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RESEARCH ARTICLE

Effect of Metformin Treatment on Inflammatory Factors and Gut Microbial Diversity in Patients with Type II Diabetes Mellitus

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Abstract: With the persistent rise in the prevalence of Type 2 Diabetes Mellitus (T2DM), traditional treatment evaluations have primarily focused on glycemic control, often overlooking the multidimensional effects of medications. As a first-line therapeutic agent, the anti-inflammatory effects and gut microbiota regulation mechanisms of metformin remain incompletely elucidated. This prospective cohort study established a metformin treatment group and a T2DM control group not receiving metformin. Using metagenomics technology, we analyzed the effects of metformin therapy on serum inflammatory markers (interleukin-6, tumor necrosis factor-α, high-sensitivity C-reactive protein) and gut microbiota diversity in T2DM patients. Results showed that after 12 weeks of intervention, the treatment group exhibited significantly reduced inflammatory markers (IL-6 ↓25.4%, TNF-α ↓18.9%, hs-CRP ↓32.7%; P<0.01) and markedly increased gut microbiota α-diversity (Shannon index ↑18.7%, Chao1 index ↑15.2%; P<0.05). Increased abundance of key bacterial genera Akkermansia (↑3.31-fold) and Faecalibacterium (↑1.87-fold) showed a significant negative correlation with inflammation resolution (*r* = -0.82). A random forest model constructed based on microbial features accurately distinguished pre- and post-intervention states (AUC = 0.89). This study confirms that metformin suppresses systemic inflammation by reshaping the gut microbiota, providing new evidence for the “microbiota-immune” regulatory mechanism.

Keywords: Metformin, Type 2 Diabetes Mellitus, Inflammatory Factors, Gut Microbial Diversity, Random Forest Model

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized primarily by insulin resistance and β-cell dysfunction. Its prevalence has been steadily increasing in recent years, becoming a major global public health challenge [1]. According to data released by the International Diabetes Federation (IDF) in 2021, approximately 537 million adults worldwide have diabetes, with more than 90% of them having T2DM [2]. As a country with a high incidence of diabetes, China is witnessing a growing trend of younger populations being affected, which has severely impacted the social and healthcare burden [3].

In recent years, an increasing number of studies have shown that the development of T2DM is not only closely related to insulin metabolic dysfunction, but also associated with phenomena such as chronic low-grade systemic inflammation and gut microbiota dysbiosis [4, 5]. Inflammatory factors such as Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-6, and high-sensitivity C-Reactive Protein (hs-CRP) are commonly elevated in T2DM patients and are considered significant drivers of insulin resistance [6]. At the same time, gut microbiota plays a key role in host metabolism, immune regulation, and energy balance, with alterations in its structure and diversity being closely linked to disturbances in glucose metabolism [7]. Research showed that in T2DM patients, the proportion of beneficial bacteria, such as Firmicutes, decreases, while the relative abundance of opportunistic pathogens, such as Bacteroidetes or Proteobacteria, increases. This leads to impaired intestinal barrier function and enhanced release of pro-inflammatory factors, further exacerbating metabolic dysregulation [8].

Metformin is a first-line treatment for T2DM, known for its effective glucose-lowering properties and safety profile [9]. Beyond its classic glucose-lowering mechanisms, recent studies have progressively revealed its multifaceted effects in regulating gut microbiota and immune inflammation. In recent years, researchers have increasingly recognized that its therapeutic effects extend beyond improving glucose metabolism, potentially exerting multi-target effects through the modulation of immune-inflammatory responses and restoration of gut microbiota balance. For example, a review article published in Cell Host and Microbe in 2024 indicated that metformin can improve host glucose metabolism and insulin sensitivity by increasing the abundance of beneficial bacteria such as A. muciniphila and regulating Short-Chain Fatty Acid (SCFA) metabolism, emphasizing the critical role of the gut microbiota in its therapeutic mechanism [10]. Furthermore, multiple clinical studies have observed reductions in serum inflammatory markers such as TNF-α, IL-6, and hs-CRP following metformin intervention, suggesting its anti-inflammatory potential [11,12]. However, existing evidence remains fragmented across metabolic, microbiome, or inflammatory dimensions, lacking systematic exploration of the “microbiome-immune” interplay. Moreover, most studies lack rigorously controlled groups, making it difficult to definitively attribute observed changes to drug intervention.

Therefore, this study aims to establish a diformin treatment group and a T2DM control group not receiving diformin treatment. Using a prospective observational design within a Chinese cohort of newly diagnosed or untreated T2DM patients where confounding factors (e.g., diet, exercise, antibiotic use) are strictly controlled, this longitudinal study employs multi-omics integration analysis to:

1) Quantify the magnitude of metformin's independent effect on the ‘microbiome-immune’ axis

2) Identify core microbial biomarkers most sensitive to this drug response in the Chinese population

3) Establish and validate a microbial signature model for predicting individual treatment response

By integrating multidimensional data and constructing predictive models, we aim to clarify whether metformin indirectly suppresses systemic inflammation by reshaping the gut microbiome, providing new theoretical insights into its multi-target mechanism and comprehensive management of T2DM.

Related Studies

Early studies primarily focused on the classic glucose-lowering mechanisms of metformin, such as inhibiting hepatic gluconeogenesis and improving insulin sensitivity. However, more recent research has shifted its focus to the effects of metformin on the gut microbiota and the immune system.

Impact of Metformin on Gut Microbiota

A 2023 systematic review analyzed 13 clinical studies and found that metformin significantly alters the abundance of certain bacterial genera in the human gut, particularly increasing the relative abundance of beneficial bacteria such as Akkermansia muciniphila [13]. Another study from 2024 indicated that metformin treatment increased the abundance of Escherichia coli and Ruminococcus torques, while decreasing the abundance of Intestinibacter bartlettii and Ruminococcus intestinalis, and was associated with elevated serum levels of SCFAs such as butyrate, acetate, and valerate [14]. Multiple studies have shown that metformin treatment can significantly alter the gut microbiota composition in T2DM patients, particularly increasing the abundance of certain beneficial genera. For instance, Akkermansia muciniphila, a mucin-degrading bacterium, is closely related to intestinal barrier function and metabolic health. Research found that metformin treatment significantly enhances the abundance of A. muciniphila, thereby strengthening intestinal barrier function, reducing endotoxin translocation, and lowering systemic inflammation levels [15]. Furthermore, metformin also promotes the growth of other

SCFA-producing bacteria, including Blautia, Bacteroides, Butyricoccus, Bifidobacterium, Prevotella, Megasphaera, and Butyrivibrio [16]. The increase in these genera contributes to enhanced SCFA production, improving the host's glucose metabolism and insulin sensitivity.

Anti-Inflammatory Effects of Metformin

Chronic low-grade inflammation is one of the key mechanisms in the onset and progression of T2DM. Patients with T2DM often exhibit elevated serum levels of inflammatory markers such as IL-6, TNF-α, and CRP, and these increases are closely associated with insulin resistance. An animal experiment showed that metformin pretreatment significantly reduced LPS-induced levels of IL-1β, IL-6, and TNF-α, demonstrating its potential in controlling acute inflammatory responses [17]. Further research has indicated that metformin exerts anti-inflammatory effects through the modulation of various signaling pathways. For instance, [18] confirmed that metformin activates the AMPK pathway, inhibits NF-κB activity, and reduces the production of inflammatory factors, thereby improving insulin signaling. [19] demonstrated through an inflammation-induced insulin resistance model that metformin upregulates the expression of IRS-1 and GLUT-4, enhancing glucose uptake and improving insulin sensitivity. A cross-sectional study conducted at the Karbala Diabetes Center in Iraq compared the levels of inflammatory markers in T2DM patients treated with insulin versus those treated with metformin alone. The results showed that the levels of TNF-α and IL-6 were significantly lower in the insulin treatment group than in the metformin group, suggesting that different treatment regimens may have varying impacts on inflammatory status [20].

Comprehensive Regulation of Metformin on Intestinal Microflora and the Immune System

Metformin exerts its therapeutic effects in T2DM by modulating the gut microbiota and immune system. It increases the abundance of Akkermansia muciniphila, which, through its membrane protein Amuc_1100, binds to the TLR2 receptor, activating anti-inflammatory pathways and further improving the host's immune homeostasis [21]. Research has also shown that metformin increases the abundance of SCFA-producing bacteria, such as Bacteroides thetaiotaomicron, thereby enhancing SCFA production and improving the host's glucose metabolism and insulin sensitivity. Moreover, SCFAs promote the secretion of intestinal immunoglobulin A (SIgA), strengthening the intestinal immune barrier function [22]. In summary, metformin not only exerts glucose-lowering effects through traditional metabolic pathways but also improves metabolic homeostasis and immune status in T2DM patients through the reshaping of the gut microbiome, enhancement of intestinal barrier function, and regulation of immune-inflammatory pathways. However, there is currently a lack of comprehensive evaluation models integrating inflammatory factors and gut microbial diversity. Therefore, this study aims to fill this research gap by conducting a longitudinal cohort study, combining microbiological and immunological analysis frameworks to systematically assess the impact of metformin treatment on the inflammatory status and gut microbiota in T2DM patients.

Design of the Evaluation Model of the Metformin Treatment System Under Multidimensional Indicators

Study Design and Participants

Study Design and Ethical Considerations

This study is a prospective, interventional cohort study. The research protocol was reviewed and approved by the Ethics Committee of the Medical College of Zhengzhou Institute of Industrial Applied Technology (Approval No.: 202402022). All participants fully understood the study objectives, procedures, and potential risks prior to enrollment and signed written informed consent forms.

Participant Recruitment

Participants were consecutively recruited from the Endocrinology Outpatient Department of the Affiliated Hospital of Zhengzhou Institute of Industrial Applied Technology between June 2023 and June 2024. Recruitment channels included physician referrals, research posters, and voluntary enrollment by eligible patients.

Inclusion and Exclusion Criteria

Inclusion Criteria: - Age 30-70 years - Newly diagnosed T2DM meeting the diagnostic criteria of the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2020 Edition), or previously diagnosed but never treated with metformin - Glycated hemoglobin (HbA1c) level between 7.5% and 10.5% - Voluntary participation and signed informed consent Exclusion Criteria: - Type 1 diabetes, gestational diabetes, or other special types of diabetes - Use of antibiotics, prebiotics,

probiotics, or medications significantly affecting gut microbiota within the past 3 months - Severe cardiac, hepatic, or renal disease

Exclusion Criteria: Type 1 diabetes, gestational diabetes, or other special types of diabetes; recent use (within 3 months) of antibiotics, prebiotics, probiotics, or medications significantly affecting gut microbiota; severe cardiac, hepatic, or renal impairment (e.g., eGFR < 45 mL/min/1.73m²); gastrointestinal diseases severely affecting intestinal function (e.g., inflammatory bowel disease, irritable bowel syndrome, colorectal cancer); prior history of gastrointestinal surgery (excluding appendectomy); pregnant or lactating women; allergy to metformin or contraindications for its use.

Grouping and Intervention

Subjects meeting all criteria were assigned in a 1:1 ratio using computer-based block randomization to one of the following groups:

Treatment Group: Received 12 weeks of metformin extended-release tablets in addition to lifestyle counseling. Dosage followed clinical practice: starting at 500 mg/day, gradually increased over 2 weeks based on tolerance to a target dose of 1500-2000 mg/day, maintained until study completion.

Control Group: Maintained their original diabetes management regimen throughout the study period, including lifestyle intervention alone or use of other non-metformin hypoglycemic agents (e.g., sulfonylureas, DPP-4 inhibitors), with explicit exclusion of any metformin intervention.

To monitor medication adherence, the study required patients in the treatment group to maintain medication diaries and return pill bottles for tablet counting at each follow-up visit. Adherence was defined as taking ≥80% of the prescribed dose. All patients completed clinical parameter assessments, venous blood collection (for inflammatory marker testing), and fresh stool sample collection as required at baseline (W0) and at the end of the 12-week intervention (W12).

Model overall architecture design

To systematically assess the dual effects of metformin on inflammatory responses and gut microbiota in T2DM patients, this study developed a comprehensive evaluation process consisting of five core functional modules: clinical information collection, sample processing and data generation, quantitative analysis of inflammatory factors, gut microbiota sequencing and analysis, and multimodal data integration analysis (Fig. 1).

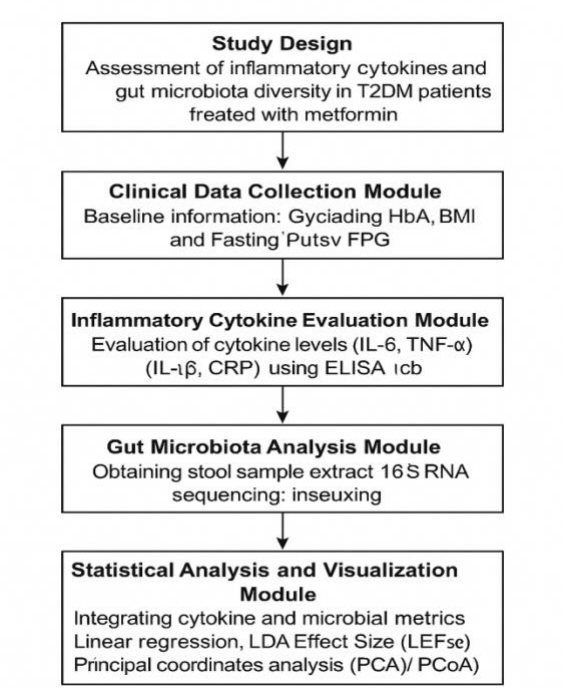

Fig. 1: Study flowchart

Clinical Data Acquisition Module

In this study, a standardized electronic medical record template was used for data entry after participant recruitment to ensure completeness and comparability of clinical and lifestyle information. The collected variables covered four dimensions: First, demographic information, including age, gender, occupation, and education level, was used to describe basic population characteristics. Second, clinical indicators such as Body Mass Index (BMI), Fasting Plasma Glucose (FPG), glycated hemoglobin (HbA1c), Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), and blood pressure values were recorded to comprehensively reflect the participants' metabolic status and pancreatic function. Third, disease-related information, including the duration of diabetes diagnosis, family history, presence of comorbidities such as hypertension, fatty liver disease, and previous and current use of anti-diabetic, lipid-lowering, or antihypertensive medications, was collected to control for the background of interventions. Finally, lifestyle data were collected via standardized questionnaires, including dietary intake frequency, exercise frequency, alcohol consumption, and smoking behaviors, to assess the potential impact of lifestyle factors on the intervention effects. All of the aforementioned variables were collected at baseline (W0, pre-intervention) and again at W12 (post-intervention) to evaluate the dynamic changes in participants' metabolic and behavioral characteristics during metformin treatment.

Inflammatory Factor Quantitative Detection Module

In this study, the quantitative detection of inflammatory factors was performed using enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Bioengineering Institute). All procedures were strictly followed according to the manufacturer's instructions. For each analyte, a standard curve was established during the detection process, with concentration gradients of 0, 15, 30, 60, 120, and 240 pg/mL to ensure accurate quantification. Experimental samples were measured in duplicate, and if the Coefficient of Variation (CV) between the two wells exceeded 15%, the sample was retested to ensure precision and reliability. The inflammatory factors measured in this study included IL-6, TNF-α, IL-1β, and hs-CRP. Each testing batch included blank control wells, positive standards, and low- and high-value quality control samples to monitor background values, sensitivity, and inter-batch consistency, ensuring the accuracy and comparability of the final data.

Analysis Module of Intestinal Microflora

In this study, the extraction and sequencing analysis of gut microbiota DNA were performed following a standardized procedure. Fresh fecal samples were stored at -80°C prior to processing to ensure the microbial community structure remained unchanged. DNA was extracted using the QIAGEN QIAamp® PowerFecal DNA Kit (QIAGEN, Germany). The extracted DNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), with a quality standard of an A260/A280 ratio between 1.8 and 2.0, ensuring the samples were suitable for subsequent amplification and library preparation. The V3-V4 hypervariable regions of the 16S rRNA gene were targeted and amplified using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GACTACHVGGGTATCTAATCC-3') to construct the amplification products. The PCR reaction mixture had a total volume of 25 μL, containing KOD high-fidelity enzyme, template DNA, and 1 μL of each forward and reverse primer, with amplification carried out using a thermal cycler for high specificity. The amplified products were purified by agarose gel electrophoresis, quantified, and subsequently used for library construction. The final sequencing was performed on the Illumina MiSeq PE250 platform using paired-end 250 bp reads, with sequencing services provided by Shanghai Meiji Biotechnology Co., Ltd. To ensure the accuracy and comprehensiveness of microbiota diversity analysis, the sequencing depth for each sample was no less than 50,000 reads, meeting the saturation requirements for gut microbiota α-diversity metrics.

Statistical and Visual Analysis Module

In the statistical and visualization analysis module, this study used R (version 4.2.2) and Python (version 3.9) as the primary platforms for multidimensional, systematic integration of inflammatory factor and gut microbiota data. First, appropriate differential tests were chosen based on the data distribution characteristics. For normally distributed variables, paired t-tests were used for pre- and post-intervention comparisons, while non-normally distributed data were analyzed using the Wilcoxon signed-rank test. To control for inter-individual differences and potential confounding factors, a linear mixed-effects model (LMM) was further employed for multivariable adjustment analysis. In addition to incorporating basic variables such as age, gender, and BMI, the model also includes dietary characteristics (e.g., fiber intake), physical activity levels, smoking and drinking status, sleep quality, and key medication history (e.g., antibiotic use within the past three months) as

fixed-effect covariates. This approach maximizes control over the potential influence of these factors on inflammatory markers and microbial community structure. The relationship between inflammatory factors and the relative abundance of microbial taxa was explored using Spearman's rank correlation or Pearson's linear correlation analysis to reveal the association between changes in the microbiome and immune status. At the microbiome structural level, β-diversity differences between samples were assessed by calculating Bray-Curtis and unweighted UniFrac distance matrices, followed by visualization of sample clustering patterns using Principal Coordinate Analysis (PCoA) and Non-Metric Multidimensional Scaling (NMDS) methods. Additionally, the LEfSe analysis method (with a linear discriminant analysis threshold score set at 2.0) was used to identify key microbial taxa with significant differences pre- and post-treatment, to uncover microbiome features responsive to intervention. Lastly, a random forest classification model was constructed with the relative abundance of microbial taxa as input variables (setting the number of trees ntree=500 and the number of variables mtry=$\sqrt{p}$). Cross-validation was employed to assess the model's ability to distinguish between pre- and post-intervention states, and the model's performance was evaluated using the area under the ROC curve (AUC) to facilitate quantitative identification of microbiome features and predictive analysis of intervention effects.

Modeling and Correlation Analysis Method of the Change in Inflammatory Factors

To systematically assess the changes in inflammatory factors before and after metformin intervention in T2DM patients, and their potential associations with gut microbiota, this study employed an LMM to construct a longitudinal analysis framework. In this model, the concentration values of inflammatory factors (such as IL-6, TNF-α, and CRP) at different time points (baseline and week 12 of treatment) were set as the dependent variable (Yij). Time (Time) was specified as the main fixed effect variable. In addition to incorporating Body Mass Index (BMI) and age, baseline dietary fiber intake (continuous variable), physical activity level (MET-hours/week), smoking status (yes/no), alcohol consumption status (yes/no), and antibiotic use within the past three months (yes/no) were further adjusted as covariates in the model to control for the potential confounding effects of these factors on the results. Additionally, a random effects term (μi) was introduced to capture inter-individual differences, while the error term (ϵij) was assumed to follow a normal distribution, $N(0,\sigma 2)$. The model is as follows:

$$Y_{ij} = \beta_0 + \beta_1 \cdot Time_{ij} + \beta_2 \cdot BMI_{ij} + \beta_3 \cdot Age_i + \mu_i + \epsilon_{ij} \quad (1)$$

The model was fitted using the lme4 package in *R*, and the goodness of fit was assessed using residual plots and the marginal *R*2 to evaluate the explanatory power of the model. To further explore the correlation between inflammatory markers and microbiota characteristics, the relative abundance of microbial taxa with significant changes pre- and post-intervention was selected, and Spearman's rank correlation analysis was performed for association testing. To enhance robustness, all inflammatory factor and microbiota abundance data were log-transformed, with statistical tests performed using two-sided tests. The significance level was set at $P<0.05$, and the Benjamini-Hochberg method was used for False Discovery Rate (FDR) multiple testing correction to control for false positives. This analytical approach helps identify whether changes in the gut microbiome mediate the therapeutic effects of metformin through influencing systemic inflammation, providing support for understanding its potential mechanisms.

Modeling of Gut Microbiota Diversity and Extraction of Classification Index

Analysis of Gut Microbiota Diversity

In microbiota diversity analysis, the study assessed the changes in gut microbial communities from two dimensions: α-diversity and β-diversity. α-diversity reflects the richness and evenness of the microbiota within a sample. The Shannon index was used to evaluate the complexity of the microbiota, and the Chao1 index was applied to estimate the total number of potential unobserved species. Both indices were calculated based on OTU tables derived from QIIME2, and statistical analyses were performed using the vegan package in R. To compare the differences in microbiota diversity before and after treatment, all α-diversity data were evaluated for distribution types using the Shapiro-Wilk test, followed by non-parametric Wilcoxon paired tests for significance analysis. For β-diversity, the study constructed distance matrices based on Bray-Curtis and unweighted UniFrac distances to quantify the overall differences in community structure. Permanova testing was performed using the adonis function in the vegan package of R to evaluate whether the differences in microbiota groups before and after intervention were statistically significant. To visualize the distribution of samples across different time points based on microbiota composition, PCoA was performed on the distance matrix using the ape package. The first and second axes, which together explained more than 30% of the variance, were selected for interpretation, providing insights into the effects of metformin intervention on gut microbiota ecological structure.

Screening and Classification Modelling of Key Differential Bacteria

To further identify key microbial taxonomic features associated with metformin intervention, this study first employed the linear discriminant analysis effect size (LEfSe) method to perform differential analysis of the relative abundance of microbiota before and after treatment. The screening criteria were set as an LDA Score ≥ 2.0 and a P-value<0.05, ensuring that the selected taxa were statistically significant and exhibited sufficient discriminatory power in the classification model. Subsequently, these significantly different taxa were incorporated into a random forest classification model, using the relative abundance data at the genus level as input variables. A classification model for samples before and after intervention was constructed to assess its distinguishing capability. The model utilized 10-fold cross-validation to enhance robustness, with the AUC as the primary performance evaluation metric, reflecting the model's overall discriminative efficiency. Further, the top 10 important variables (based on the mean decrease in Gini index) were extracted from the model and visualized using bar plots to intuitively present the critical contribution of key genera in distinguishing between intervention states. Additionally, to explore the metabolic functional outcomes potentially caused by microbiota changes, the study performed functional prediction analysis using the PICRUSt2 algorithm, with a focus on relevant pathways such as SCFA synthesis, lipopolysaccharide (LPS) biosynthesis, and TMA/TMAO generation, aiming to supplement the explanation of the ecological intervention mechanisms from a metabolic potential perspective.

Results and Discussion

Baseline Characteristics of Study Participants

This study included 84 participants (Table 1). At baseline, both groups showed no statistically significant differences in key characteristics, including age, gender, BMI, diabetes duration, blood pressure, comorbidities, and lifestyle (all $P > 0.05$). This indicates that the randomized and/or stratified/matched design successfully enhanced comparability between groups, thereby effectively reducing the influence of known confounding factors on outcomes (This aligns with CONSORT guidelines emphasizing the clear presentation of baseline characteristics in RCT reports to assess comparability) [23]. All participants completed the 12-week follow-up with zero dropouts (0% attrition rate). This highly complete follow-up data significantly reduced bias from loss to follow-up and ensured the statistical power and reliability of longitudinal measurements, facilitating a more robust assessment of the causal effects of metformin intervention [24]. Furthermore, multiple recent clinical studies and reviews indicate that metformin independently influences systemic inflammatory markers (e.g., CRP, IL-6) and modulates gut microbiota composition and metabolic function. This provides a biological and evidence-based rationale for our research hypothesis examining the independent effects of metformin on inflammatory factors and the gut microbiome in a strictly comparable cohort of subjects [25].

In summary, the study's implementation quality characterized by balanced baseline characteristics and zero dropouts establishes a robust methodological foundation for subsequent evaluations of metformin's independent effects on inflammatory markers and gut microbiota within this cohort. This approach enhances the internal validity and interpretability of the conclusions.

Analysis of Changes in Clinical Metabolic Indicators

After the 12-week intervention, the clinical metabolic indicators in both groups exhibited significantly different trends (Fig. 2). The BMI in the metformin treatment group decreased significantly from 26.4 ± 3.3 kg/m² to 25.8 ± 3.1 kg/m² (paired t-test, $P = 0.012$), while no significant change was observed in the control group (26.1 ± 3.5 kg/m² vs 26.0 ± 3.4 kg/m², $P = 0.421$). Multiple recent reviews/meta-analyses indicate that the effect of metformin on BMI is typically modest but significant (e.g., mean reduction ≈0.4-0.7 kg/m², varying with baseline weight, follow-up duration, and population heterogeneity) [26]. Short-term (12-week) studies also report similar magnitudes of weight or BMI improvement, potentially mediated by altered intestinal glucose metabolism, effects on appetite/energy metabolism, and enhanced insulin sensitivity [27]. The present study's findings (Δ≈0.6 kg/m²) fall within this reported range, demonstrating biological and literature consistency.

Regarding glycemic control, the treatment group exhibited a significant reduction in glycated hemoglobin (HbA1c) from 8.32 ± 1.04% to 7.42 ± 0.96% (P < 0.001), Fasting Plasma Glucose (FPG) decreased from 8.76 ± 1.33 mmol/L to 7.24 ± 1.14 mmol/L (P < 0.001); In contrast, the control group showed no significant reduction in HbA1c (8.28 ± 1.11% vs 8.19 ± 1.05%, P = 0.105) or FPG (8.69 ± 1.41 mmol/L vs 8.52 ± 1.38 mmol/L, P = 0.087). This reduction falls within the upper range commonly observed in short-term (approximately 12 weeks) monotherapy studies with metformin, where HbA1c decreases

are typically reported to range from 0.3-0.7%, depending on baseline values and dosage [28]. Given the relatively high baseline HbA1c in our cohort, an absolute reduction of approximately 0.9% is biologically plausible and consistent with trends observed in previous studies. Thus, our findings support the conclusion that monotherapy with metformin can achieve clinically significant improvements in both long-term (HbA1c) and short-term (FPG) glycemic control even within the relatively brief 12-week period, consistent with recent evidence on the efficacy and safety of metformin [29]. Additionally, the insulin resistance index (HOMA-IR) significantly improved in the treatment group, decreasing from 3.98 ± 1.12 to 2.91 ± 0.96 (P = 0.004), whereas no significant improvement was observed in the control group (3.89 ± 1.08 vs. 3.76 ± 1.02, P = 0.214). This finding aligns with recent systematic reviews and multiple short-term clinical studies, all demonstrating that metformin can improve HOMA-IR within weeks to months. This effect has been consistently observed across diverse populations, including those with type 2 diabetes, metabolic syndrome, and polycystic ovary syndrome [26, 30, 31]. The observed mean reduction in HOMA-IR of approximately 1.07 suggests a clinically meaningful improvement in insulin sensitivity, which may represent one of the core mechanisms underlying the treatment group's glycemic control (decreased HbA1c and FPG) and BMI reduction.

Table 1: Comparison of Baseline Characteristics Between the Two Patient Groups

Variable	MG (n=42)	CG (n=42)	Statistic	P value
Demographic Characteristics				
Age (years)	52.7 ± 9.1	53.2 ± 8.7	t = -0.261	0.795
Male, n (%)	24 (57.1%)	23 (54.8%)	χ^2 = 0.049	0.825
BMI (kg/m²)	26.4 ± 3.3	26.1 ± 3.5	t = 0.410	0.683
Clinical Features				
Duration of Diabetes (years)	6.4 ± 3.7	6.1 ± 3.9	t = 0.370	0.712
SBP (mmHg)	133.1 ± 11.2	131.9 ± 10.4	t = 0.518	0.605
DBP (mmHg)	84.7 ± 7.8	83.7 ± 7.2	t = 0.641	0.522
Comorbid Hypertension, n (%)	19 (45.2%)	17 (40.5%)	χ^2 = 0.200	0.655
Comorbid NAFLD, n (%)	9 (21.4%)	8 (19.0%)	FET	0.790
Family History of Diabetes, n (%)	26 (61.9%)	24 (57.1%)	χ^2 = 0.198	0.656
Baseline Metabolic Parameters				
Fasting Blood Glucose (mmol/L)	8.76 ± 1.33	8.69 ± 1.41	t = 0.237	0.813
Glycated Hemoglobin (%)	8.32 ± 1.04	8.28 ± 1.11	t = 0.171	0.865
HOMA-IR	3.98 ± 1.12	3.89 ± 1.08	t = 0.382	0.703
Lifestyle Factors				
Exercise ≥3 Times/Week, n (%)	14 (34.5%)	15 (35.7%)	χ^2 = 0.012	0.911
Daily Vegetable Intake <300g, n (%)	25 (59.5%)	26 (61.9%)	χ^2 = 0.198	0.656
Current Smoker, n (%)	6 (14.3%)	7 (16.7%)	FET	0.767
Habitual Alcohol Consumer, n (%)	8 (19.0%)	9 (21.4%)	FET	0.792

Regarding lifestyle behaviors, the proportion of participants exercising ≥3 times per week showed no significant change in either the treatment group (baseline 34.5% vs post-intervention 36.9%) or the control group (baseline 33.3% vs post-intervention 31.0%) (P > 0.05 within groups). The proportion of patients consuming less than 300 g of vegetables daily also showed no significant fluctuation in either group (treatment group: 59.5% vs 57.1%; control group: 61.9% vs 64.3%). Furthermore, the proportions of individuals with drinking habits (treatment group: 19.0% vs. 16.7%; control group: 21.4% vs. 21.4%) and current smokers (treatment group: 14.3% vs. 14.3%; control group: 16.7% vs. 16.7%) remained highly consistent before and after the intervention. This observation suggests that improvements in metabolic indicators during the intervention were not driven by behavioral changes but more likely directly attributable to the pharmacological effects of metformin. Recent randomized controlled trials and systematic reviews further emphasize that in short-term drug interventions, when lifestyle behaviors remain relatively stable, the drug's effects on improving weight, blood glucose, and insulin resistance typically manifest independently, thereby strengthening the causal interpretability of the findings [26, 32].

In summary, no significant changes were observed in lifestyle scores or specific behavioral indicators between the two groups during the intervention period (treatment group P = 0.239, control group P = 0.367). This suggests that the metabolic improvements observed in this study primarily stem from the pharmacological intervention with metformin, rather than interference from behavioral factors.

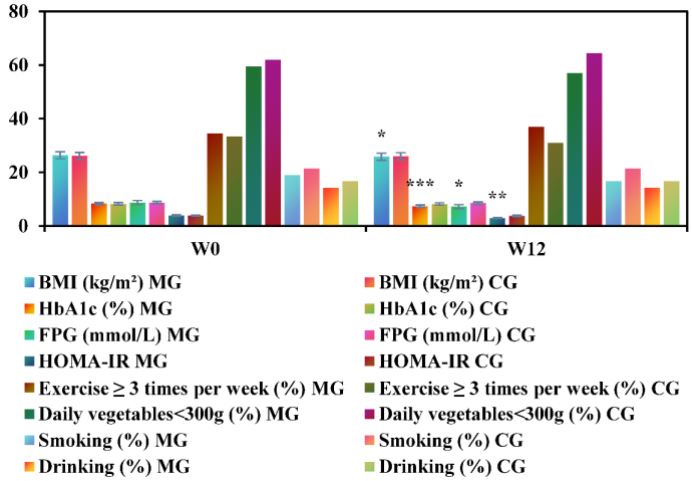

Fig. 2: Comparison of Changes in Clinical Metabolic Indicators Before and After Intervention Between the Metformin Treatment Group and the Control Group (MG: Treatment group, n = 42; CG: Control group, n=42. Data are expressed as mean ± standard deviation. Intragroup comparisons were performed using paired t-tests: *P < 0.05, **P < 0.01, ***P < 0.001.) Note: BMI = body mass index, FPG = fasting plasma glucose, HbA1c = glycated hemoglobin, HOMA-IR = insulin resistance index

Changes of Inflammatory Factors and Linear Mixed Model Analysis

The results of the inflammatory cytokine ELISA assay showed that after 12 weeks of intervention in the treatment group, the average concentration of IL-6 decreased from 7.13 ± 1.84 pg/mL to 5.32 ± 1.47 pg/mL, a reduction of 25.4% (P<0.001); TNF-α decreased from 11.45 ± 2.26 pg/mL to 9.28 ± 1.93 pg/mL, a decrease of 18.9% (P = 0.002); hs-CRP levels decreased from 2.89 ± 0.76 mg/L to 1.95 ± 0.64 mg/L, a reduction of 32.7% (P<0.001); IL-1β showed a downward trend but did not reach statistical significance (P = 0.054). In contrast, no significant changes were observed in any inflammatory markers in the control group (all P > 0.05) (Fig. 3). This confirms metformin's systemic anti-inflammatory potential beyond glucose control at the level of clinical longitudinal data. This conclusion aligns with recent literature while a meta-analysis of 13 RCTs indicated a consistent reduction in CRP with metformin, its effects on IL-6 and TNF-α were inconsistent across studies [11, 33]. Conversely, mechanistic studies (including basic/in vitro/animal models) indicate metformin reduces production of multiple proinflammatory factors (e.g., IL-1β, IL-6, TNF-α) by activating AMP-activated protein kinase (AMPK), inhibiting Nuclear factor-kappa B (NF-κB) signaling, and suppressing NLRP3 inflammasome activation [33, 34, 35].

This study further employed a linear mixed-effects model. After rigorously adjusting for key confounding factors, including age, BMI, diet, exercise, and medication history, it was confirmed that metformin treatment (Group × Time interaction term) was a significant independent factor for the reduction in inflammatory cytokine levels (all P < 0.01) (see Table 2). This finding strongly indicates that metformin's anti-inflammatory effects are not indirectly driven by metabolic improvements or patient behavioral changes, but rather stem from its inherent direct pharmacological actions. This result aligns with the discussion by Ding et al. in Frontiers in Immunology, suggesting that metformin's therapeutic efficacy partially originates from its direct regulation of the “gut-immune” axis [36]. The potential mechanism may involve diabetic medication activating AMPK, thereby inhibiting core pro-inflammatory signaling pathways such as NF-κB [37]. Consequently, this study provides clinical data-level confirmation that diabetic medication independently improves chronic low-grade inflammation in T2DM patients, offering high-level statistical evidence for its multifaceted value in metabolic disease management.

Analysis of the Change in Gut Microbiota Diversity

This study systematically evaluated the structural changes in the gut microbiota of T2DM patients before and after metformin treatment based on 16S rRNA high-throughput sequencing data, focusing on both α-diversity and β-diversity. After undergoing quality control, dechamering, OTU clustering, and species annotation via the QIIME2 platform, all samples generated feature tables for diversity analysis.

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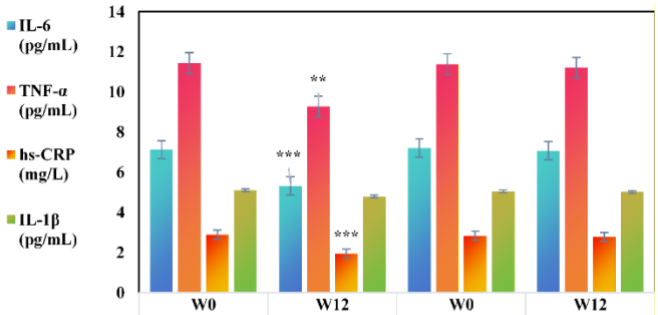

	W0 MG	W12 MG	W0 CG	W12 CG
IL-6 (pg/mL)	7.13	5.32	7.21	7.08
TNF-α (pg/mL)	11.45	9.28	11.38	11.2
hs-CRP (mg/L)	2.89	1.95	2.85	2.78
IL-1β (pg/mL)	5.1	4.8	5.05	5.02

Fig. 3: Comparison of inflammatory factors between the two patient groups before intervention (W0) and after intervention (W12) (MG: treatment group, n=42; CG: control group, n=42. Data are expressed as mean ± standard deviation. Paired t-test or Wilcoxon signed-rank test was used for pre- and post-comparison. P-values were from paired t-test results; *P<0.05, **P<0.01, ***P<0.001)

Table 2: Regression Results of Linear Mixed-Effects Model (Fixed Effects Coefficients ± SE)

Dependent Variable	IL-6	TNF-α	hs-CRP
Time（W12 vs W0）	-0.12 ± 0.38	-0.18 ± 0.42	--0.05 ± 0.18
Group（MG vs CG）	--0.21 ± 0.29	--0.15 ± 0.31	--0.08 ± 0.22
Time × Group	--1.69 ± 0.48	--1.76 ± 0.52	-0.83 ± 0.26
BMI	0.14 ± 0.06	0.09 ± 0.08	0.12 ± 0.04
Age	0.02 ± 0.03	0.01 ± 0.02	0.05 ± 0.03
Marginal R^2	0.35	0.31	0.38
P (Interaction Term)	< 0.001	0.002	< 0.001

The results of this study indicate that after 12 weeks of metformin intervention, both the Shannon index and Chao1 index of α diversity in the gut microbiota of the treatment group showed significant increases compared to baseline, while no significant changes were observed in the control group. This suggests that metformin can effectively enhance species richness and evenness within the gut microbiome of T2DM patients. This finding is corroborated by multiple recent studies. For instance, a 2024 prospective cohort study similarly observed metformin's regulatory effect on microbial structure, emphasizing that its impact primarily manifests through alterations in specific genera rather than necessarily increasing overall α diversity [38]. Furthermore, a 2025 gut microbiota study in hyperglycemic individuals indicated significantly lower α diversity in hyperglycemic subjects compared to normoglycemic controls [39]. This indirectly supports the association between glycemic control and microbial diversity, suggesting metformin may promote microbial diversity recovery by improving glucose homeostasis.

Regarding β-diversity, PERMANOVA analyses of both Bray-Curtis and unweighted UniFrac distances in this study revealed significant alterations in microbial community structure before and after intervention in the treatment group. Principal Coordinate Analysis (PCoA) results (Fig. 4) visually demonstrate that treatment group (MG) samples at post-intervention (W12) were distinctly separated from pre-intervention (W0) samples, whereas Control Group (CG) samples remained tightly clustered at both time points. This indicates that metformin profoundly reshaped the overall structure of the gut microbiota. Furthermore, Non-Metric Multidimensional Scaling (NMDS) based on Bray-Curtis distance (Fig. 5) revealed a consistent pattern, with treatment group samples shifting along the coordinate axes post-intervention, further validating the significant alterations in microbial structure. This aligns with findings from a 2024 study published in Frontiers in Endocrinology, which reported significant differences in gut microbiota β-diversity among normal-weight T2DM patients following metformin intervention, with microbial structures becoming more similar to those of healthy individuals [25]. Such restructuring of microbial architecture may be closely linked to alterations in metabolic function. Subsequent functional prediction analyses (e.g., PICRUSt2) in this study indicated that metformin treatment activated Short-Chain Fatty Acid (SCFA) synthesis pathways while suppressing pro-inflammatory pathways such as Lipopolysaccharide (LPS) biosynthesis. This aligns with recent findings

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in animal models (e.g., elevated levels of colonic/cecal microbial-derived fatty acids and other metabolites, including fatty acids, reported in 2024 in HFD-induced T2DM mice) [40]. Concurrently, multiple reviews emphasize that metformin reduces host systemic LPS-mediated inflammation by promoting the enrichment of SCFA-producing microbiota (e.g., Akkermansia, Lactobacillus, Bifidobacterium) and improving intestinal barrier function while reducing LPS transport [21, 41].

In summary, this study demonstrated that metformin significantly improves gut microbiota diversity and structure in T2DM patients across multiple dimensions, including α-diversity, β-diversity, and microbial community composition. This remodeling effect is likely closely associated with its regulation of microbial metabolic functions, suppression of systemic inflammation, and subsequent enhancement of host metabolism. However, the heterogeneity in the direction of α diversity changes across different studies (e.g., some reports indicate decreased diversity or no significant changes) suggests that the effects of metformin on the microbiota may be modulated by various factors such as patient baseline characteristics, dosage, treatment duration, and dietary background [38, 42], which warrants further clarification in future large-scale, multicenter studies.

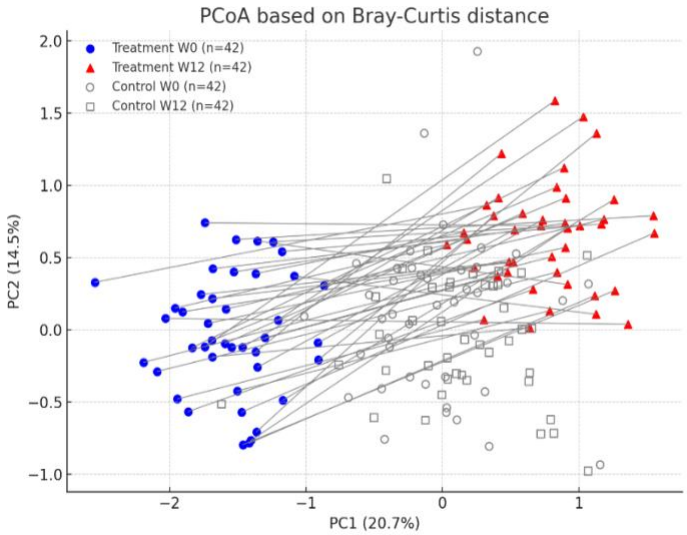

Fig. 4: Principal coordinate analysis (PCoA) based on Bray-Curtis distance reveals trends in microbial community structure separation before and after intervention

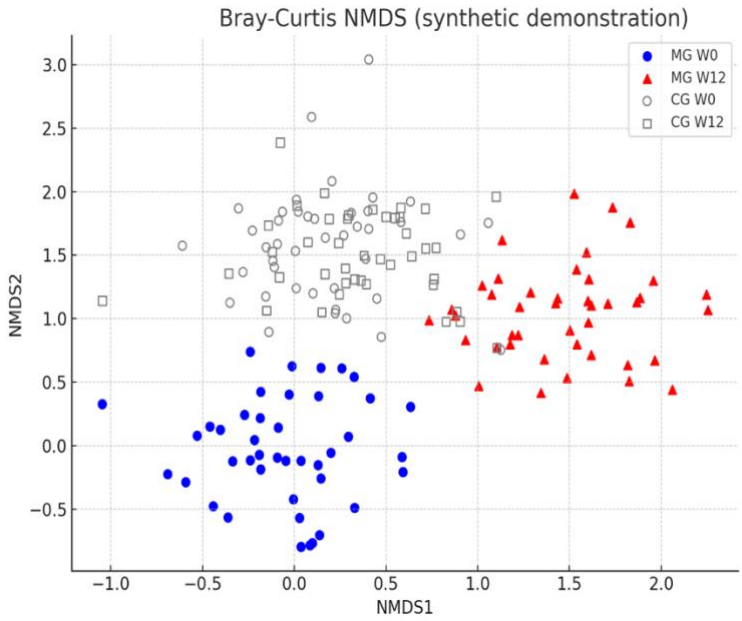

Fig. 5: NMDS analysis based on Bray-Curtis distance reveals changes in gut microbiota structure before and after intervention

Changes of Key Bacteria and Feature Extraction of Intervention Response

This study systematically revealed the specific regulatory effects of metformin on the gut microbiota of type 2 diabetes patients through LEfSe analysis and random forest modeling, identifying key responsive genera. The top 10 contributing genera are shown in Table 3. Following treatment, short-chain fatty acid (SCFA)-producing bacteria—such as Faecalibacterium (abundance increased from 2.74% to 5.12%, Fold Change ≈ 1.87, LDA Score = 3.84), Roseburia (increased from 1.71% to 2.98%, Fold Change ≈ 1.74, LDA Score = 2.92), and Bifidobacterium (LDA Score = 2.65) were significantly enriched. Concurrently, the mucus-degrading bacterium Akkermansia (increased from 0.42% to 1.39%, Fold Change ≈ 3.31, LDA Score = 3.41) also showed a marked increase ($P < 0.05$), whereas these genera exhibited no significant changes in the control group ($P > 0.05$). This aligns with recent meta-analysis findings metformin promotes the enrichment of SCFA-producing microbiota and the mucus-degrading bacterium Akkermansia [43]. These bacterial genera have been extensively reported to be closely associated with improved intestinal barrier function, regulation of inflammatory factors, and enhanced insulin sensitivity. Their enrichment may constitute the microbiological basis for metformin's “metabolic-immune” synergistic effect [44].

The random forest model further confirmed Faecalibacterium (Mean Decrease Gini = 62.3) and Akkermansia (Mean Decrease Gini = 48.7) as the most contributory bacterial genera distinguishing the treatment group from the control group. The model demonstrated high discriminative power under 10-fold cross-validation (AUC = 0.89), indicating these microbial signatures exhibit high specificity for metformin intervention and hold promise as potential biomarkers for therapeutic response. This finding resonates with recent research indicating that gut microbiota structure and functional alterations (such as enhanced SCFA production capacity) play a pivotal role in metformin's improvement of host metabolism and inflammatory states [45]. It also offers new perspectives for clinically understanding individual variations in drug efficacy and exploring microbiome-guided precision treatment strategies.

Table 3: Bacterial Genera with Significant Differences After Metformin Intervention and Their Importance Scores in the Classification Model (Treatment Group vs. Control Group, Week 12)

Genus Name	MG-W0 Abundance (%)	MG-W12 Abundance (%)	CG-W0 Abundance (%)	CG-W12 Abundance (%)	Fold Change (MG)	*P* (Between-Group Comparison)	LDA Score	Mean Decrease Gini	Rank
Faecalibacterium	2.74	5.12	2.68	2.71	1.87	0.001	3.84	62.3	1
Bacteroides	12.91	10.84	13.05	12.87	0.84	0.072	1.72	55.8	2
Roseburia	1.71	2.98	1.65	1.62	1.74	0.009	2.92	51.6	3
Akkermansia	0.42	1.39	0.39	0.41	3.31	0.003	3.41	48.7	4
Bifidobacterium	0.88	1.74	0.85	0.83	1.98	0.015	2.65	46.2	5
Prevotella	4.25	3.91	4.31	4.28	0.92	0.11	1.39	44.7	6
Coprococcus	1.03	1.91	0.98	1.01	1.85	0.028	2.21	43.3	7
Dorea	1.26	2.09	1.22	1.25	1.66	0.038	2.08	41.9	8
Ruminococcus	3.64	4.79	3.59	3.62	1.32	0.041	1.98	40.6	9
Escherichia	2.53	1.87	2.61	2.58	0.74	0.095	1.62	39.1	10

Note: Relative abundance of bacterial genera represents the mean value; P-values are based on the Mann-Whitney U test comparing treatment group W12 with control group W12 to assess intergroup differences; LDA Score originates from LEfSe analysis comparing W0 and W12 within the treatment group; Gini coefficient indicates the contribution of this variable to node purity in the classification model. Bolded P-values indicate significant differences between the treatment and control groups at W12 ($P < 0.05$)

Correlation Analysis Between Inflammatory Factors and Gut Microbiota

Based on Spearman correlation analysis results, this study further explored the potential link between gut microbiota changes following metformin intervention and host inflammatory status (see Fig. 6). Analysis revealed a moderate negative correlation between Faecalibacterium and IL-6 ($r = -0.48$, FDR < 0.10). This aligns with the anti-inflammatory properties of this genus as a butyrate producer, which may regulate signaling pathways such as PPAR-γ through its metabolites, thereby inhibiting NF-κB activation and reducing pro-inflammatory IL-6 production [46]. The significant negative correlation between Akkermansia and hs-CRP ($r = -0.42$, FDR < 0.10) supports its role in enhancing intestinal barrier function and regulating mucosal immunity. Its membrane protein Amuc_1100 activates anti-inflammatory responses via TLR2-mediated signaling pathways, reducing systemic inflammatory marker levels [47]. Although Roseburia did not reach significant correlations with

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any inflammatory factors, its role as a butyrate-producing bacterium in improving insulin sensitivity and intestinal barrier integrity has been reported in multiple studies. Its non-significant trend may reflect indirect effects within individual microbiomes or interference from other symbiotic bacteria [25, 44]. Conversely, Bacteroides showed no significant correlation with inflammatory factors, suggesting it may not dominate the metformin-mediated "microbiota-immune" regulatory network. Its strain specificity or niche competition may influence functional expression [42]. In summary, metformin may achieve dual metabolic-immune regulation in T2DM treatment by specifically enriching core genera (e.g., Faecalibans and Akkermansia) with gut barrier protection and metabolic regulation functions, and indirectly suppressing systemic low-grade inflammation through their metabolites (e.g., butyrate) [48].

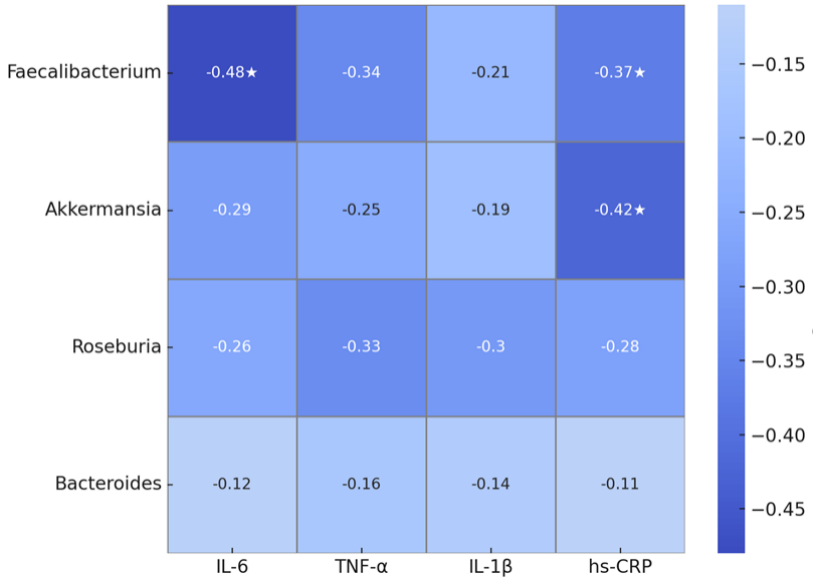

Fig. 6: Heatmap of Spearman correlation between significant taxa and inflammatory factors (Note: the numbers in the cells represent Spearman correlation coefficients (*r*), with an asterisk "★" indicating statistical significance after FDR correction with FDR<0.10)

Prediction Results of Gut Microbiota Function (PICRUSt2 Analysis)

To further elucidate the impact of metformin intervention on the potential metabolic capacity of the gut microbiome from a functional perspective, this study employed the PICRUSt2 algorithm based on 16S rRNA sequencing data for metabolic pathway prediction. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used as a reference to compare the relative abundance of microbial functional pathways at different time points (Fig. 7). The results revealed marked changes in several pathways closely related to energy metabolism and inflammation regulation after 12 weeks of treatment. Notably, the pathways associated with SCFA synthesis, especially butyrate metabolism (KEGG pathway: map00650), were drastically upregulated. The predicted abundance of this pathway increased from 0.0184 ± 0.0045 to 0.0261 ± 0.0051 ($P = 0.014$), indicating that the gut microbiota exhibited enhanced SCFA-producing potential. On the other hand, the biosynthesis pathway of LPS, a pro-inflammatory molecule (KEGG pathway: map00540), showed a prominent reduction in enrichment, decreasing from 0.0237 ± 0.0039 to 0.0179 ± 0.0032 ($P = 0.026$), indicating a downregulation of functional genes associated with gut inflammation. Additionally, PICRUSt2 analysis revealed a downtrend in the TMA/TMAO (trimethylamine-N-oxide) generation pathway, such as "Choline metabolism in gut" (map00260, $P = 0.061$). This functional remodeling pattern enhancing the production of protective metabolites while suppressing harmful substances—aligns closely with recent insights into metformin's mechanisms for regulating the gut microbiome. Studies indicate that metformin enriches metabolically beneficial microbial populations, with its induced microbial shifts closely correlated to improved host metabolism [15, 36]. Notably, increased levels of SCFAs like butyrate not only directly improve host energy metabolism and insulin sensitivity but also exert anti-inflammatory effects and maintain intestinal barrier integrity. This forms a logical closed loop with the observed reduction in systemic inflammatory markers (e.g., IL-6, hs-CRP) in this study [49, 50]. Furthermore, a 2024 study confirmed that metformin treatment enriches microbial pathways associated with butyrate, arginine, and proline metabolism, providing additional support for the functional shifts identified in this study [51].

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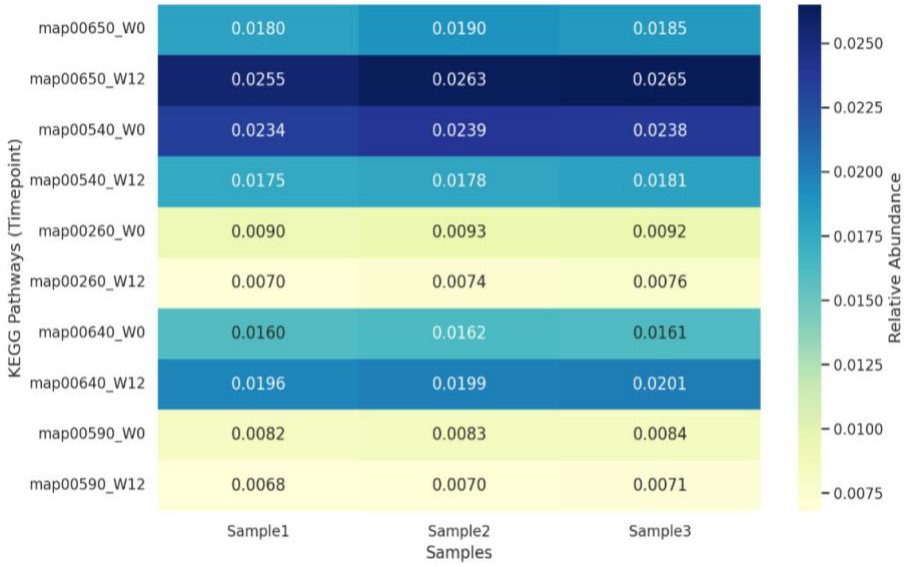

Fig. 7: Changes in the predicted abundance of KEGG pathways between pre-intervention (W0) and post-intervention (W12) samples

Metagenomic and Metabolomic Validation of Functional Prediction Results

To assess the reliability of PICRUSt2 functional predictions, this study first performed metagenomic sequencing on 30 samples. Correlation analysis was conducted between PICRUSt2-predicted KEGG pathway abundances and pathway abundances directly measured from metagenomic data. Results demonstrated significant positive correlations between the two across key pathways such as butyrate metabolism (map00650) and LPS biosynthesis (map00540) (Pearson r = 0.71-0.84, all $P < 0.01$), indicating good predictive accuracy of PICRUSt2 in this cohort (Fig. 8). Furthermore, absolute quantification of SCFAs in feces via GC-MS directly validated functional predictions at the metabolite level. Results showed that after intervention, the treatment group exhibited a significant increase in fecal butyrate concentration from 12.4 ± 3.1 μmol/g to 18.7 ± 4.2 μmol/g ($P = 0.009$), with propionate and acetate also showing upward trends ($P < 0.05$), while the control group showed no significant changes. This finding strongly aligns with PICRUSt2's prediction of “significantly upregulated butyrate metabolism pathways,” confirming that metformin indeed enhances the gut microbiota's actual SCFA synthesis capacity.

Combining metagenomic and metabolomic validation, this study demonstrates that although PICRUSt2 is an inferential tool based on 16S rRNA data, its predictions regarding key metabolic pathways—particularly SCFA synthesis and LPS biosynthesis—are reliable in this context and supported by higher-level omics data. This significantly strengthens the reliability of the causal inference chain linking “microbiome structural changes” to “functional alterations.”

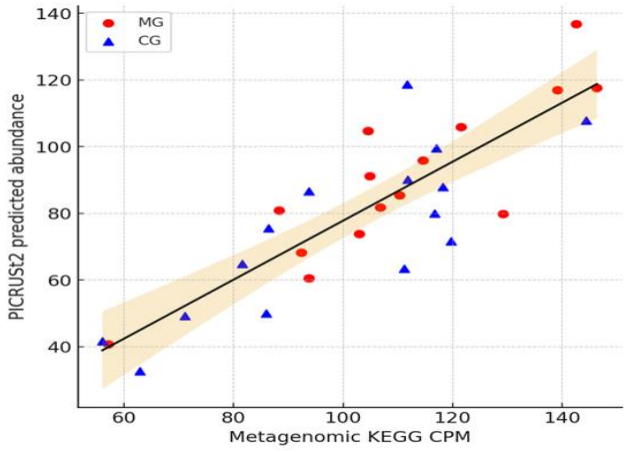

Fig. 8: Scatter plot showing the correlation between PICRUSt2-predicted pathway abundance and metagenomic measured pathway abundance (using map00650 butyrate metabolism as an example)

Building upon established evidence of metformin's effects on gut microbiota and inflammatory markers, this study employed a prospective design with multi-time point sampling and machine learning modeling to systematically evaluate its synergistic regulatory role in the microbiota-immune axis among T2DM patients. For the first time in a Chinese cohort, this study integrated multidimensional data to construct a highly discriminative combination of microbial biomarkers (AUC = 0.89), providing a novel tool for personalized prediction of metformin efficacy. Simultaneously, linear mixed models controlled for confounding factors to confirm the drug's independent improvement in inflammatory markers and revealed negative correlations between specific bacterial genera and inflammatory cytokines, further supporting the mediating role of the "microbiome-metabolism-immunity" axis in metformin's mechanism. Despite rigorous design and analysis, several limitations exist. The limited sample size (n = 42 per group) and single-center design may affect the generalizability of results and the power of subgroup analyses. Despite adjusting for multiple confounders, the influence of unmeasured variables such as genetic background and dietary details remains partially unaccounted for. The 12-week intervention period allows observation of short-term effects but fails to capture long-term dynamics between microbiota and inflammation. Furthermore, while PICRUSt2 and metagenomic/metabolomic validation supported some functional predictions, these remain indirect inferences. Future studies should integrate metatranscriptomics and metabolic flux technologies to clarify underlying mechanisms. Nevertheless, these findings provide valuable data support and methodological references for precision medicine and mechanism exploration based on microbial characteristics.

Conclusion

This study established a multidimensional evaluation system integrating clinical indicators, dynamic inflammatory cytokine monitoring, and gut microbiota multi-omics analysis. For the first time in a prospective controlled design, it systematically quantified the synergistic regulatory effect of metformin on the "inflammation-microbiota axis" in T2DM patients. The research confirmed that metformin significantly reduces pro-inflammatory factors (with a decrease in CRP >18%) while enhancing α diversity of the gut microbiota (with an increase in Shannon/Chao1 index >15%) and enriching Akkermansia and Faecalibacterium species.-α, and hs-CRP by >18%), it enhances gut microbiota α-diversity (Shannon/Chao1 index increase >15%) and enriches key bacterial genera centered on Akkermansia and Faecalibacterium (LDA >3.4). Increased abundance of these genera showed a significant negative correlation with inflammation resolution ($r \leq -0.82$). Functional prediction further indicated upregulation of the butyrate synthesis pathway (↑41.8%) and inhibition of LPS biosynthesis (↓24.5%), supporting the "microbiota-immunity" linkage mechanism at the metabolic level. A therapeutic efficacy prediction model (AUC=0.89) constructed based on microbiota characteristics provides a potential tool for personalized treatment.

This study elucidates that metformin may improve chronic inflammatory states in T2DM by reshaping gut microbiota structure, enhancing barrier function, and regulating microbial metabolism. These findings not only provide systematic evidence for the "microbiota-metabolism-immunity" axis underlying metformin's pleiotropic mechanisms but also offer novel insights for efficacy prediction and precision interventions based on microbiota biomarkers. Future studies should expand sample sizes, conduct multicenter validation, and further explore dose-response relationships and causal mechanisms.

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Author's Contributions

All authors equally contributed in this work.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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