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Research Article

Metformin Contributes to the Therapeutic Effects of Acne Vulgaris by Modifying the Gut Microbiome

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Background. Considering the increasing side effects of the first-line treatment for acne vulgaris, metformin was developed to be an effective adjunct therapy, but its mechanism of action is poorly defined. Recent evidence shows that the gut microbiota is a site of metformin action. The aim of this study was to evaluate the effects and mechanism of action for metformin in the adjuvant treatment of acne vulgaris by regulating gut microbiota. *Methods*. First, untreated acne patients were randomly allocated into two treatment groups. Both groups were treated with isotretinoin, but only one was additionally treated with metformin, for three months. Sprague Dawley (SD) rats were used as acne models, and they were also separated into groups that received isotretinoin, metformin, a combination of isotretinoin and metformin, and the vehicle, respectively. Then, the fecal samples from drug-intervention rats were transferred to germ-free rats with acne. The severity of the disease was evaluated using the Global Acne Grading System (GAGS) scoring for patients, and the number of comedones and mononuclear cells in pathological sections was used for rats. The composition of the gut microbiota was detected using gene sequencing for 16S rDNA. *Results*. Metformin had strong effects on the composition and function of the gut microbiota, and this correlated with the reduction in the severity of acne in both humans and rats. The fecal transfer to pseudo-germ-free rats improved both the inflammatory phenotype and comedones of acne in recipients of metformin-altered microbiota. *Conclusion*. The results suggest that metformin improves the symptoms of acne vulgaris by modulating the gut microbiota.

1. Introduction

Acne vulgaris is a cutaneous chronic inflammatory disorder that affects approximately 85% of adolescents. This condition can cause permanent physical scarring, distortion of the facial appearance of patients, and an overall reduction of the quality of life [1, 2]. According to the care guidelines for the management of acne vulgaris, a combination of topical therapies and systemic agents such as antibiotics and isotretinoin is recommended for the treatment of moderate to severe cases [3]. Due to the concerns that are related to the increasing antibiotic resistance, new approaches have been developed as promising treatments for acne vulgaris. The adverse effects of isotretinoin, such as cheilitis, xerosis, acne

flare, photophobia, elevated liver enzymes, and depressed mood, also raised the need for new treatment methods for acne vulgaris [1, 4]. Interestingly, metformin, which is an oral antihyperglycemic agent, is one of the new treatment approaches that was recently reported to significantly reduced acne severity in women with polycystic ovary syndrome (PCOS) [5, 6]. The relationship between acne vulgaris and insulin resistance in females with PCOS could be the explanation for this groundbreaking finding. Recent studies also revealed that metformin is an effective adjunct therapy for the treatment of moderate to severe acne vulgaris in patients who have not been previously diagnosed with PCOS or androgen excess [7–9]. The study suggested that metformin's mechanism of action in the treatment of acne is not completely attributed to its direct action against insulin resistance.

It is well known that the oral administration of metformin in humans could improve glycemia, but intravenous administration does not yield similar results. This implies that the action of metformin could be associated with the gut. In recent years, there has been accumulating evidence suggesting that metformin alters the gut microbiota by increasing the mucin-degrading*Akkermansia muciniphila*, as well as several short-chain fatty acids (SCFAs). The microbial metabolites such as butyrate and propionate may then increase intestinal gluconeogenesis, thereby controlling glycemic control [10]. Additionally, metformin was reported to restore hippocampal neurogenesis, as well as learning and memory by regulating the gut microbiota [11]. On the other hand, acne vulgaris has long been reported to affect some gastrointestinal mechanisms [12–15]. However, the notion that metformin treatment could improve acne vulgaris by modulating the gut microbiota has not been explored. Moreover, the underlying mechanism of action by metformin is also relatively unknown, but this will be discussed in this study based on results from both human and animal models.

2. Materials and Methods

2.1. Population. From June 2019 to April 2020, 46 patients who were diagnosed with moderate to severe acne vulgaris and 20 healthy controls were enrolled in this study. All participants aged 18 to 30 years came from universities in Luzhou city, Sichuan province. The severity of acne vulgaris was determined using the GAGS score, where GAGS≥ 19 was defined as moderate to severe acne vulgaris. The exclusion criteria were as follows: other dermatoses such as atopic dermatitis, eczema, psoriasis, ichthyosis, and inflammatory bowel syndrome; systemic diseases such as obesity, fatty liver disease, diabetes mellitus, and malignancy; addiction to smoking and alcohol. All patients included in this study were treatment-naïve and were required not to take any systemic antibiotics, retinoids, corticosteroids, or immunosuppressive agents within a period of six months. Before the study procedures, all participants signed written informed consent forms that emphasized the use of data and samples for scientific purposes. The study was approved by the ethics committees of the hospital, affiliated with Southwest Medical University (no: KY2019139).

2.2. Clinical Trail Design. Patients were randomly selected to receive the isotretinoin capsule (0.25 mg/kg/d) [16] either with (named the AVM group) or without (named the AVP group) the metformin hydrochloride tablet (500 mg) daily, for 12 weeks. The demographic data, including age, gender, body mass index (BMI), and course of disease, were recorded before enrollment. The GAGS score, porphyrin, and red areas obtained using the VISIA-CR™ imaging system (Canfield Scientific Inc., USA) were used to evaluate the level of improvement in the acne patients [17]. Fresh blood and stool samples were taken at the baseline and also 12 weeks after treatment. Blood investigations included the full blood count, renal and liver function tests, fasting blood glucose (GLU), serum insulin (INS), and blood lipids. Fresh stool samples from each participant were collected in sterile containers, immediately homogenized, divided into five aliquots of 220 mg each, frozen at −80°C for 30 minutes, and then prepared for 16S rDNA sequencing. Patients were followed up every month, and pills were counted during each visit to confirm adherence to the study protocol. The full, detailed clinical trial protocol has been registered (ChiCTR2200058107).

2.3. Acne Model Construction. Three-week (100 ± 20 g) and six-week (200 ± 20 g) male SD rats were purchased, and all experiments commenced one week after adaptive feeding. All experiments were repeated three times. The right auricle of the SD rats was chosen as the modeling site. First, 0.3 ml of the oleic acid extract (purity ≥99.8%) was applied evenly on the auricle daily [18]. Meanwhile, an inactivated *Cutibacterium acnes* (*C. acnes*) suspension (1×10^7 cells/ml) (ATCC6919; Guangzhou Zuoke Biotechnology Development Co., Ltd., China) [19] was injected subcutaneously into the right auricle at a dose of 50 μl/200 g, once every two days, for two weeks.

2.4. Drug Intervention in SD Rats. Seven-week-old male SD rats were randomly treated with metformin (200 mg/kg/d) [20], isotretinoin (1 mg/kg/d) [21], metformin combined with isotretinoin, and a soya oil vehicle (0.25 ml/d) [22] through gavage feeding for two weeks. The SD rats' right auricle that had been injected with normal saline served as the model control.

2.5. Preparation of Fecal Microbiota Suspension from Donor Rats. Seven-week-old male SD rats were randomly treated with metformin (200 mg/kg/d) [20], isotretinoin (1 mg/kg/d) [21], metformin combined with isotretinoin, and soya oil vehicle (0.25 ml/d) [22] through gavage feeding for two weeks. The right auricle of SD rats injected with normal saline served as the model control. The five groups were named as follows: Met (metformin + acne model), Iso (isotretinoin + acne model), Met + Iso (isotretinoin + metformin + acne model), Acne (vehicle + acne model), and Con (blank control) group, respectively. Each group had three rats, and every experiment was repeated three times. Before and after

treatment, the stool samples and auricle tissues in all groups were collected for subsequent experiments and detection.

2.6. Pseudo-Germ-Free Rat Modeling and FMT. The experiment for pseudo-germ-free rat modeling and FMT were carried out as reported in the literature, with slight modification [23, 24]. Briefly, four-week-old male SD rats were treated with fresh broad-spectrum antibiotics (neomycin sulfate (1 g/L), metronidazole (1 g/L), and ampicillin (1 g/L)) in drinking water (renewed every two days) for two weeks. This was carried out to make the gut sterile. The stored FMS mixture (2 mL/d, at room temperature) was placed in a 37°C-water bath for four to five hours before being transferred to the pseudo-germ-free rats by gavage feeding for seven days. The same volume of saline was gavaged into the control rats at the same time. The acne model was then set up, and FMT was continuously performed every day for 14 days. The FMT groups were named as follows: Met-Tr, Iso-Tr, (Met + Iso)-Tr, Acne-Tr, and Con-Tr, according to donor grouping. The SD rats in the Con-Tr group received saline by injection into the right auricle.

2.7. Evaluation of the Acne Phenotype in the Rat Model. At the beginning and end of the acne modeling procedure, SD rats in each group were photographed and the auricle thickness was measured using an electronic vernier caliper. The auricle swelling rate (%) = ((auricle thickness after acne modeling-auricle thickness before acne modeling)/auricle thickness before acne modeling) × 100%. Based on the histopathological section with hematoxylin and eosin (HE) staining, the number of comedones and monocytes was calculated per field of vision.

2.8. 16S rDNA Amplicon Sequencing and Sequence-Based Analysis. DNA was extracted from stool samples (200–300 mg) using the Stool Genomic DNA Extraction Kit (Solebo Technology Co., LTD., Beijing, China) according to the manufacturer's instructions. The V3-V4 hypervariable regions of the bacteria's 16S rRNA gene were amplified with barcode-indexed primers (341F 5′-CCTACGGGRSGCAG-CAG-3′) and (806R 5′-GGACTACVVGGGTATCTAATC-3′). The KAPA HiFi Hotstart ReadyMix PCR kit was used for high-fidelity amplification. Then, amplicons were quantified using the QuantiFluor™-ST blue fluorescence quantitative system (Promega, USA). The purified amplicons were pooled in equimolar concentration, and paired-end sequencing was performed using an Illumina Miseq instrument (Illumina, San Diego, California, USA). The data analysis was carried out using the Majorbio Cloud Platform (https://www.majorbio.com/), which is a free online platform.

2.9. Statistical Analysis. All data were analyzed using SPSS version 21. Over 90% of the variables showed abnormal distribution, according to the Shapiro–Wilk test. Therefore, the variables were then analyzed using the Kruskal–Wallis rank-sum and Wilcoxon rank-sum tests. Results were reported as mean ± standard deviation (SD). Statistical significance was defined as $P < 0.05$. The GraphPad Prism 8.0 software package was used for plotting the graphs.

3. Results

3.1. Background and Efficacy of Metformin Treatment in Patients with Moderate to Severe Acne. Although a total of 46 patients and 20 healthy controls were initially allowed into the study, only 40 patients were analyzed. Three patients in the AVM group and one case in the AVP group withdrew because they were unable to adhere to the stipulations of the study. Two individuals in the AVP quit the trial due to severe cheilitis. Please note that the demographic data of all participants are shown in Table 1. Compared with the baseline, the GAGS scores in both AVP and AVM decreased significantly ($P < 0.001$ and $P < 0.001$), and the indexes of the red area (%) and porphyrin (%) improved ($P < 0.05$) after treatment in both groups. There was no difference in the GAGS scores of the AVM and AVP groups at the baseline. After treatment, the GAGS scores for the AVM group were lower than those for the AVP group ($P = 0.018$), and this indicated the effective adjunct therapy of metformin.

3.2. Metformin Restored the Gut Microbiota of Patients with Acne. To determine the effects of metformin on the gut microbiome, we performed 16S rDNA sequencing on 100 fecal samples. However, three participants from the AVM group, three from the AVP group, and five from the control group (CG) were removed due to the failure of the fecal bacteria DNA extraction. On average, we obtained 30,826 paired-end reads for each sample. The 83 samples resulted in a total of 1092 OTUs at a 3% dissimilarity cutoff, and this meant about 109 to 396 OTUs per sample. Compared with the CG, the microbial diversity significantly decreased in both the AVP and AVM groups at the baseline, based on the calculations that were carried out using the Shannon diversity index ($P = 0.0009$ and $P = 0.0004$) and Simpson diversity index ($P = 0.012$ and $P = 0.007$) (Figures 1(a) and 1(b)). There was no significant difference in microbial diversity between the AVP and AVM groups at the baseline (Shannon: $P = 0.496$ and Simpson: $P = 0.185$). After treatment, the Shannon diversity index ($P = 0.036$) was higher, while the Simpson index ($P = 0.026$) was lower in the AVM group compared to the AVP group (Figures 1(a) and 1(b)). Moreover, the Shannon ($P = 0.046$) and Simpson ($P = 0.066$) diversity indexes in the AVM group improved after treatment. However, there was no difference in microbial diversity in the AVP group before and after treatment (Figures 1(a) and 1(b)).

The PCoA analysis was carried out to assess the discrepancies based on the OTUs with different relative abundances. From the PCoA plot, we observed a tendency to form two clusters at the baseline which were the AVP and AVM cluster (red and green dots) and CG cluster (blue dots)

Table 1: Clinical and laboratory findings of moderate to severe acne patients and healthy controls.

Factors	Before treatment			After treatment	
	CG ($n = 20$)	AVP_1 ($n = 20$)	AVM_1 ($n = 20$)	AVP_2 ($n = 20$)	AVM_2 ($n = 20$)
Gender (F/M, n)	6/14	3/17	7/13	3/17	7/13
Age (years)	$25.40 \pm 2.64^{\#}$	20.90 ± 1.94	20.30 ± 2.56	—	—
BMI (kg/m^2)	20.08 ± 1.61	21.02 ± 2.47	20.52 ± 1.81	—	—
Disease duration (months)	—	52.45 ± 31.08	51.60 ± 24.35	—	—
Facial GAGS	—	28.60 ± 4.96	28.80 ± 4.65	$18.70 \pm 5.17^{*\bigstar}$	$15.55 \pm 4.79^{\blacktriangle}$
Red area (%)	$67.98 \pm 17.30^{\#}$	29.32 ± 15.13	30.78 ± 20.19	$47.62 \pm 20.37^{*}$	$43.87 \pm 21.61^{\blacktriangle}$
Porphyrin (%)	$75.27 \pm 8.32^{\#}$	45.45 ± 20.52	57.58 ± 23.27	$74.18 \pm 20.85^{*}$	$77.10 \pm 19.90^{\blacktriangle}$
Cholesterol (mmol/L)	3.99 ± 0.46	3.88 ± 0.55	3.82 ± 0.56	3.96 ± 0.59	4.00 ± 0.69
Triglyceride (mmol/L)	0.95 ± 0.43	0.86 ± 0.32	0.89 ± 0.26	1.00 ± 0.36	1.06 ± 0.49
LDL (mmol/L)	2.30 ± 0.36	2.17 ± 0.50	2.24 ± 0.73	2.42 ± 0.61	2.42 ± 0.77
HDL (mmol/L)	1.43 ± 0.27	1.41 ± 0.37	1.36 ± 0.31	1.27 ± 0.27	1.30 ± 0.35
GLU (mmol/L)	4.59 ± 0.38	4.78 ± 0.30	4.60 ± 0.21	4.64 ± 0.29	4.69 ± 0.30
INS (pmol/L)	7.85 ± 2.98	6.65 ± 3.29	7.36 ± 2.55	6.84 ± 2.11	8.02 ± 3.02
Home-IR	1.61 ± 0.67	1.43 ± 0.73	1.51 ± 0.53	1.41 ± 0.44	1.67 ± 0.65
ALT (U/L)	20.80 ± 9.79	23.83 ± 7.44	22.32 ± 15.80	20.61 ± 12.65	20.99 ± 13.92
AST (U/L)	20.18 ± 3.98	18.03 ± 5.27	23.58 ± 15.10	20.88 ± 6.06	21.82 ± 10.33

Note. Data were presented as mean ± SD. ALT: alanine transaminase, AST: aspartate aminotransferase, CG: healthy control, AVP_1: AVP group before treatment, AVP_2: AVP group after treatment, AVM_1: AVM group before treatment, and AVM_2: AVM group after treatment. $^{\#}$(CG vs. AVP_1 and AVM_1): $^{\#}P < 0.05$, *(AVP1 vs.): $^{*}P < 0.05$, $^{\blacktriangle}$(AVM_1 vs.): $^{\blacktriangle}P < 0.05$, and $^{\bigstar}$(AVM_2 vs. AVP_2): $^{\bigstar}P < 0.05$.

($P = 0.007$) (Supplementary Figure S1A). After treatment, the plots of the AVM group obviously separated from the ones at the baseline ($P = 0.044$) (Figure 1(c)), though the before and after treatment plots of the AVP group overlapped ($P = 0.445$) (Figure 1(d)).

3.3. Metformin Alters the Gut Microbiota at Both Phylum and Genus Levels in Patients with Acne Vulgaris. A total of 612 bacterial taxa were analyzed in our samples, with 305 taxa being at the genus level. At the phylum level, the ratio of the Bacteroidetes and Firmicutes (B/F) was lower in acne patients than it was in CG. When compared with the baseline, the ratio increased in the AVM group after 12 weeks of taking metformin ($P = 0.006$). However, there was no change in the ratio in the AVP group before and after treatment (Figure 2(a)). Additionally, the relative abundance of *Desulfobacterota* ($P = 0.001$ and $P < 0.001$) was significantly lower and that of *Proteobacteria* ($P = 0.001$ and $P = 0.005$) was higher in both the AVP and AVM groups before treatment, compared to the CG. When metformin was used to treat the AVM group, the relative abundances of *Desulfobacterota* and *Proteobacteria* significantly increased and declined, respectively, compared with those at the baseline ($P < 0.001$ and $P < 0.001$, Figures 2(b) and 2(c)). The relative abundances of *Proteobacteria* and *Desulfobacterota* did not change in the AVP group after treatment with isotretinoin alone ($P > 0.05$, Figures 2(b) and 2(c)). At the genus level, the relative abundances of Bacteroides ($P = 0.047$ and $P = 0.028$) and *Bifidobacterium* ($P = 0.063$; $P = 0.065$) were relatively lower, but *Ruminococcus* ($P = 0.063$ and $P = 0.001$) was higher in both the AVP and AVM groups at the baseline than in the CG group (Figures 2(d)–2(g)). After treatment, the relative abundances of Bacteroides ($P < 0.001$, Figure 2(d)) and *Bifidobacterium* ($P = 0.031$, Figure 2(e)) increased, while those of *Ruminococcus* ($P < 0.001$, Figure 2(f)) and *Romboutsia* ($P < 0.001$, Figure 2(g)) reduced in the AVM group. However, there was no difference in the relative abundances of Bacteroides, *Bifidobacterium*, *Ruminococcus*, and *Romboutsia* before and after treatment in the AVP group ($P > 0.05$, Figures 2(d)–2(g)).

Based on the KEGG analysis, the pathways that improved as a result of metformin treatment were the butanoate metabolism, propanoate metabolism, AMPK signaling pathway, secondary bile acid biosynthesis, and PPAR signaling pathway (Supplementary Figures 2A and 2B).

3.4. Metformin Reduces the Inflammatory Phenotype in Acne Models of SD Rats. To confirm the role of metformin treatment on acne, we administered isotretinoin, metformin, isotretinoin + metformin, or the vehicle in the acne models of SD rats. Besides the negative control group, the Met + Iso group showed the lightest redness and swelling of the right auricle after two weeks of administering the interventions (Figure 3(a)). After treatment the auricle swelling rates in Iso (1.734 ± 0.288), Met (1.537 ± 0.218), and Met + Iso (1.462 ± 0.167) groups were lower than what was observed in the Acne group (4.150 ± 1.364) ($P < 0.001$; $P < 0.001$; $P < 0.001$) (Figure 3(b)). Please note that the swelling rate in the Met + Iso group was the lowest ($P = 0.038$).

The results from the histological examination of the right auricle tissues by HE staining are shown in Figure 3(a). The acne group had the most comedones and mononuclear cells per field of vision at 40 magnifications, compared to the other groups. The average number of comedones in the Iso (13.185 ± 3.024) and Met + Iso (12.926 ± 2.493) groups were both significantly lower than that in the Met (20.685 ± 4.984) ($P = 0.003$ and $P = 0.001$) (Figure 3(c)). However, the number of mononuclear cells

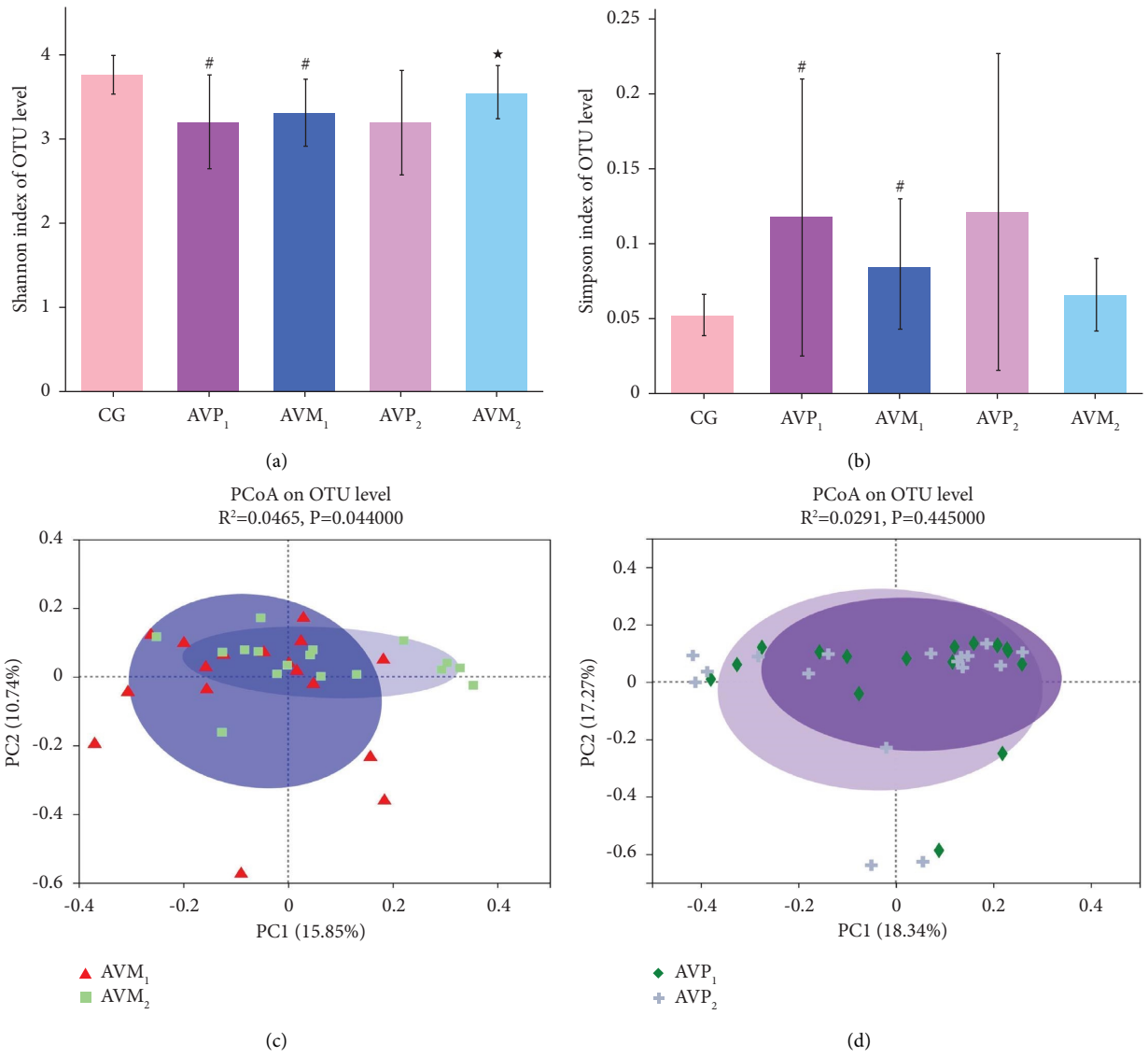

Figure 1: The alpha and beta diversity of the gut microbiota for healthy controls and patients with moderate to severe acne vulgaris before and after treatment: (a) comparison of the Shannon index between the healthy samples and patients before and after treatment, (b) comparison of the Simpson index between the healthy samples and patients before and after treatment, (c) PCoA analysis of the microbiota composition in the AVM group before (AVM_1) and after treatment (AVM_2), and (d) PCoA analysis of the microbiota composition in the AVP before (AVP_1) and after treatment (AVP_2). $^{\#}$(CG vs.): $^{\#}P < 0.05$; *(AVP_1 vs. AVP_2) and (AVM_1 vs. AVM_2): $^{\star}P < 0.05$.

in both the Met (18.648 ± 2.856) and Met + Iso (17.907 ± 7.128) groups was lower than that in the Iso group (26.556 ± 5.701) ($P = 0.003$ and $P = 0.024$) (Figure 3(d)) when metformin was administered.

3.5. Metformin Alters the Gut Microbiota Composition of SD Rats. Our next question was whether metformin treatment could alter the gut microbiota composition in SD rats. After the metformin intervention, the microbial diversity in Met (Shannon: $P = 0.002$ and Simpson: $P = 0.0002$) and Met + Iso (Shannon: $P = 0.023$) was higher than that at the baseline (Figures 4(a) and 4(b)). However, there was no change in both the Shannon and Simpson indexes in the Iso group before and after treatment. The PCoA analysis also showed that the plots before and after treatment separated in both the Met and Met + Iso groups ($P = 0.003$ and $P = 0.007$) (Figures 4(c) and 4(d). Nevertheless, there were no significant differences in the Iso group before and after treatment ($P = 0.334$) (Supplementary Figures S1B). To further identify gut microbes that were altered by metformin in rats, the ratio of B/F, the phylum of Proteobacteria and Desulfobacterota, and the genus of Bacteroides, Bifidobacterium, Ruminococcus, and Romboutsia were compared among the five groups. The results that were obtained were similar to the reports from the clinical data and the KEGG-based functional analysis (Supplementary Figures S3A to S3G, S2C and S2D).

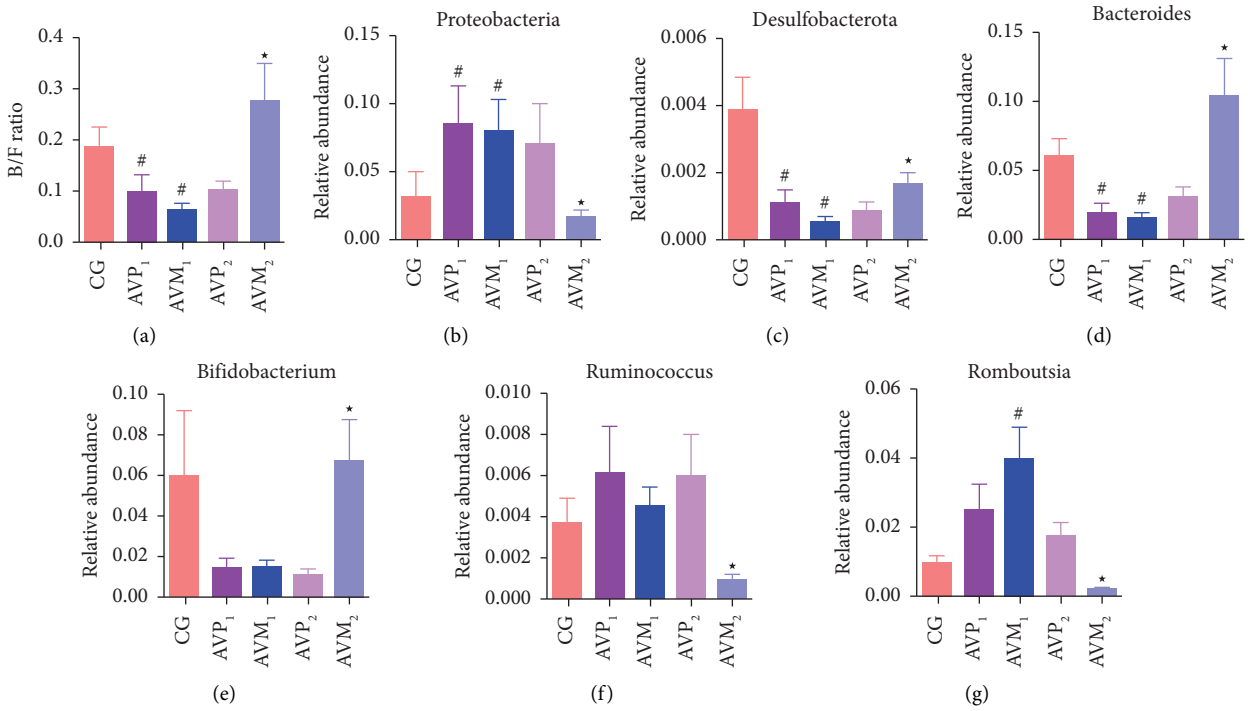

Figure 2: The difference in microbial composition for patients before and after treatment at both phylum and genus levels. (a) Comparison of the relative abundance ratios of Bacteroidetes and Firmicutes (B/F) for patients before and after treatment; (b, c) the difference in microbial composition for patients before and after treatment at the phylum level; (d–g) the difference in microbial composition for patients before and after treatment at the genus level. CG: healthy control, AVP_1: AVP group before treatment, AVP_2: AVP group after treatment, AVM_1 group before treatment, and AVM_2 group after treatment. $^{\#}$(CG vs.): $^{\#}P < 0.05$; *(AVP_1 vs. AVP_2) and (AVM_1 vs. AVM_2): $^{\star}P < 0.05$.

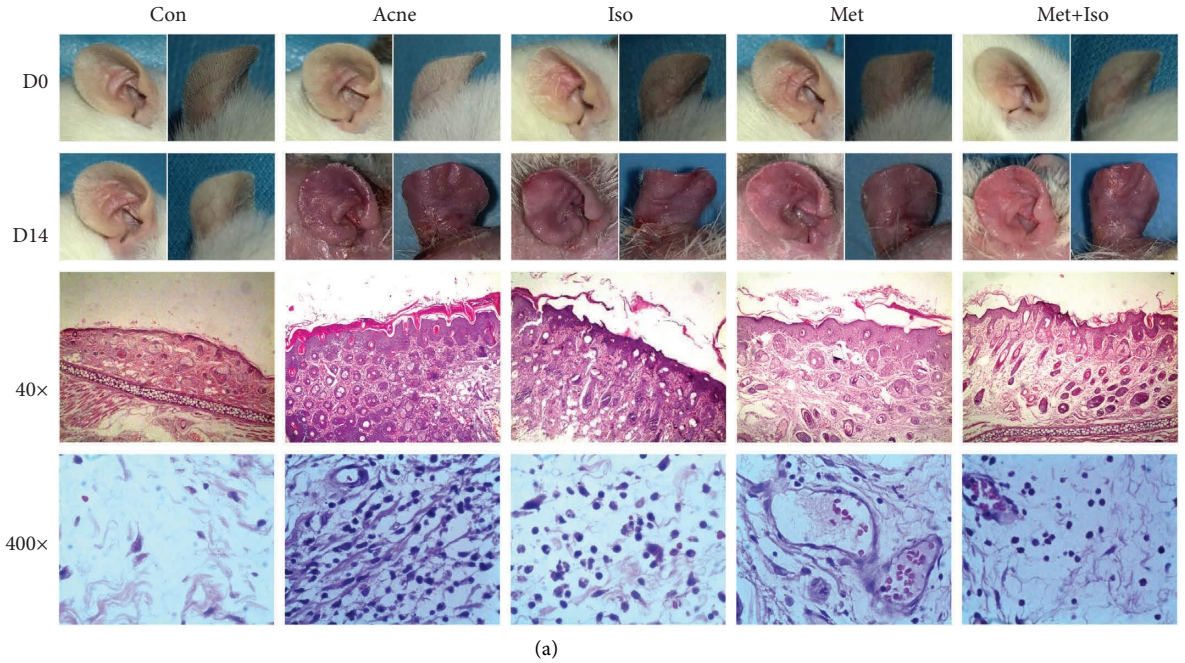

Figure 3: Continued.

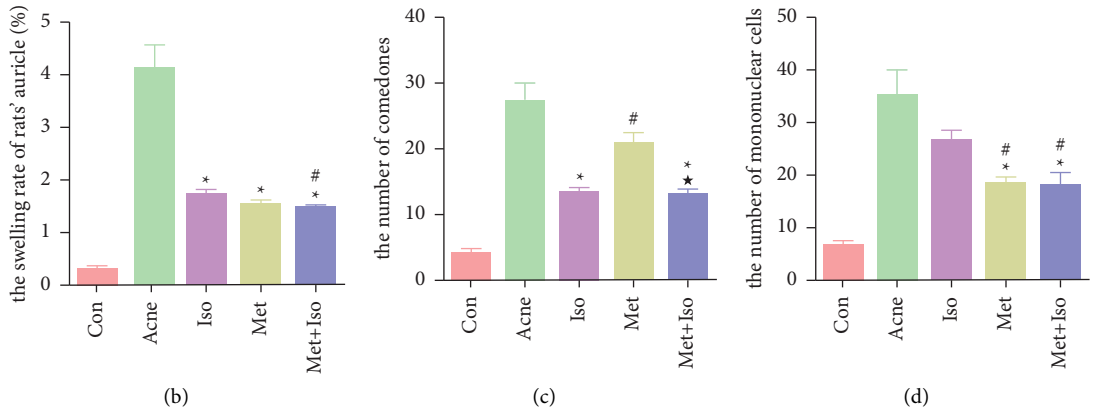

FIGURE 3: Effects of metformin administration on skin lesions and the pathological manifestation of acne in SD rats. (a) Skin appearance and histological observations of the right auricle in the five groups on the first and 14th days of the experiment; (b) comparison of the auricle swelling rate in SD rats in the five groups after treatment; (c, d) comparison of the number of comedones and mononuclear cells in the five groups after treatment. *(Acne vs.): $^{*}P < 0.05$, $^{\#}$ (Iso vs.): $^{\#}P < 0.05$, and $^{\bigstar}$(Met vs. (Met + Iso)): $^{\bigstar}P < 0.05$.

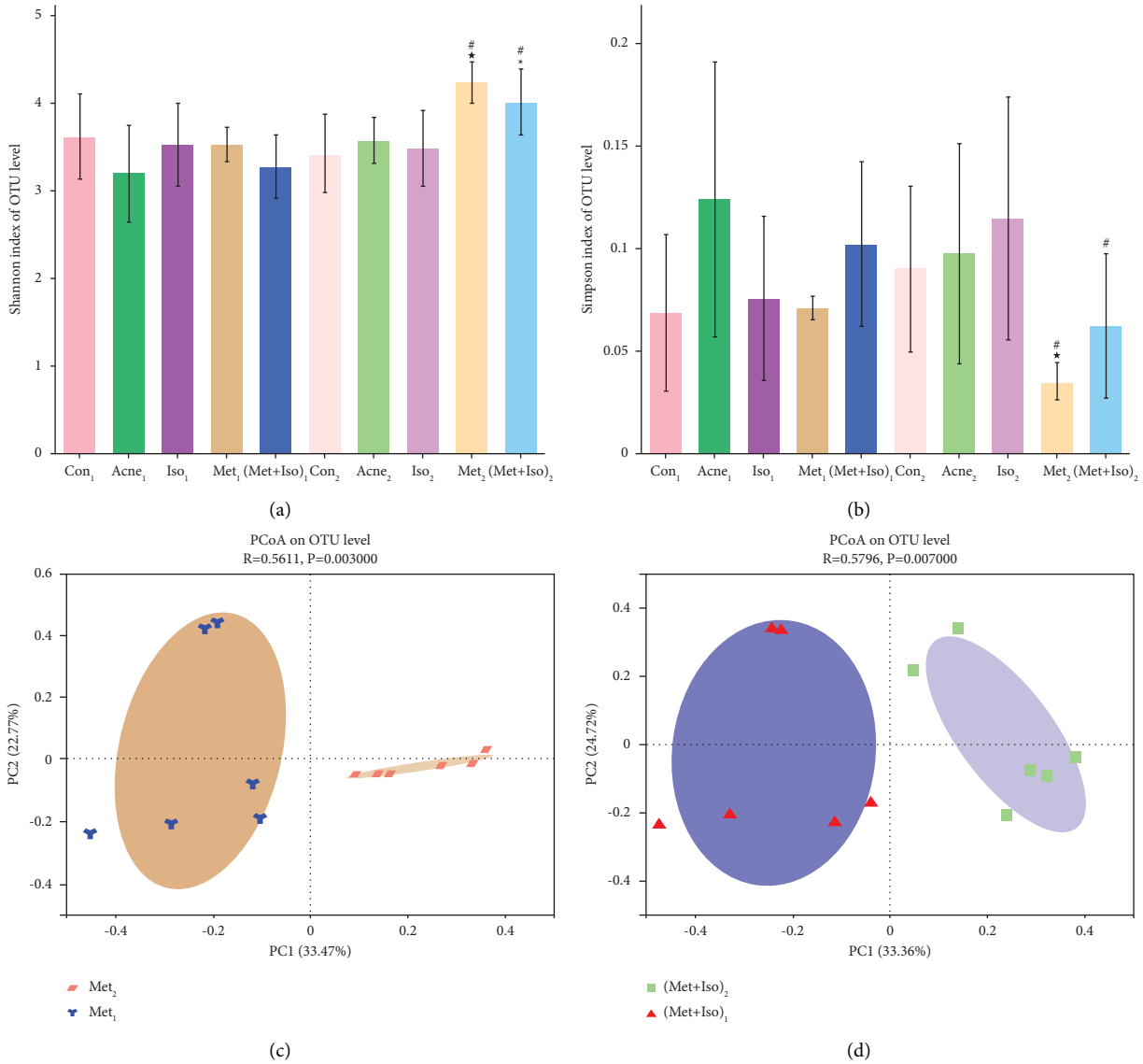

FIGURE 4: The alpha and beta diversity of gut microbiota in SD rats before and after different drug interventions: (a) comparison of the Shannon index before and after different drug interventions, (b) comparison of the Simpson index before and after different drug interventions, (c) PCoA analysis of the microbiota composition in SD rats in the Met group before and after treatment, and (d) PCoA analysis of the microbiota composition in SD rats in the Met + Iso group before and after treatment. $^{\#}$ (Iso_2 vs.): $^{\#}P < 0.05$, $^{\bigstar}$(Met_1 vs. Met_2): $^{\bigstar}P < 0.05$, and *($(Met + Iso)_1$ vs. $(Met + Iso)_2$: $^{*}P < 0.05$.

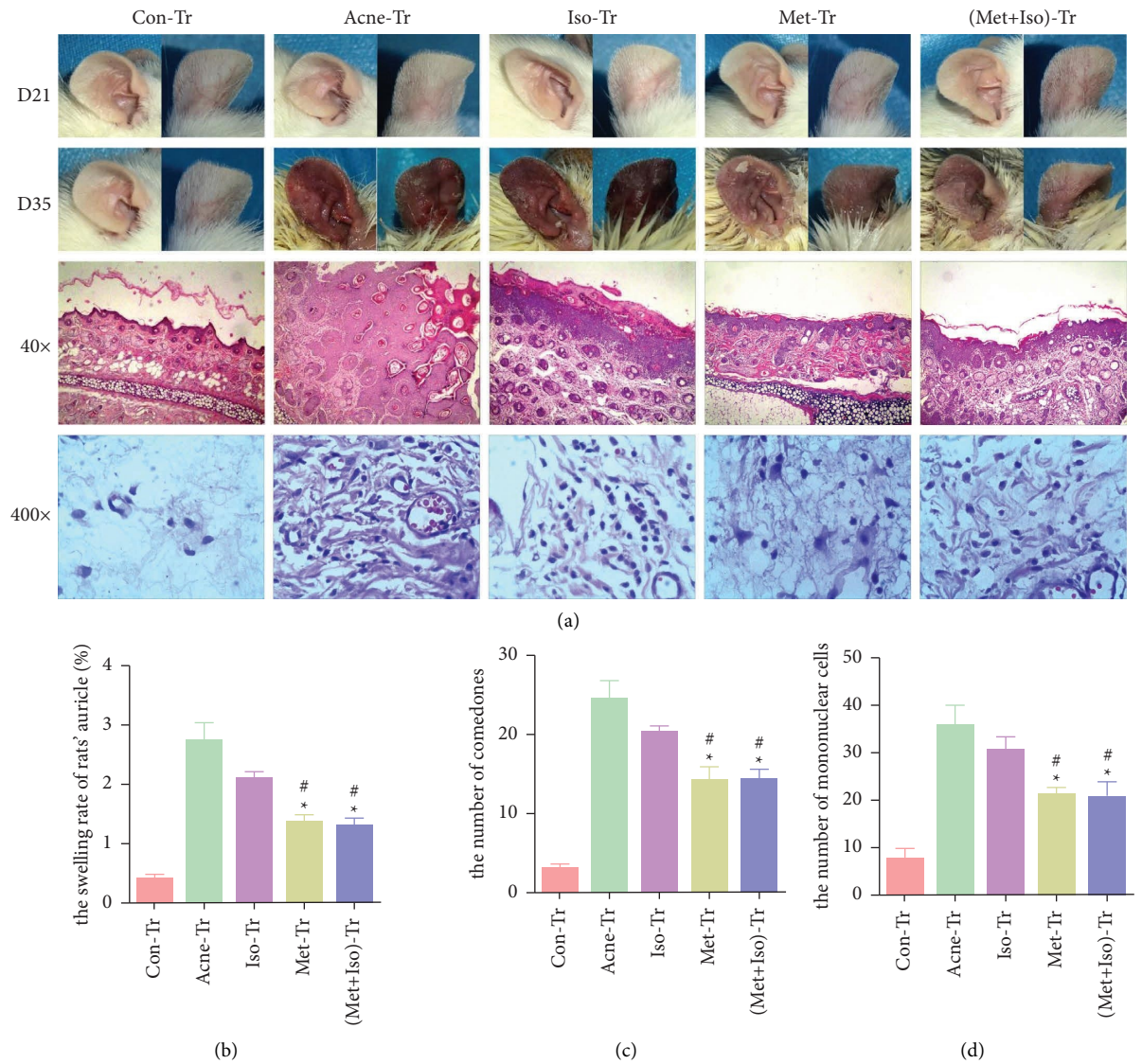

Figure 5: Effects of the gut microbiota that had been modified by different drugs on skin lesions and the pathological manifestation of acne in SD rats: (a) skin appearance and histological observations of the right auricle among the five groups after gut microbiota transplantation (original magnification, ×40, ×400); (b) the auricle swelling rate of SD rats; (c, d) comparison of the number of comedones and mononuclear cells among the five groups after gut microbiota transplantation. *(Acne-Tr vs.): $^{*}P < 0.05$, #(Iso-Tr vs.): $^{\#}P < 0.05$.

3.6. Transplantation of the Metformin Altered-Gut Microbiota Attenuated the Acne Phenotype in the Rat Model. To further verify that the metformin-adapted gut microbiota had a therapeutic effect on acne vulgaris, we carried out the FMT experiment. The success of the intestinal pseudo sterility and FMT was confirmed by 16s rDNA sequencing (Supplementary Figure S4A to S4T). After the intervention, we observed that the auricles of the SD rats showed increased redness and ear swelling in the Acne-Tr and Iso-Tr groups but less in the Met-Tr and (Met + Iso)-Tr groups (Figure 5(a)). Also, FMT treatment resulted in a decrease in the rate of auricle swelling in the Met-Tr (1.350 ± 0.383) and (Met + Iso)-Tr (1.301 ± 0.365) groups compared with the Acne-Tr and Iso-Tr groups (2.715 ± 0.982; 2.094 ± 0.273) ($P < 0.001$) (Figure 5(b)).

The number of comedones in the Acne-Tr group (24.704 ± 6.738) was similar to that in the Iso-Tr group (20.259 ± 2.482) ($P = 0.111$). Compared with the Acne-Tr and Iso-Tr groups, the Met-Tr (14.074 ± 5.275) and (Met + Iso)-Tr groups (14.148 ± 4.327) showed a significant decrease in the number of comedones ($P < 0.05$) (Figure 5(c)). Additionally, the number of mononuclear cells in the Acne-Tr (35.407 ± 12.506) and Iso-Tr (30.148 ± 9.314) groups still showed no significant differences ($P = 0.102$). Compared with the Acne-Tr and Iso-Tr groups, the number of mononuclear cells in the Met-Tr (20.963 ± 3.878) and (Met + Iso)-Tr (20.593 ± 8.966) groups significantly reduced ($P = 0.005$ and $P = 0.047$) (Figure 5(d)).

4. Discussion

In this study, we first performed a randomized controlled trial in individuals with acne vulgaris treating them with isotretinoin alone or in combination with metformin. The results showed that metformin, but not isotretinoin, had effects on the composition and function of the gut microbiota in parallel with the reduction of the severity of acne. This result was then confirmed by the animal experiments that were carried out. Additionally, fecal transfer to pseudo-germ-free mice resulted in improved inflammatory phenotypes and comedones of acne in recipients who received metformin-altered microbiota. This indicated that the metformin-adapted microbiota contributed to the positive effects of metformin in acne vulgaris patients.

After performing 16s rDNA sequencing of fecal samples, we observed significantly decreased microbial diversity in individuals with acne vulgaris, which then increased to health levels after three months of the metformin therapy. A decreased diversity in gut microbiota in patients with acne and other inflammatory skin diseases, such as Behçet's disease and psoriasis, was also reported in previous studies [25, 26]. It has been reported that individuals who have low bacterial richness have a more pronounced inflammatory phenotype [27]. However, the biodiversity of the intestinal flora lost during inflammation could be restored by metformin treatment [28, 29].

At the phylum level, the B/F ratio in the sample from patients with acne vulgaris could be enhanced by metformin treatment as discovered in this present study. The two predominant phyla, Bacteroidetes and Firmicutes, are mainly determined by the type of diet that one eats, in addition to genetic and environmental factors [30]. If there is an imbalance in the microbiota, dysbiosis will occur. The modification in the B/F ratio is an eventual indicator of changes in the microbiota's composition. Accumulating evidence has proven that the decreased B/F ratio is critical in the occurrence and progression of the inflammatory disease, obesity, diabetes, and cardiovascular disorders [31–35]. In this study, the lower ratio of B/F was also found in acne patients at the baseline, and this could be increased by metformin treatment. It was reported that metformin intervention could ameliorate atherosclerosis, in addition to suppressing inflammation in the hippocampus of HFD-fed mice. In this case, inflammation is suppressed by significantly downregulating the expression of the proinflammatory cytokines by increasing the B/F ratio. This results in an increase in bacterial diversity in the gut [29]. We also observed that the relative abundance of Bifidobacterium, a well-known beneficial microbe, was lower in acne patients and was elevated by metformin treatment. In fact, Bifidobacterium genus has been processed and used as an intestinal regulator for many years. It was also reported to have beneficial effects on its hosts by improving dysbiosis of the gut microbiota [36]. Bifidobacterium could produce SCFA, which provides energy to intestinal cells, inhibits intestinal inflammation, maintains the gut barrier, and prevents lipopolysaccharide (LPS) translocation from the intestinal barrier [37–39]. It was reported that metformin treatment can increase the abundance of SCFA-producing bacteria such as Bifidobacterium and Lactobacillus in rats, as well as in type 2 diabetes mellitus (T2DM) patients [40, 41]. The SCFA then inhibits inflammation by targeting the mammalian G protein-coupled receptor pair of GPR41 and GPR43, as well as regulating the activity of histone deacetylase (HDAC) [42, 43]. Additionally, metformin could reduce the absorption of bile acids. It could also control inflammation by increasing the abundance of beneficial microbes and promoting secondary bile acid production [44, 45].

Besides the observed changes in Bifidobacterium, treatment with metformin significantly increased the relative abundancy of Bacteroidetes while reducing that of Proteobacteria, Romboutsia, and Ruminococcus. In a Japanese study that involved 31 patients with T2DM, there was also a significant increase in the relative abundance of Bacteroides at the genus level after the administration of metformin [46]. Bacteroides are increasingly used as model gut commensals in cocolonization studies with enteropathogens, where they can regulate the intestinal microenvironment of the host, as well as directly or indirectly enhance the resistance to colonization and resilience against infection in the intestines [47]. Also, the gut microbiome maintains the gut integrity and systemic host homeostasis, where optimal control of intestinal LPS activity may play an important role. LPS are mainly produced by Proteobacteria in the gut, and they are well known for promoting impaired intestinal epithelial barriers and immune function, as well as inducing strong inflammatory responses, even causing septic shock or death in animals and humans [48–50]. It has been reported that metformin treatment in atherosclerosis can reduce LPS [31]. In this study, we also observed the decreasing LPS biosynthesis and increased SCFA metabolism signaling using the KEGG analysis in the metformin-treating group. This suggests that metformin may inhibit the inflammation of acne vulgaris by promoting the growth of SCFA-producing bacteria and by reducing the LPS-producing and proinflammatory bacteria in the gut.

In summary, our work shows that metformin could treat moderate to severe acne by regulating the gut microbiota. However, additional studies that combine untargeted metabolomics and metaproteomics are recommended to further identify the microbial metabolites or proteins that are involved. Determining how these proteins interact with the host targets in improving acne vulgaris is also of paramount importance.

Data Availability

Our sequence data have been submitted to SRA databases under accession number PRJNA885589. Requests for access to the clinical data can be submitted through the Chinese Clinical Trial Registry (ChiCTR) site at https://www.chictr.org.cn. For detailed data on animal experiments, please contact dengyongqiong1@126.com.

Ethical Approval

The studies involving human participants were reviewed and approved by the Ethics Committees of the hospital affiliated with Southwest Medical University (no: KY2019139).The animal experiment was approved by the animal experiment management committee of the Southwest Medical University (no: swmu20210426).

Consent

The patients/participants provided their written informed consent before participating in this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yongqiong Deng was involved in conceptualization, methodology, writing, reviewing, and editing; Shiyu Jiang was involved in writing the original draft, formal analysis, and software; Yaxin Huang was involved in software and formal analysis; Xiaoqi Tan was responsible for investigation and data curation; Yukun Huang was responsible for visualization; Lingna Chen was involved in resources and visualization; Jixiang Xu was involved in collecting resources; Xia Xiong was involved in supervision and funding acquisition; Jiaqiang Zhou was responsible for supervision project administration; Yong Xu was involved in supervision and project administration. Authors Yongqiong Deng and Shiyu Jiang contributed equally to this article.

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Supplementary Materials

Supplementary Figure 1: PCoA analysis of fecal microbiota diversity in humans and SD rats before and after drug intervention. (A) PCoA analysis of microbiota composition in humans at the baseline among three groups (CG, AVP, and AVM); (B) PCoA analysis of the microbiota composition in SD rats in the Iso group before and after treatment. 1: group before treatment and 2: group after treatment. Supplementary Figure 2: KEGG signaling pathways based on different gut microbiota. (A, B) KEGG signaling pathways based on changed fecal metabolism in the AVP and AVM groups before and after treatment, respectively; (C–E) KEGG signaling pathways based on changed fecal metabolism between different drug interventions, respectively ((Iso vs. Met), (Iso vs. Met + Iso), and (Met vs. Met + Iso)). Enrichment represented the value of-log10 (P value). 1: group before treatment and 2: group after treatment. Supplementary Figure 3: the differential gut microbial community in fecal samples of SD rats among the five groups after treatment. (A) Comparison of the ratio of the Bacteroidetes and Firmicutes (B/F) in the five groups with different interventions; (B, C) the difference in gut microbiota among the five groups at the phylum level; (D–G). The difference in gut microbiota among five groups at the genus level. *(Acne vs.): $^{*}P < 0.05$, $^{\#}$(Iso vs.): $^{\#}P < 0.05$, and $^{\bigstar}$(Met vs. (Met + Iso)): $^{\bigstar}P < 0.05$. Supplementary Figure 4: the Alpha diversity of gut microbiota for recipient and donor rats before and after treatment. (A–E) The Shannon index of the gut microbiota in the recipient rats on the 0th, 7th, 14th, and 35th experimental days; (F–J) the Simpson index of the gut microbiota in the recipient rats on the 0th, 7th, 14th, and 35th experimental days; (K–O) the Shannon index between the donor rats and recipient rats after treatment; (P–T) the Shannon index between donor rats and recipient rats after treatment. $^{\#}$(D0 vs. D14): $^{\#}P < 0.05$ and $^{\bigstar}$(D14 vs. D35): $^{\bigstar}P < 0.05$. (*Supplementary Materials*)

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