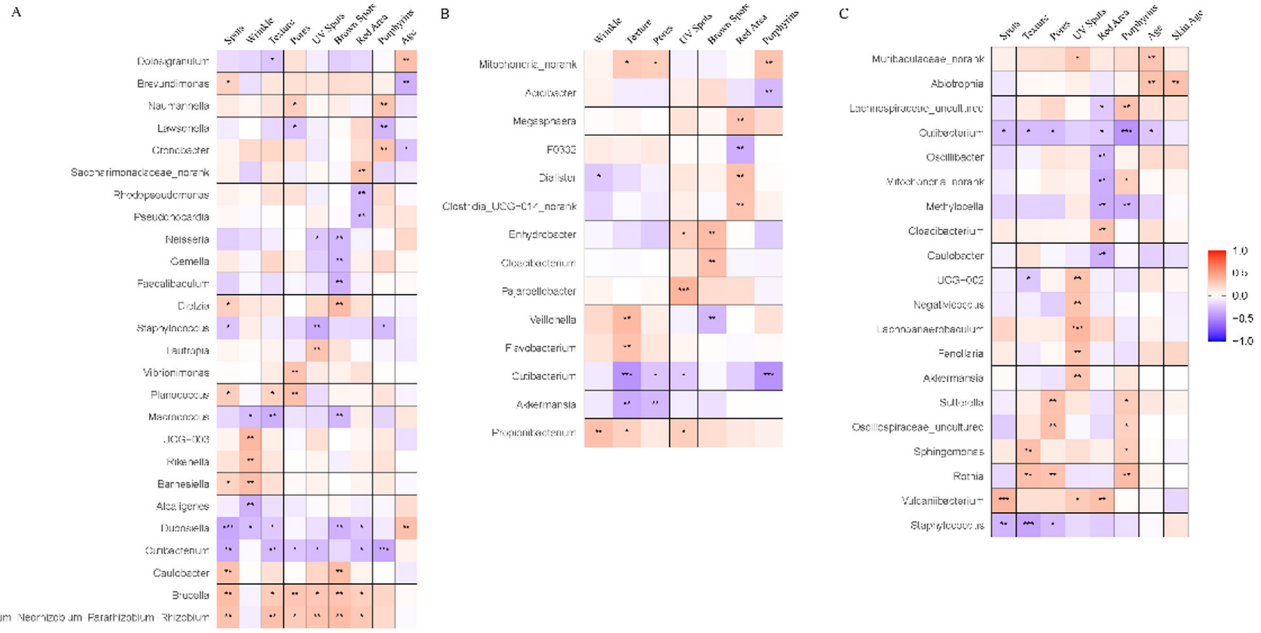

Fig. 8 Correlation analysis between the VISIA index and the skin microbiota on the (A) left-side cheek, (B) forehead, and (C) right-side cheek facial skin. Data were analyzed by calculating the Spearman correlation coefficient. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

ko00625 (chloroalkane and chloroalkene degradation), ko00071 (fatty acid degradation), and ko00643 (styrene degradation). No significant changes in the pathways were observed in either the O or the T group before and after the intervention. Following intervention in the OT group, K02025 (encoding multiple sugar transport system permease protein), K02026 (encoding multiple sugar transport system permease protein), K03496 (encoding chromosome partitioning protein) and K08884 (encoding serine/threonine protein kinase, bacterial) exhibited a significant increase. Conversely, no significant change was observed for KO within the O and T groups before and after the intervention (Fig. S6 and S7†).

3.4 Correlation between the VISIA index and skin microbiota

The Spearman analysis was utilized to investigate the correlation between key genera and fundamental clinical cutaneous indexes. *Staphylococcus* was significantly negatively correlated with the score of spots, pores, and textures ((Fig. 8), $p < 0.05$). Notably, the strongest correlation was observed specifically between the abundance of *Staphylococcus* and the score of pores. Pores and porphyrins are commonly found in the sebaceous gland region, which is predominantly inhabited by *Staphylococcus.*[9] *Cutibacterium* exhibited a significant negative correlation with the score of textures, pores and porphyrins in three-side facial skin, with the strongest correlation observed for porphyrins. Porphyrins can be synthesized and stored by *C. acnes*,[53] and our findings support the conclusion that *C. acnes* has the capability to synthesize porphyrins. Targeting porphyrin synthesis could be a promising approach for treating acne by selectively inhibiting the growth of acne-related strains without affecting non-acne strains or symbiotic organisms.[54] These results demonstrated the potential of combined treatment with oral and topical administration of XOS to decrease the abundance of lipophilic microorganisms such as *Cutibacterium*, thereby reducing porphyrins and mitigating the acne risk in women.

4. Conclusion

Dual intervention targeting the gut and skin microbiota could increase the proportion of participants with skin rejuvenation, along with attenuating the facial pores. Following the intervention, decreased cutaneous *Cutibacterium* abundance and an enrichment in the intestinal *Bifidobacterium* were observed compared with oral or topical intervention. Function prediction analysis revealed a downregulation of K02770, which encodes fructose-1-phosphate kinase involved in *de novo* lipid synthesis from fructose under dual intervention. These findings suggest that the inhibition of lipophilic *Cutibacterium* may contribute to the reduction of facial pores associated with aging. The impact on the skin microbiota by the gut microbiota provides a novel perspective to comprehend the skin–gut axis. Future researchers could pay more attention to the roles of skin and gut microbiota in resisting skin aging and create novel formulas based on the gut–skin axis.

Author contributions

Liujing Zhang: investigation, writing – original draft, and writing – review & editing. Shun Yu: methodology. Yin Guan: investigation and formal analysis. Dan Wang: visualization. Ju

Yang: project administration. Jingling Li: investigation. Wei Zhao: conceptualization and supervision. Feng Zhang: conceptualization.

Conflicts of interest

There are no conflicts to declare.

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