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Original Article

Probiotic Bifidobacterium reduces serum TMAO in unstable angina patients via the gut to liver to heart axis

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ABSTRACT

Background and aims: Studies indicate that the gut microbiota and its metabolites are involved in the progression of cardiovascular diseases, and enterohepatic circulation plays an important role in this progression. This study aims to identify potential probiotics for the treatment of unstable angina (UA) and elucidate their mechanisms of action.

Methods: Initially, the gut microbiota from patients with UA and control was analyzed. To directly assess the effects of *Bifidobacterium* supplementation, 10 patients with UA were enrolled and administered *Bifidobacterium* (630 mg per intake twice a day for 1 month). The fecal metagenome, serum trimethylamine N-oxide (TMAO) levels, and other laboratory parameters were evaluated before and after *Bifidobacterium* supplementation.

Results: After supplementing with *Bifidobacterium* for 1 month, there were statistically significant differences ($P < 0.05$) in TMAO, aspartate aminotransferase, total cholesterol, and low-density lipoprotein compared to before. Additionally, the abundance of *Bifidobacterium longum* increased significantly, although the overall abundance of *Bifidobacterium* did not reach statistical significance. The gut microbiota, metabolites, and gut-liver axis are involved in the progression of UA, and potential mechanisms should be further studied.

Conclusions: Metagenomic analysis demonstrated a reduced abundance of *Bifidobacterium* in patients with UA. Supplementation with *Bifidobacterium* restored gut dysbiosis and decreased circulating TMAO levels in patients with UA. This study provides evidence that *Bifidobacterium* may exert cardiovascular-protective effects through the gut–liver–heart axis.

Clinical trial number: ChiCTR2400093946.

1. Introduction

The World Health Organization reports that cardiovascular (CV) diseases (CVDs) are responsible for 17.9 million deaths annually, accounting for 31% of all deaths.[1] Of these deaths, 85% are directly related to stroke and heart attack.[1] Therefore, significant efforts have been made to investigate and identify new therapeutic risk factors for atherosclerosis that can be exploited to enhance primary and secondary prevention of CVDs. Recent studies have proposed that environmental factors, particularly nutrition and the intestinal microbiota, are important contributors to CVD development.[2–4] Additionally, intestinal dysbiosis, a significant risk factor for CVDs, has been linked to obesity and diabetes development.[5,6] Nevertheless, the mechanisms by which the gut microbiota influences the pathogenesis and development of CVDs are still being investigated.

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Previous studies have demonstrated that trimethylamine N-oxide (TMAO) plays an important role in digestive system diseases, particularly in fatty liver and metabolic liver diseases.[7,8] Moreover, some studies have shown that elevated TMAO levels are associated with the severity of liver diseases.[9,10] TMAO is a metabolite linked to the intestinal microbiota, produced jointly by the gut flora and liver.[11] The biosynthetic pathway of TMAO involves interactions between diet, gut microbiota, and the liver. Trimethylamine (TMA) is generated by the gut flora through the metabolism of compounds such as choline, phosphatidylcholine, carnitine, gamma-butyrobetaine, betaine, crotonobetaine, and glycerol phosphorylcholine, which are abundant in fish, beef, and eggs. TMA is then absorbed through the portal vein circulation and transported to the liver, where it is oxidized by hepatic flavin monooxygenase to form TMAO. TMAO is both hydrophobic and hydrophilic, regulates protein activity and stability, increases foam cell production, and inhibits cholesterol reverse transport. Given that TMAO production depends on the diversity and composition of the gut microbiota, its levels fluctuate with dysbiosis. A recent study also indicated that liver-generated TMAO might exert a strong effect on CVDs.[12] However, the underlying regulatory mechanism remains to be explored.

The interaction between metabolic disease, *e.g.*, metabolic dysfunction-associated fatty liver disease (MAFLD) and CVDs, has elicited increasing research attention. A recent Meta-analysis reported that patients with NAFLD have a nearly 50% higher risk of CV events.[13] The risk of adverse CV events increases with the severity of NAFLD. Furthermore, this risk is independent of age, sex, obesity measures, diabetes, and other common myocardial metabolic risk factors for CVDs. Mechanistically, TMAO can modulate the immune system by stimulating TXNIP—NLRP3 inflammasomes, leading to the production of inflammatory markers.[14] High TMAO levels also negatively affect lipid profiles and contribute to a 43% increase in the risk of developing coronary artery disease (CAD).[15] Moreover, TMAO is associated with high levels of C-reactive protein and endothelial dysfunction caused by increased gut permeability and higher serum levels of lipopolysaccharide endotoxin.[16] Recent studies have found that TMAO, as an important liver-regulated metabolite, may play critical roles in disease onset and development.[17–19] However, the underlying mechanisms remain to be elucidated.

A previous study found that the gut microbiota can directly affect hypercholesterolemia and CAD development by producing metabolites such as bile acids, indoles, and short-chain fatty acids.[20] Furthermore, the gut microbiota can contribute to CAD development through indirect mechanisms, such as immune system modulation.[21] Dietary patterns may modulate gut microbiota composition and regulate the production of secondary metabolites. Choline, betaine, phosphatidylcholine, lecithin, and L-carnitine, primarily found in red meat, contribute to the production of TMAO,[22–26] which has been established as a factor that can affect CV health in various ways. However, the interaction between the gut microbiota and TMAO generation, as well as their effects on the pathogenesis and progression of CAD, are still unexplored.

Herein, this study aimed to identify potential probiotics for patients with CVDs by exploring alterations in gut microbiota. Through metagenomic analysis of the gut microbiota in the exploration cohort, we identified *Bifidobacterium* as a potential probiotic in patients with unstable angina (UA). To further validate the clinical application of *Bifidobacterium* in these patients, a validation cohort of 10 patients who received *Bifidobacterium* supplementation along with standard secondary prevention medications was established. By integrating gut microbiota and serum TMAO alterations, we discovered that *Bifidobacterium* can serve as a probiotic that can lower serum TMAO levels and promote long-term CV benefits. This study provides insights into the potential of utilizing *Bifidobacterium* as a probiotic therapy for patients with CVDs.

2. Materials and methods

2.1. Ethical approval

All procedures involving human participants were conducted in accordance with the ethical standards of the institutional or national research committee, as well as the Helsinki Declaration and its later amendments or comparable ethical standards. This pilot study was registered with the Chinese Clinical Trial Register (https://www.chictr.org.cn/indexEN.html, registration number: ChiCTR2400093946). This study was approved by the Ethics Committee of Bayannur City Hospital (approval number: 20200117002) and the First Affiliated Hospital of Xi'an Jiaotong University (approval number: XJTU1AF2018LSL-025), and written informed consent was obtained from each participant.

2.2. Study population

A total of 30 patients with UA and 30 healthy individuals from Bayannur City Hospital and the First Affiliated Hospital of Xi'an Jiaotong University were recruited in the exploration cohort from August 2020 to May 2022. The validation cohort consisted of 10 patients with UA who received *Bifidobacterium* supplementation along with standard secondary prevention medications. The present study is a pilot study. *Bifidobacterium* triple live bacteria enteric-coated capsules (Jincheng Haise Pharmaceutical Co., Ltd., Shanxi, China) were administered twice daily at a dose of 630 mg per intake for 1 month (Fig. 1).

The inclusion criteria were as follows: (i) patients newly diagnosed with UA who were undergoing coronary angiography and had a Gensini score of $\geq$40, (ii) age 45–75 years, (iii) Han ethnicity without blood relationship, (iv) relatively stable long-term residence, (v) regular lifestyle and dietary habits without significant changes, and (vi) normal bowel movements and absence of recent diarrhea, constipation, or changes in bowel frequency or habits. The exclusion criteria were as follows: (i) history of diabetes, hyperlipidemia, hyperuricemia, cerebral infarction, and cerebral hemorrhage; (ii) severe liver diseases, renal dysfunction, and malignant tumors; (iii) digestive system diseases; (iv) intake of traditional Chinese medicines, formulas, antibiotics, and other medications that may have affected the gut microbiota within the past months; and (v) individuals with poor compliance and those currently participating in other clinical trials.

The samples were selected by voluntary participation, and the validation cohort was distinct from the exploration cohort.

2.3. Sample collection

Both fasting venous blood and stool samples were collected from the same individual in the exploration cohort for the analysis of baseline laboratory parameters and fecal metagenome. In the validation cohort, fasting venous blood and stool samples were obtained from each participant before and after 1 month of *Bifidobacterium* supplementation to analyze laboratory parameters, fecal metagenome, and TMAO levels. Peripheral venous blood samples were obtained in the morning while participants were hospitalized or enrolled. Collected samples were centrifuged at 1000×*g* for 10 min at 4 °C and stored at −80 °C until further analysis. Stool samples were collected on the same day, snap-frozen with liquid nitrogen, and stored at −80 °C until further testing.

2.4. Metagenomic and 16S rRNA gene sequencing

The TMAO and its related metabolites' standards and 2 stable isotope-labeled standards were obtained from ZZ Standard Co., Ltd. (Shanghai, China). Ammonium acetate was of analytical grade and

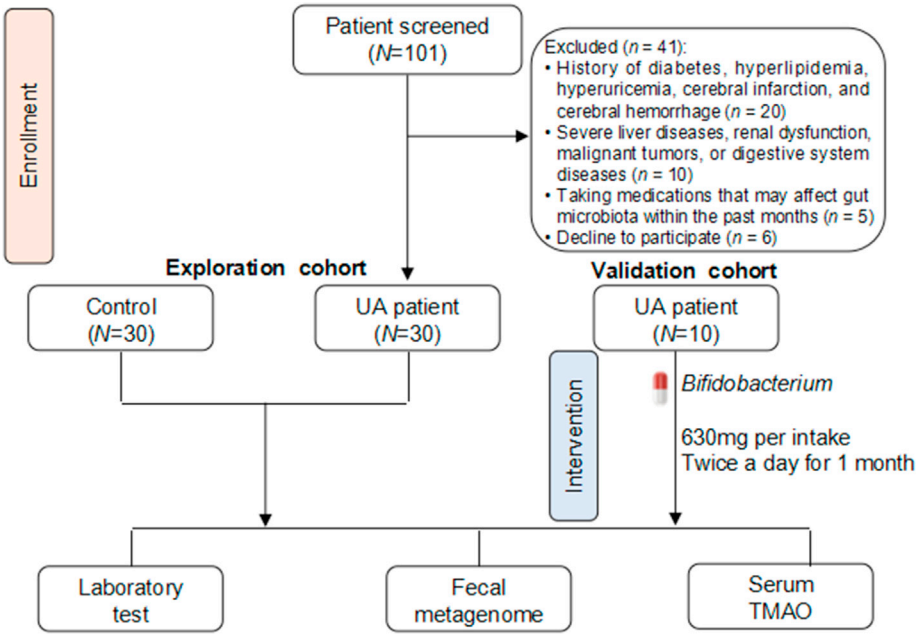


Fig. 1. Schematic of the study design. A total of 101 patients diagnosed with UA were screened, of whom 41 were excluded for various reasons. The exploration cohort comprised 30 patients with UA and 30 healthy individuals. The validation cohort consisted of 10 patients with UA who were administered *Bifidobacterium* along with standard secondary prevention medications. Baseline laboratory parameters, fecal metagenome, and serum TMAO levels were evaluated for all participants in the exploration cohort and patients with UA in the validation cohort, both before and after *Bifidobacterium* supplementation. Abbreviations: TMAO, trimethylamine N-oxide; UA, unstable angina.

obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (Optima LC-MS) was purchased from Thermo-Fisher Scientific (Fair Lawn, NJ, USA) for metagenomic sequencing. Sequencing was performed on the Illumina NovaSeq platform. The abundance and diversity of intestinal flora were determined by Illumina HiSeq sequencing (Novogene Co., Ltd., Beijing, China), after amplification and purification of the V3–V4 region of bacterial 16S rRNA genes. More details were described previously.[27]

2.5. TMAO measurement

Serum levels of TMAO-related metabolites and amino acids were measured via isotope dilution liquid chromatography–tandem mass spectrometry.

2.6. Statistical analyses

GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics version 22.0 (IBM Corp, Armonk, NY, USA) were used for statistical analyses. Data are presented as frequencies or percentages for categorical variables and means ± SEM for continuous variables. Shapiro-Wilk test was used to determine the normality of the data distribution. To compare differences between two independent groups, a simple *t*-test was used for normally distributed continuous variables, the Chi-square test for categorical data, and the Mann–Whitney *U* test for non-normally distributed continuous data. For paired comparisons (*e.g.,* before and after *Bifidobacterium* supplementation), the Wilcoxon signed rank test was used for non-normally distributed variables. Correlations were conducted using Spearman's test or Pearson's correlation analysis. KEGG enrichment analysis was performed using R 3.5.1 and iPath 3 (https://pathways.embl.de/). Advanced Diff Redundancy analysis and LEfSe were performed using OmicStudio tools (https://www.omicstudio.cn/tool). The significance of differences was determined using a two-tailed test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Metagenomic analysis revealed a reduced abundance of Bifidobacterium in patients with UA

As shown in Table 1, statistically significant differences in red blood cell count and hemoglobin were observed between the UA and the control groups after excluding drug interference, whereas no differences existed in age and renal functions. Furthermore,

Table 1
Baseline characteristics of the exploration cohort.

Characteristics	Control (n = 30)	Unstable angina (n = 30)	*P*-value
Age (years)	56.43 ± 8.49	57.97 ± 8.25	0.481
Female, n (%)	19 (63.3)	11 (30.0)	**0.009**
WBC ($\times 10^9$/L)	6.13 ± 1.67	6.32 ± 1.32	0.351
Neu ($\times 10^9$/L)	4.23 ± 1.29	3.97 ± 1.30	0.438
Ly ($\times 10^9$/L)	1.98 ± 0.52	1.91 ± 0.66	0.678
RBC ($\times 10^{12}$/L)	4.52 ± 0.44	4.88 ± 0.46	**0.004**
Hb (g/L)	136.77 ± 15.70	146.27 ± 14.78	**0.019**
Plt ($\times 10^9$/L)	227.9 ± 51.25	200.33 ± 40.21	0.866
ALT (U/L)	19.83 ± 11.52	27.2 ± 12.04	**0.019**
AST (U/L)	19.67 ± 7.97	21.3 ± 10.59	0.494
TC (mmol/L)	4.32 ± 0.93	4.36 ± 1.22	0.888
HDL (mmol/L)	1.15 ± 0.26	1.24 ± 0.62	0.470
TG (mmol/L)	1.71 ± 0.97	1.60 ± 0.73	0.631
LDL (mmol/L)	2.63 ± 0.69	2.66 ± 1.01	0.882
VLDL (mmol/L)	0.34 ± 0.19	0.61 ± 0.80	0.073
sLDL (mmol/L)	0.72 ± 0.41	0.73 ± 0.49	0.948
eGFR (mL/min/1.73 m^2)	92.50 ± 14.13	96.00 ± 46.13	0.695
Cre (μmol/L)	64.45 ± 14.48	65.45 ± 13.27	0.900

Data are expressed as means ± SEM or n (%). $P < 0.05$ for equality between control and CAD. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; Cre, creatinine; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ly, lymphocyte; Neu, neutrophil; Plt, platelet; RBC, red blood cell; sLDL, small dense low-density lipoprotein; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein; WBC, white blood cell.

metagenome sequencing showed distinct microbiome composition between the UA and control groups (Fig. 2A). No significant difference was observed in the alpha diversity of the gut microbiome of the UA and control groups, indicating that the UA group had similar bacterial diversity to the control group (Fig. 2B). Interestingly, abundance of *Bifidobacterium* was reduced in the UA group (Fig. 2C and D). These observations suggest that *Bifidobacterium* may serve as a potential probiotic in patients with UA.

3.2. Supplementation with Bifidobacterium restored gut dysbiosis in patients with UA

To directly test the effects of *Bifidobacterium* supplementation, 10 patients with UA were enrolled and administered *Bifidobacterium* capsules twice daily for 1 month (Fig. 3A). The 10 UA patients were all male with an average age of 57.36 ± 9.06. Plasma levels of total cholesterol and low-density lipoprotein decreased in the UA group after *Bifidobacterium* supplementation (P = 0.042 and 0.036, respectively); however, no significant difference in the levels of other lipids, including high-density lipoprotein and triglycerides, was found. Notably, AST levels increased post-intervention (Table 2).

Furthermore, fecal 16S rRNA gene sequencing analysis was conducted in the UA group before and after *Bifidobacterium* supplementation (Fig. 3B and C). Figure. 3B shows the results of a phylogenetic tree analysis of the microorganisms before and after *Bifidobacterium* supplementation. Principal coordinate analysis revealed that the gut microbiota changed after *Bifidobacterium* supplementation (Fig. 3C). After 1 month of treatment, patients with UA showed an increase in the abundance of *Bifidobacterium*; however, this change was not statistically significant (Fig. 3D). In contrast, *Bifidobacterium longum* demonstrated a statistically significant increase (Fig. 3E). These findings suggest that *Bifidobacterium* may restore gut dysbiosis in patients with UA.

3.3. Bifidobacterium supplementation modulated metabolic pathways and TMAO levels in patients with UA

To better understand the potential mechanisms through which *Bifidobacterium* may influence the occurrence of UA, we functionally characterized the altered gut microbiome before and after *Bifidobacterium* supplementation and examined its relationships with serum TMAO levels and other parameters. Functional alterations in the microbiome of patients with UA following *Bifidobacterium* supplementation were associated with various metabolic categories, including carbohydrate, amino acid, and nucleotide metabolism (Fig. 4A). Additionally, *Bifidobacterium* abundance was correlated with clinical indicators at both the genus and species levels; of note, negative correlation between TMAO and *bifidobacterium bifidum* and *bifidobacterium dentium* and positive correlation between TMAO and *bifidobacterium longum* and *bifidobacterium pseudolongum* were identified (Fig. 4B).

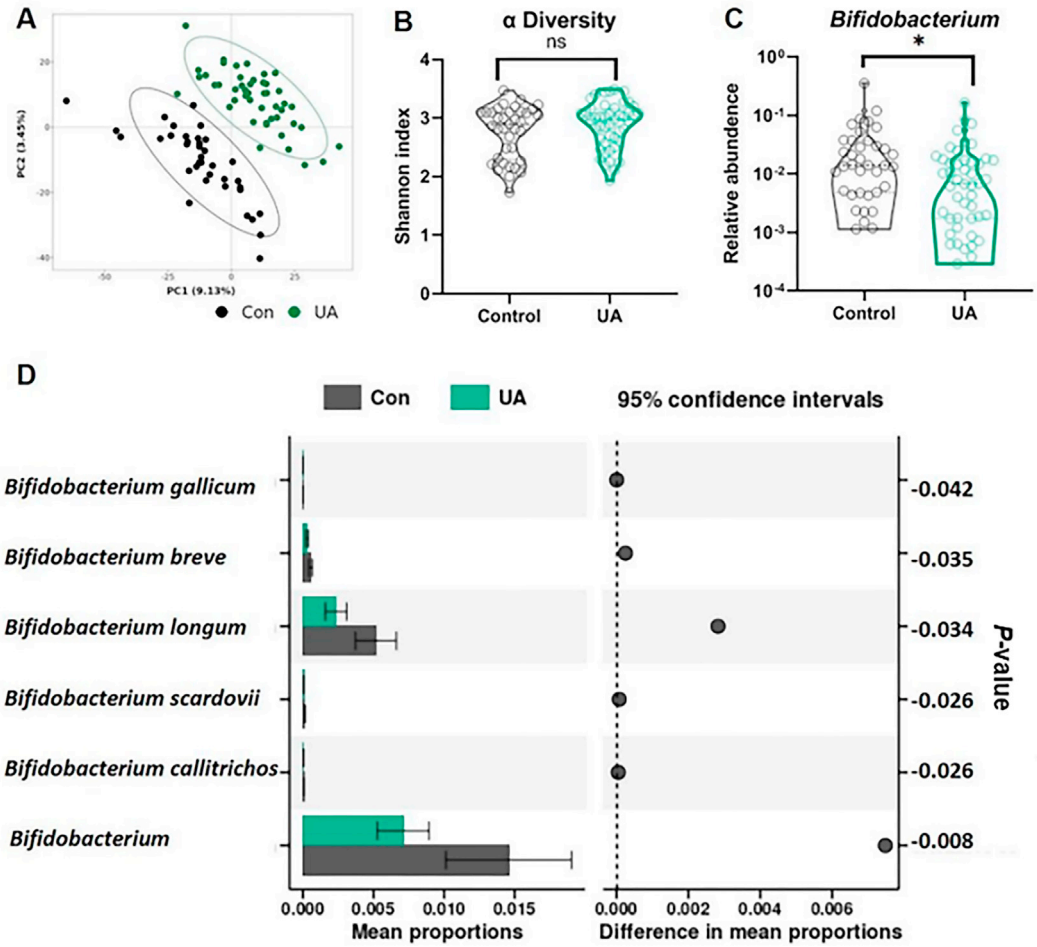


Fig. 2. Metagenomic analysis indicated a reduced abundance of *Bifidobacterium* in patients with UA. (A) PLS-DA shows the metabolites of patients in the UA and con groups. **(B)** α-diversity and **(C)** the abundance of *Bifidobacterium* metabolites are compared between the UA and Con groups. **(D)** A stamp plot displays the *Bifidobacterium* genus and species. *P < 0.05; ns, not significant. Abbreviations: Con, control; PLS-DA, partial least squares discriminant analysis; UA, unstable angina.

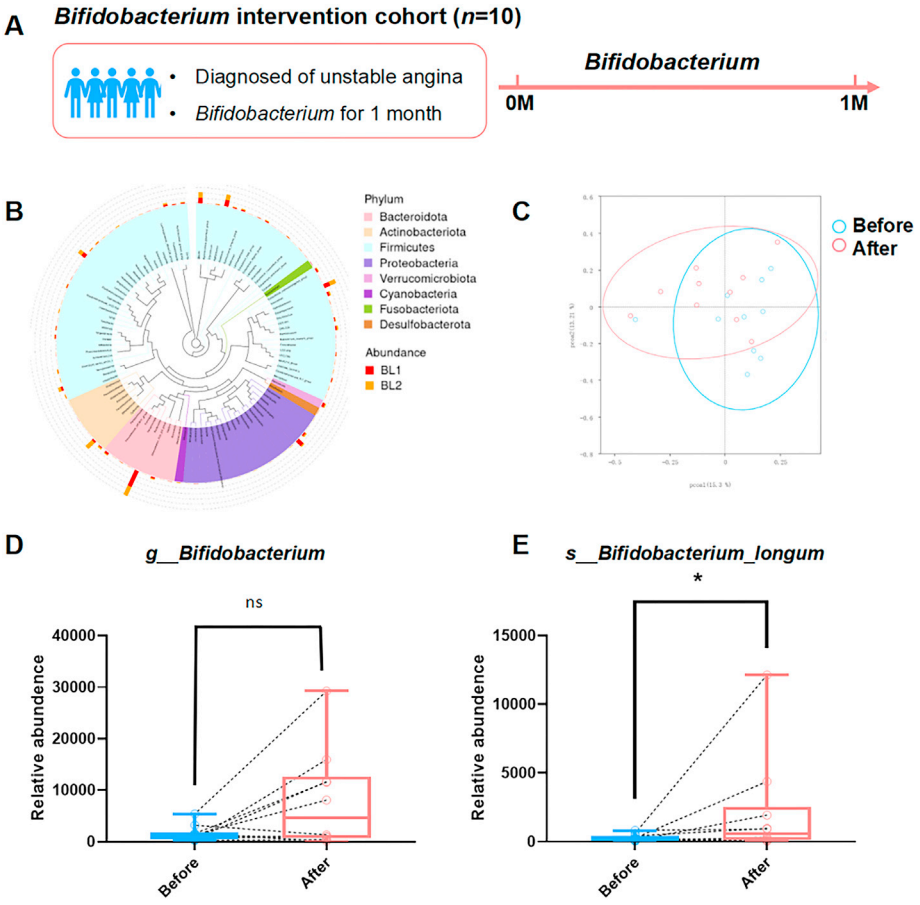


Fig. 3. Effect of *Bifidobacterium* supplementation on gut microbiota in patients with unstable angina. **(A)** Flowchart showing *Bifidobacterium* supplementation in patients with unstable angina. **(B)** Genus-level evolutionary tree of the study cohort. **(C)** PCoA of the gut microbiota before and after *Bifidobacterium* supplementation. **(D)** Abundance of *Bifidobacterium* at the genus levels after supplementation. **(E)** Abundance of *Bifidobacterium* at the species levels after supplementation. *$P < 0.05$; ns, not significant.

Table 2
Characteristics of the participants before and 1 month after *Bifidobacterium* supplementation.

Characteristics	Before ($n = 10$)	After ($n = 10$)	*P*-value
WBC ($\times 10^9$/L)	7.76 ± 1.62	7.03 ± 1.69	0.342
Neu ($\times 10^9$/L)	5.23 ± 1.55	4.77 ± 1.78	0.544
Ly ($\times 10^9$/L)	1.84 ± 0.38	1.62 ± 0.31	0.189
RBC ($\times 10^{12}$/L)	4.92 ± 0.31	4.84 ± 0.39	0.603
Hb (g/L)	151.00 ± 8.83	146.80 ± 10.18	0.338
Plt ($\times 10^9$/L)	211.30 ± 29.80	231.70 ± 75.19	0.436
ALT (U/L)	23.50 ± 11.62	32.60 ± 13.16	0.119
AST (U/L)	18.00 ± 3.94	22.70 ± 5.69	**0.046**
TC (mmol/L)	4.15 ± 0.81	3.46 ± 0.57	**0.042**
HDL (mmol/L)	0.95 ± 0.21	0.94 ± 0.27	0.923
TG (mmol/L)	2.06 ± 0.91	1.91 ± 0.76	0.683
LDL (mmol/L)	2.54 ± 0.76	1.89 ± 0.48	**0.036**
VLDL (mmol/L)	0.41 ± 0.18	0.38 ± 0.15	0.687
sLDL (mmol/L)	0.74 ± 0.33	0.59 ± 0.23	0.270
eGFR (mL/min/1.73 m^2)	92.30 ± 12.86	95.40 ± 6.86	0.510
Cre(μmol/L)	79.80 ± 11.06	77.30 ± 7.39	0.560

Data are expressed as means ± SEM or *n* (%). $P < 0.05$ for equality between control and CAD. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; Cre, creatinine; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ly, lymphocyte; Neu, neutrophil; Plt, platelet; RBC, red blood cell; sLDL, small dense low-density lipoprotein; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein; WBC, white blood cell.

3.4. Bifidobacterium supplementation decreased circulating TMAO levels in patients with UA

Finally, circulating TMAO levels were evaluated in 10 patients with UA before and after *Bifidobacterium* supplementation. *Bifidobacterium* significantly reduced serum TMAO levels in patients with UA (Fig. 5A). Additionally, TMAO levels were significantly correlated with clinical indicators both before and after supplementation (Fig. 5B). Overall, the composition and function of the gut microbial community differed between the control and UA groups, and *Bifidobacterium* supplementation may lower TMAO levels and exert a CV protective effect (Fig. 5C).

4. Discussion

In this study, metagenomic analysis of the exploration cohort revealed a decreased abundance of *Bifidobacterium* in patients with UA. However, ethnic or regional differences must be considered during sample enrollment, which could become confounding factors that affect the internal and external validity of the present results. We also examined differences in gut microbiota and TMAO levels between patients who received *Bifidobacterium* supplementation and the control group. The findings showed that an increase in *Bifidobacterium* concentration correlates with a reduction in

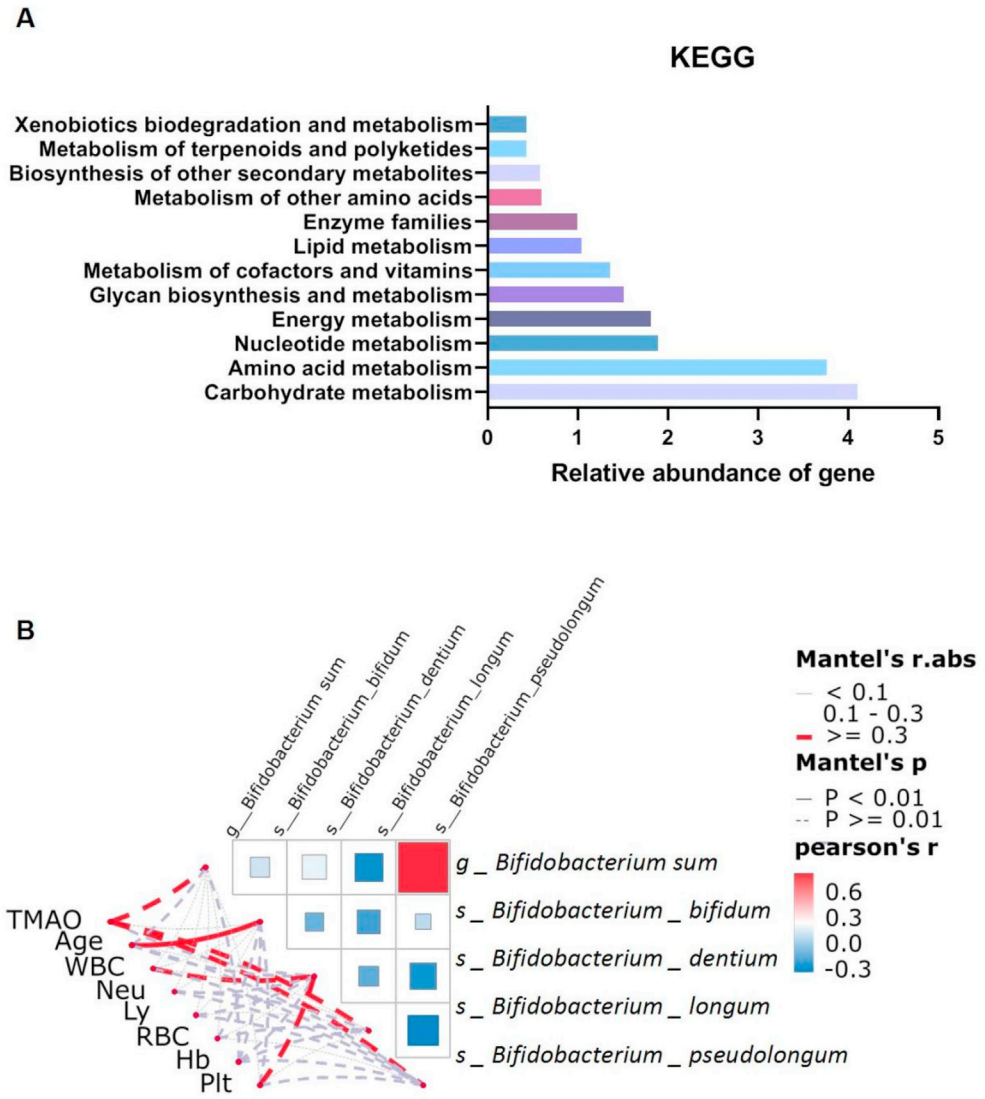


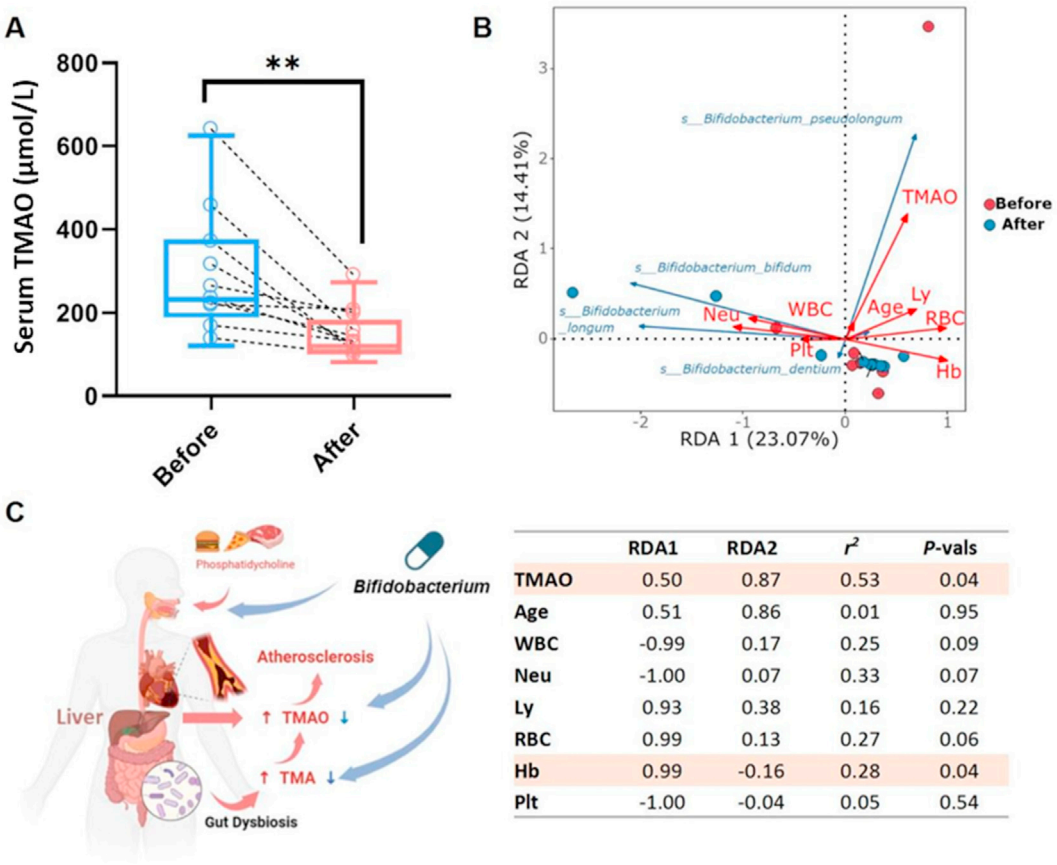
Fig. 4. *Bifidobacterium* supplementation altered metabolic pathways and was correlated with clinical indicators in patients with unstable angina. (A) KEGG enrichment analysis shows marked enrichment in the carbohydrate metabolism pathway. **(B)**. A correlation network heatmap was created to illustrate the relationship between *Bifidobacterium* and clinical indicators. Abbreviations: Hb, hemoglobin; Ly, lymphocyte; Neu, neutrophil; Plt, platelet; RBC, red blood cell; TMAO, trimethylamine N-oxide; WBC, white blood cell.

TMAO levels. The mediation of liver-generated TMAO by the intestinal microbiota has emerged as a promising therapeutic target for atherosclerosis treatment. This study provides novel evidence that *Bifidobacterium* potentially confers CV protection via the gut–liver–heart axis.

The gut microbiota plays a pivotal role in host metabolism and indirectly affects lipid metabolism by influencing cholesterol and bile acid metabolism. For instance, certain probiotics can increase the conversion of cholesterol to bile acids, promote cholesterol excretion, and reduce cholesterol levels in the blood. The gut microbiota also supports the integrity of the gut barrier, preventing the leakage of toxins into the bloodstream. Additionally, an imbalance in the gut microbiota can result in inflammation that is associated with insulin resistance and metabolic syndrome. Understanding the intricate relationship between gut microbiota and host metabolism is critical for developing therapeutic strategies for diabetes, cardiometabolic diseases, and other metabolic disorders. This study showed a decreased abundance of *Bifidobacterium* in patients with UA. *Bifidobacterium* supplementation restored gut dysbiosis and reduced plasma total cholesterol and low-density lipoprotein levels in patients with UA. These findings provide evidence for the potential of *Bifidobacterium* as a probiotic adjuvant therapy for UA.

The liver-generated metabolite TMAO plays a critical role in CVDs by modulating intestinal flora. Probiotics are ingestible microorganisms that reach the intestinal lumen, where they can play functional roles in host physiology.[28] The oral administration of adequate amounts of probiotics has been reported to provide CV benefit.[29,30] Moreover, the treatment of apolipoprotein E (ApoE)-deficient mice with *Bacteroides vulgatus* and *Bacteroides dorei*, two species potentially exhibiting lower abundance in patients with CAD, inhibited the formation of atherosclerotic plaques.[31] This study underscores the potential of TMAO therapy to exert beneficial effects on CAD prevention and treatment.

This study provides further evidence that changes in gut microbiota after *Bifidobacterium* supplementation lowered TMAO levels in patients with UA. *Bifidobacterium* indirectly influences immune homeostasis by regulating the intestinal environment and microbial community structure. *Bifidobacterium*'s bile salt hydrolase can hydrolyze bile salts into free bile acids, thereby enhancing



	RDA1	RDA2	r^2	*P*-vals
TMAO	0.50	0.87	0.53	0.04
Age	0.51	0.86	0.01	0.95
WBC	-0.99	0.17	0.25	0.09
Neu	-1.00	0.07	0.33	0.07
Ly	0.93	0.38	0.16	0.22
RBC	0.99	0.13	0.27	0.06
Hb	0.99	-0.16	0.28	0.04
Plt	-1.00	-0.04	0.05	0.54

Fig. 5. *Bifidobacterium* supplementation decreased circulating TMAO levels in patients with unstable angina. (A) Circulating TMAO levels before and after *Bifidobacterium* supplementation. **(B)** RDA shows significant correlations between *Bifidobacterium* and clinical indicators at different time points. **(C)** Proposed mechanism. $^{**}P < 0.01$. Abbreviations: Hb, hemoglobin; Ly, lymphocyte; Neu, neutrophil; Plt, platelet; *P*-vals, *P*-values; RBC, red blood cell; RDA, redundancy analysis; TMA, trimethylamine; TMAO, trimethylamine N-oxide; WBC, white blood cell.

its antibacterial activity against species such as *Bacteroidetes*, *Bacillus*, *Ruminococcus*, *Clostridium*, and *Escherichia coli*.[32–34] These bacteria further metabolize bile components into secondary bile acids. The transformation of primary bile acids into secondary bile acids is a pivotal intestinal process that affects metabolism and immune function, thereby helping maintain intestinal homeostasis.[35,36] Furthermore, five species, namely, *Eubacteria*, *Anaeroplasma*, *Roseburia*, *Oscillospira*, and *Dehalobacteria*, were found to be effective in preventing atherosclerosis in a previous animal study.[37] Similarly, experiments performed by Stepankova *et al*.[38] demonstrated the protective effects of intestinal bacteria on the progression of atherosclerotic lesions.

Furthermore, a correlation was found between exogenous supplementation of *Bifidobacterium* and TMAO levels. Long-term dietary habits can alter the composition of the intestinal flora, ultimately affecting TMAO generation. Studies have indicated that TMAO plays a significant role in the onset and progression of various diseases, including coronary heart disease and fatty liver, and dietary interventions can notably reduce CV risk.[39,40] A high-fiber diet has been reported to increase the proportion of acetate-producing microbiota, reduce blood pressure, and alleviate heart hypertrophy and fibrosis[41–44] However, an ongoing debate exists regarding whether TMAO acts as a trigger for the onset and progression of human diseases or simply serves as a potential pathological marker. Interestingly, our study revealed that different species of *Bifidobacterium* exhibit distinct correlations with TMAO. The potential reasons are speculated as follows: *Bifidobacterium bifidum* and *Bifidobacterium dentium* may reduce TMAO levels by competitively inhibiting other TMA-producing microorganisms or by directly metabolizing TMA. As the gut microbiota is a complex ecosystem, the interactions among different *Bifidobacterium* species as well as with other microbes may influence TMAO levels. *Bifidobacterium longum* and *Bifidobacterium pseudolongum* may have synergistic effects with other TMA-producing microorganisms, or their presence may not be sufficient to counteract the TMAO-promoting effects of other microbes. Further research is required to elucidate the relationship between TMAO and *bifidobacteria*. If the full potential of TMAO can be realized, it could emerge as a novel therapeutic target for improving patient prognosis.

This study has several limitations. First, the sample size was relatively small, including only 10 patients with UA who were administered *Bifidobacterium*. This limitation may constrain the identification of potential risk factors in microbial community analysis through 16S rRNA gene sequencing. Therefore, large-scale, multicenter randomized controlled trials with long-term follow-up are needed to determine how *Bifidobacterium* might exert its long-term CV benefits in patients with CAD. Second, inconsistencies in sex composition might have led to variations in certain indicators. Although this study enrolled patients diagnosed with UA, which has a higher prevalence in men, this issue of sex composition could not be easily avoided and should be considered a confounding factor. Third, alterations in the patients' gut microbiota and TMAO levels may not be solely attributed to *Bifidobacterium*. These changes could also be influenced by variations in gut permeability following dietary changes, coronary stent implantation, and

modifications in blood flow. Therefore, further in-depth studies are needed to elucidate the potential mechanisms by which *Bifidobacterium* lowers circulating TMAO levels.

5. Conclusions

This study evaluated changes in the gut microbiota and TMAO levels in patients with UA. We found that the abundance of *Bifidobacterium* was significantly decreased in patients with UA. Probiotic intervention via *Bifidobacterium* supplementation decreased the levels of the liver-generated metabolite TMAO, which could represent a new strategy for improving UA outcomes in the future.

Authors' Contribution

Zhihong Zhou, Lizhe Sun, and Wei Zhou contributed equally to this paper and should be considered co-first authors. **Zhihong Zhou:** Visualization, Software, Methodology, Investigation. **Lizhe Sun:** Writing – original draft, Software, Methodology. **Wei Zhou:** Formal analysis, Data curation. **Wen Gao:** Data curation. **Xiao Yuan:** Investigation, Data curation. **Huijuan Zhou:** Methodology. **Yuzhen Ren:** Data curation. **Bihua Li:** Data curation. **Yue Wu:** Writing – review & editing, Project administration. **Jianqing She:** Writing – review & editing, Supervision, Project administration, Funding acquisition. All of the authors approved the final manuscript.

Data availability

The study protocol, standard operating procedures, and data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that there is no conflicts of interest.

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