International Journal of Biology and Life Sciences
ISSN: 2957-9511 | Vol. 11, No. 2, 2025

Effects of Cinnamaldehyde on the Gut Microbiota Composition and Function in T1DM Model Mice

Honghong Duan [1], Jie Luo [1], Shilong Xie [1], Nana Liu [1], Jie Zhang [1], Dianchun Xie [1],

Honglei Zhao [2,*]

[1] The Third People's Hospital of Longgang, Clinical Institute of Shantou University Medical College（The Third People's Hospital of Longgang District Shenzhen）, Shenzhen, Guangdong, China
[2] Fuwai Shenzhen Hospital, Chinese Academy of Medical Sciences, Shenzhen, Guangdong, China
*** Corresponding author:** Honglei Zhao (Email: zhaohonglei@nsqyljtzb.cn)

Abstract: Objective To investigate the effects of cinnamaldehyde on the gut microbiota composition and function in T1DM model mice. **Methods** Following cinnamaldehyde intervention, total fecal DNA was extracted from experimental mice. The extracted DNA underwent quality assessment, library construction, sequencing, and bioinformatic analysis to obtain raw data. Subsequently, metagenomic analysis was performed: Kneaddata was used for quality control and host genome removal; MetaPhlAn2 was applied for taxonomic profiling, and HUMAnN2 for functional annotation. Strain-level resolution was achieved using StrainPhlAn based on MetaPhlAn2 results. **Results** Cinnamaldehyde intervention significantly increased *Lactobacillus johnsonii* abundance in healthy mice, rising to 43.56% and 38.88%. Additionally, *Parasutterella excrementihominis*, *Dorea* sp. 5_2, *Burkholderiales* bacterium 1_1_47, and *Mucispirillum schaedleri* showed marked enrichment in healthy mice. In T1DM model mice, intestinal *Enterococcus faecalis*, *Lactobacillus reuteri*, and *Enterorhabdus caecimuris* levels increased, while *Lactobacillus murinus* decreased. **Conclusion A**t the functional level, multiple metabolism-associated genes were altered post-cinnamaldehyde treatment. Cinnamaldehyde modulated the expression of functional genes and metabolic pathway enrichment in murine microbiomes. Specifically, it promoted branched-chain amino acid (BCAA) synthesis and upregulated cell growth factor-related pathways in healthy mice, enhancing growth metabolism. In T1DM mice, cinnamaldehyde elevated TCA cycle-related genes, glycosaminoglycan synthesis, and signaling molecules (e.g., N-acetylneuraminic acid), facilitating host degradation and utilization of glucose/lipids. Correlation analyses revealed significant associations between blood glucose levels and 88 microbial gene functions/pathways (positive) and two pathways (negative); 11 pathways negatively correlated with insulin resistance.

Keywords: Cinnamaldehyde; Gut Microbiota; Type 1 Diabetes Mellitus.

1. Introduction

In recent years, research on the gut microbiota has become a hot topic in the pathogenesis and therapeutic targets of diabetes(Liu et al., 2022)[1].Studies have found that the composition and diversity of the gut microbiota in type I diabetes mice have changed significantly compared with normal mice. The relative abundances of Bacteroides, Rossiella, Faecalibacterium, Akkermansia, and Bifidobacterium in the gut microbiota are closely related to the occurrence and development of diabetes; Bifidobacterium lactis promotes glycogen synthesis and glucose absorption, thus affecting the host's glucose metabolism(Li et al., 2020)[2]. Currently, research on the pathogenic mechanism of the gut microbiota in T1DM mainly focuses on the structure and function of the microbiota. The relationship with glucose metabolism is evaluated by observing changes in microbiota diversity and microbiota metabolites(Del Chierico et al., 2022)[3]. In a comparative study of 16 children with T1DM and 16 healthy children, significant differences were found in the gut microbiota structure between healthy children and children with T1DM. The abundances of Actinobacteria and Firmicutes in children with T1DM, as well as the ratio of Firmicutes to Bacteroidetes, were lower than those in healthy children. At the genus level, there were more Lactobacillus, Bifidobacterium, Blautia coccoides/Eubacterium rectale, and Prevotella in the gut of healthy children; there were more Clostridium, Bacteroidetes, and Veillonella in the gut of children with T1DM. The abundances of Haemophilus, Lachnospira, Dialister, and Acidaminococcus increased, while the abundance of Blautia decreased significantly, indicating that the diversity and stability of the gut microbiota are related to the development of T1DM(Tamahane et al., 2024)[4]. Therefore, the stability of the gut microbiota and the high ratio of Firmicutes to Bacteroidetes are considered to be early diagnostic indicators of T1DM. Changes in gut microbiota diversity are usually accompanied by changes in the expression of microbiota functional genes, resulting in up - regulation and down - regulation of microbiota metabolites and signaling molecules at the pathway level. Metabolites enter the systemic circulation through intestinal epithelial cells, inducing the regulation of pathways related to glucose, lipids, bile acids, etc., and inflammatory responses, which may be the possible molecular mechanism by which the gut microbiota affects the onset and development of diabetes(Zhao et al., 2023)[5].

High - throughput sequencing technology can quickly and accurately obtain information related to microbial diversity and function, and it is an important means for studying the mechanism of action between the gut microbiota and diseases. Targeted regulation of the gut microbiota by traditional Chinese medicine may provide new drug treatment options for patients with T1DM. To further study the relationship

between the characteristics of microbiota structure changes after cinnamaldehyde intake and T1DM phenotypes, fecal samples of mice during the cinnamaldehyde intervention experiment were analyzed by metagenomic sequencing to simultaneously obtain the changes in both the diversity and function of the mouse gut microbiota. The main targets and pathways of cinnamaldehyde intake in regulating the gut microbiota were evaluated, and the potential molecular mechanism of cinnamaldehyde in improving diabetes was explored.

2. Materials and Methods

After the cinnamaldehyde intervention experiment, the mice were euthanized by excessive CO_2 inhalation. 200 mg of fecal samples were collected from the colon tissues of mice in each group. The total DNA of mouse feces was extracted according to the experimental steps provided by the QIAamp DNA Stool Mini Kit, and the extracted DNA was detected, subjected to library construction, sequencing, and information analysis to obtain the raw data. After obtaining the raw sequencing data, bioBakery Workflows developed by the Huttenhower Laboratory at Harvard University was used for metagenomic analysis. First, Kneaddata was used for quality control and removal of the host genome. Then, MetaPhlAn2 was imported for microbial community classification annotation and HUMAnN2 for functional annotation. The results of MetaPhlAn2 can be analyzed at the strain level using StrainPhlAn.

2.1. Data Quality Control

The original data were processed using the Kneaddata pipeline to filter out bases with a quality value less than 20 and a minimum length less than 50% of the read length, as well as adapters. By comparing with the default database, human - derived gene contamination was filtered out to obtain valid data.

2.2. Species Identification and Annotation

For the data that passed the quality inspection, MetaPhlAn2 was used for species identification of metagenomic data. In this experiment, multi-threading was employed to process the sequencing files of each sample one by one and then merge them into a species abundance information table.

2.3. Gene Functions and Metabolic Pathways

The HUMAnN2 software (Franzosa et al., 2018) was used to identify and evaluate the enrichment degree of functional genes in the gut microbiota and the expression status of genes involved in metabolic pathways. A hierarchical search strategy was adopted to determine the species, align to the pan - genome, and quantify gene families and metabolic pathways, thereby obtaining the annotated abundance matrix table of functional genes in the gut microbiota of mice in different treatment groups. The associations between strains in the gut microbiota structure within functional pathways under different treatment conditions were compared, and further, the associations between the microbiota structure and the expression levels of functional genes were established. Univariate and multivariate statistical analysis methods were employed to evaluate the abundance characteristics of the diversity of major metabolic functions and the expression difference characteristics of different functional genes in metabolic pathways. During the intervention and regulation period of cinnamaldehyde, the ternary response of gut microbiota diversity - abundance - function was established to re - understand the functional composition of the microbiome and the relationships among species from the perspective of diversity.

3. Experimental Results

3.1. Analysis of Gut Microbiota Diversity

The Alpha diversity of the gut microbiota in mice during the cinnamaldehyde intervention experiment period. First, the differences in the gut microbial diversity among four groups of mice (Groups A, B, C, and D) were analyzed and evaluated from two aspects: Alpha and Beta diversity. Alpha diversity reflects the species diversity within a single sample, and four indices were used, namely the Shannon index, chao1 index, Simpson index, and Inverse Simpson index. As shown in the figure, after normal mice were fed with cinnamaldehyde (Group B), the Alpha diversity showed a relatively convergent characteristic. The medians of both the Shannon and Inverse Simpson indices were higher than those in Group A, indicating that compared with normal mice, the intake of cinnamaldehyde could promote the increase in the richness of the gut microbiota in normal mice. The intervention results in T1DM model mice showed that in Group D, which was given cinnamaldehyde, the four indices (Simpson, Shannon, chao1, and Inverse Simpson) of gut microbiota were higher than those in Group C. In terms of the median values, the Alpha diversity indices of Group D were the highest among the four treatment groups. The results suggest that cinnamaldehyde can improve the Alpha diversity of the microbiota to a certain extent. Through analysis of variance (ANOVA), it was found that there were no significant differences in Alpha diversity among Groups A, B, C, and D ($p > 0.05$). Therefore, the Alpha diversity of T1DM model mice was higher than that of normal mice, and the Alpha diversity of the cinnamaldehyde intervention group was higher than that of the control group, but these differences were not statistically significant. The results suggest that the intake of cinnamaldehyde can promote the improvement of the structural diversity of the gut microbiota in T1DM model mice.

Beta diversity of the gut microbiota in mice during the cinnamaldehyde intervention experiment period. PC1 and PC2 explained 65% and 21% of the variance respectively, clearly showing the differences in the microbiota structure among the four treatment groups. Compared with Group A, the projections of Group B were more convergent, which was consistent with the alpha diversity results described above, indicating that cinnamon intake could directionally regulate the gut microbiota of normal mice, leading to a decrease in the inter - group differences in the cinnamaldehyde - treated groups. Further comparison of the results of Group B, Group C, and Group D showed that although Groups C and D both belonged to T1DM model mice, the projections of Group B and Group D had a higher degree of overlap, suggesting that the gut microbiota structure after cinnamon treatment tended to be similar, while the community structures of Group A (vehicle - treated) and Group C were more similar. The PCOA analysis in Figure 3 - 4B also showed a distribution pattern consistent with that of PCA. The NMDS analysis in Figure 3 - 4C revealed that there were differences in the gut microbiota structure between T1DM model mice and normal mice. The gut microbiota of T1DM model mice had higher heterogeneity and a more discrete spatial distribution. As shown in Figure 3 - 4B, the overall community structure was

directionally adjusted after cinnamaldehyde treatment. The DAPC analysis in Figure 12D further revealed that the community structures of samples from different treatments presented four modules. The difference between Group B and Group D was small, and the microbiota was mainly driven by genera represented by Parabacteroides. It can be inferred that there were significant differences in Beta diversity between mice treated with cinnamaldehyde and those treated with the vehicle, while the differences in Beta diversity between T1DM model mice and normal mice were small. The results suggest that cinnamon intake can directionally drive changes in the abundance of important genera represented by Parabacteroides, thereby causing directional regulation of the overall community structure in normal mice and T1DM model mice. The differences in microbiota structure caused by cinnamaldehyde intervention were greater than the background community structure differences between T1DM and normal mice.

3.2. Analysis of Gut Microbial Composition

The biological composition of the mouse gut microbiota at different taxonomic levels during the cinnamaldehyde intervention experiment period is shown in Tables 1, 2, 3, and 4. At the phylum level, the gut microbial flora is composed of six major categories: Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Deferribacteres, and Actinobacteria. Among them, Firmicutes is the predominant phylum, with an average relative abundance of 73.27% in all samples. The results of one - way analysis of variance show that before and after the intervention of the excipient and cinnamaldehyde, the relative abundance of Deferribacteres in Group A of normal mice is higher than that in the T1DM model mouse treatment group (Figure 3 - 5B), and there are no significant differences or changes in other microbial flora at the phylum level (Table 1).

Table 1. Statistical table of the dominant gut microbiota of four groups of mice at the phylum level.

	One-way ANOVA				Mean abundances			
Phylum	P	FDR	P adjustec	F Value	A	B	C	D
Deferribacteres	0.0068	0.048	0.048	5	1.1	0.058	0.018	0.019
Bacteroidetes	0.067	0.16	0.38	2.7	8.12	7.51	13.97	4.98
Proteobacteria	0.35	0.55	1	1.1	5.71	3.5	4.38	8.45
Actinobacteria	0.41	0.55	1	0.99	7.89	5.69	8.34	14.12
Verrucomicrobia	0.47	0.55	1	0.87	2.01	1.09	2.94	0.064
Firmicutes	0.59	0.59	1	0.65	73.14	81.91	70.17	72.14

Table 2. Statistical table of the dominant gut microbiota of four groups of mice at the class level.

	One-way ANOVA				Mean abundances			
Class	P	FDR	P adjust	F Value	A	B	C	D
Betaproteobacteria	0.000035	0.00049	0.00049	12	0.52	1.38	0.074	0.018
Clostridia	0.00028	0.002	0.0036	8.9	3.82	2.41	0.86	1.15
Deltaproteobacteria	0.005	0.023	0.06	5.4	0.22	0.2	0.01	0.097
Deferribacteres	0.0068	0.024	0.075	5	1.1	0.058	0.018	0.019
Gammaproteobacteria	0.015	0.042	0.15	4.2	0.068	0.044	3.06	7.44

At the class level, Clostridia, Betaproteobacteria, and Deltaproteobacteria were more abundant in normal mice, while Gammaproteobacteria, Bacteroidia, and Actinobacteria were more abundant in T1DM model mice. As shown in Figure 14A, in normal mice, the abundances of Deferribacteres and Epsilonproteobacteria in the vehicle - intervention group were higher than those in the cinnamon - intervention group, and their enrichment levels were also higher than those in T1DM model mice. The results of one - way ANOVA statistical analysis showed that Gammaproteobacteria were significantly enriched in the intestines of T1DM model mice, and the enrichment level in the cinnamon - treatment group (Group D) was significantly higher than that in the vehicle group (Group C) ($p < 0.05$), indicating that cinnamaldehyde intervention had a significant impact on the composition of bacterial classes in T1DM.

Furthermore, the differences in the microbial community structure among different groups were analyzed at the order level. Pseudomonadales, Burkholderiales, Deferribacterales, Virusesnoname, and Campylobacterales had relatively high abundances in the intestines of normal mice, while Erysipelotrichales, Caudovirales, and Enterobacteriales were more abundant in the T1DM model mice. One - way analysis of variance (ANOVA) revealed significant changes in five microbial groups. Among them, Enterobacteriales were significantly enriched in the T1DM model mice ($p < 0.05$), while Desulfovibrionales, Deferribacterales, Clostridiales, and Burkholderiales were enriched in the intestines of normal mice. It is worth noting that the effects of cinnamon intervention on the microbial community structure in groups B and D were inconsistent, indicating that normal mice and T1DM model mice have different feedback characteristics in response to cinnamon intake.

At the family level, there were many differences in the gut microbiota structure between normal mice and T1DM model mice. As shown in Figure 16A, the abundances of Oscillospiraceae, Prevotellaceae, Eubacteriaceae, Desulfovibrionaceae, unclassified Burkholderiales, Sutterellaceae, Ruminococcaceae, Deferribacteraceae, and Lachnospiraceae were significantly higher in the gut microbiota of normal mice. The abundances of Leuconostocaceae, Clostridiaceae, Enterococcaceae, Enterobacteriaceae, and Bifidobacteriaceae were higher in T1D mice. Among mice in the same group but with different treatments, the gut microbiota structure also showed differences. In normal mice, the abundances of Oscillospiraceae, Peptpstreptococcaceae, Prevotellaceae, and Pseudomonadaceae decreased in the cinnamaldehyde - intervention group, while the abundances of Burkholderiatales - noname and Sutterellaceae increased after cinnamaldehyde intervention. The differences among T1DM model mice with different treatments were relatively small.

One - way ANOVA statistical analysis showed that there were significant differences in the abundances of 13 families of bacteria at the family level. Specifically, cinnamaldehyde intervention in groups C and D induced more than a five - fold increase in the abundances of Streptococcaceae and Enterobacteriaceae. The abundances of Ruminococcaceae and Lachnospiraceae indicated that the difference in the baseline values of gut microbiota between normal mice and T1DM model mice was greater than the fluctuations in microbiota abundance caused by cinnamaldehyde intake regulation. This suggests that the target of cinnamaldehyde intake in improving the host's blood glucose and lipid levels lies in the abundance regulation of functional microbiota represented by Streptococcaceae and Enterobacteriaceae.

Table 3. Statistics of the dominant intestinal flora of four groups of mice at the order level

	One-way ANOVA				Mean abundances			
Order	P	FDR	P adjustec	F Value	A	B	C	D
Burkholderiales	0.000035	0.00066	0.00066	12	0.52	1.38	0.074	0.018
Clostridiales	0.00028	0.0027	0.005	8.9	3.82	2.41	0.86	1.15
Desulfovibrionales	0.005	0.032	0.085	5.4	0.22	0.2	0.01	0.097
Deferribacterales	0.0068	0.032	0.11	5	1.1	0.058	0.018	0.019
Enterobacteriales	0.015	0.057	0.22	4.2	0.052	0.038	3.06	7.44

At the genus level, the clustering results of the gut microbiota structures of T1DM model mice and normal mice showed differential characteristics. Anaerotruncus, Oscillibacter, Subdoligranulum, unclassified Lachnospiracea, Olsenella, Roseburia, Desulfovibrio, Mucispirillum, Faecalibacterium, and Prevotella had relatively high abundances in the intestines of normal mice. Among them, Parasutterella, Odoribacter, Lachnospiraceae - noname, and Burkholderiales - noname were enriched in both Group A and Group B of normal mice under different treatments. In contrast, the abundances of Gammaretrovirus, Belaretrovirus, Helicobacter, Dorea, and Mucispirillum showed a downward trend in the cinnamaldehyde - intervention group compared with the excipient - control group. In the intestines of T1DM model mice, the abundances of Parasutterella, Odoribacter, Lachnospiraceae - noname, and Burkholderiales - noname were significantly lower than those in normal mice. The difference between the cinnamon - intervention group and the excipient - intervention group was relatively small. The main difference was reflected in the enrichment of Enterococcus and Enterorhabdus in Group D, while the relative abundances of Bacteroides, Allstipes, and Lactococcus showed a downward trend in Group D. Further statistical analysis using one - way analysis of variance (ANOVA) revealed that there were significant differences in the abundance distribution characteristics of a total of 22 genera. The most typical enriched genera in Group D were Parabacteroides, Lactococcus, and Escherichia, indicating that cinnamon intake could significantly regulate the abundances of these two genera. This result was consistent with the previous DAPC analysis of the beta - diversity of the microbiota, which showed that the main driving factors for the four - module characteristics were Parabacteroides and Escherichia. It suggested that cinnamaldehyde intake could increase the relative abundances of functional genera at the genus level, thereby regulating the succession of the entire community structure.

Table 4. Statistical table of the dominant gut microbiota of four groups of mice at the family level.

	One-way ANOVA				Mean abundances			
Family	P	FDR	P adjustec	F Value	A	B	C	D
Ruminococcaceae	2.1E-06	0.000071	0.000071	17	1.44	0.88	0.038	0.082
Sutterellaceae	0.000018	0.00031	0.00059	13	0.19	0.59	0.037	0.003
Burkholderiales_noname	0.000061	0.00069	0.002	11	0.32	0.79	0.038	0.013
Oscillospiraceae	0.0016	0.014	0.05	6.7	0.085	0.048	0.002	0.01
Lachnospiraceae	0.0021	0.014	0.063	6.4	1.84	1.07	0.71	0.44
Eubacteriaceae	0.0046	0.024	0.13	5.5	0.097	0.17	0.002	0.028
Desulfovibrionaceae	0.005	0.024	0.14	5.4	0.22	0.2	0.01	0.097
Enterococcaceae	0.0056	0.024	0.15	5.2	0	0	0.077	0.54
Deferribacteraceae	0.0068	0.026	0.18	5	1.1	0.058	0.018	0.019
Prevotellaceae	0.011	0.037	0.27	4.5	0.023	0.008	0.007	0.005
Enterobacteriaceae	0.015	0.046	0.36	4.2	0.052	0.038	3.06	7.44
Bacteroidaceae	0.029	0.082	0.67	3.5	0.46	0.58	1.74	0.32
Streptococcaceae	0.038	0.099	0.84	3.2	0.0033	0.002	7.96	1.44

We compared the characteristics of gut microbiota composition between healthy mice and T1DM model mice at the **species level**. The results revealed that *Anaerotruncus* sp. G3 2012, *Lachnospiraceae* bacterium 8157FAA, *Desulfovibrio desulfuricans*, *Mucispirillum schaedleri*, *Bacteroides stercoris*, *Alistipes putredinis*, *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Prevotella copri*, and *Lachnospiraceae* bacterium 3146FAA were enriched in the gut microbiota of healthy mice. In contrast, *Bacteroides xylanisolvens* and *Lactobacillus animalis* exhibited increased abundance in the intestinal microbiota of T1DM model mice. Further statistical analysis using one-way ANOVA identified significant differences in the abundance distribution patterns of 25 bacterial genera. Notably, cinnamaldehyde supplementation significantly elevated the abundance of *Lactobacillus johnsonii* in both Group B and Group D mice. Specifically, the relative abundance of *L. johnsonii* increased from 10.3% (healthy control) and 3.75% (T1DM) to 43.56% and 38.88%, respectively, representing 4.2-fold and 10.4-fold increases. Additionally, *Parasutterellaexcrementihominis*, *Dorea* sp.52, *Burkholderiales* bacterium 1147, and Mucispirillum schaedleri showed significantly higher

abundance in healthy mice. Conversely, Enterococcus faecalis, Lactobacillus reuteri, and Enterorhabdus caecimuris were significantly enriched in T1DM model mice. It is noteworthy that another dominant lactic acid bacterium, Lactobacillus murinus, displayed a decreasing trend following cinnamaldehyde intervention, with statistically significant differences observed in T1D mice ($p<0.05$).

In conclusion, the intake of cinnamaldehyde and excipients affects the gut microbiota structure of normal mice and T1DM model mice respectively, and regulates the enrichment of different genera. This includes the influence on the abundances of Lactobacillus johnsonii, Lactobacillus murinus and a variety of other functional strains, thereby regulating the structure of the entire gut microbiota community. During the intervention period, it shows the characteristics of directional succession, and finally forms the "enterotype" modules in the PCoA analysis.

3.3. Correlation analysis between gut microbial structure and clinical indicators

The correlation between the succession characteristics of the diversity of the intestinal flora structure in mice and clinical indicators during the cinnamaldehyde intervention experiment period is shown in Tables 1 - 4. The correlation between the intestinal flora and blood glucose and insulin tolerance was evaluated by the method of Spearman correlation analysis. As can be seen from Figure 19A, the clustering results of blood glucose indicators show that the two indicators of post - meal blood glucose and blood glucose level at 120 minutes of the glucose tolerance test are highly correlated and negatively correlated with insulin resistance. At the genus level, Lactococcus, Klebsiella, Escherichia, Parabacteroides, Enterococcus, Butyricimonas, and Blautia are significantly positively correlated with the two indicators of post - meal blood glucose and blood glucose level at 120 minutes of the glucose tolerance test, while Odoribacter, Faecalibacterium, Parasutterella, Subdoligranulum, Burkholderiales_noname, Enterorhabdus, Desulfovibrio, unclassified Lachnospiraceae, Eubacterium, and Oscillibactera are significantly negatively correlated (Spearman correlation coefficient > 0.35, $p < 0.05$, positive samples > 15). At the **species level**, postprandial blood glucose and blood glucose levels at 120 minutes during the oral glucose tolerance test (OGTT) exhibited **significantly positive correlations** with *Lactococcus garvieae*, *Escherichia coli*, *Enterococcus faecalis*, *Butyricimonas synergistica*, *Lactococcus lactis*, and *Ruminococcus torques* (Figure 19B). Conversely, they showed **significantly negative correlations** with *Lactobacillusjohnsonii*, *Faecalibacteriumprausnitzii*,*Parasutterellaexcrementihominis*,*Burkholderiales* bacterium1147, *Enterorhabduscaecimuris*, *Desulfovibrio desulfuricans*, *Lachnospiraceae* bacterium 3146FAA, and *Eubacterium plexicaudatum* (Spearman correlation coefficients $|r| > 0.35$, $p < 0.05$, positive sample sizes> 15). Recently candidates *Akkermansia* and *Bifidobacterium* demonstrated weak correlations with blood glucose metrics, suggesting that cinnamaldehyde intervention primarily targets *Parabacteroides* and *Lactobacillus* species as key modulatory points.

4. Discussion

The gut microbiota is closely related to the development of T1DM(Abuqwider et al., 2023)[7].Goffau's research found that the comparison between T1DM patients and the control group showed a decrease in the number of butyrate - or lactate - producing bacteria. In addition, the microbiota structure of the T1DM group was characterized by the lack of the two most abundant species, Bifidobacterium adolescentis and Bifidobacterium pseudocatenulatum(Blok et al., 2025)[8]. From the perspective of alpha diversity, the median results of the three indices, Shannon, chao1, and Inverse Simpson, jointly indicated that the intake of cinnamon could promote the improvement of gut microbiota richness. The analysis of beta diversity further revealed that cinnamon intake could directionally regulate the gut microbiota of normal mice, resulting in a decrease in the inter - group differences in the microbiota structure between groups B and D of the cinnamon intervention group. The community structure presented four modules, with a small difference between groups B and D, and the microbiota was mainly driven by multiple genera represented by Parabacteroides(Zhao et al., 2021)[9].At the level of functional genera, this study found that seven genera represented by Lactococcus were significantly positively correlated with post - meal blood glucose indicators. Among them, the abundance of Lactobacillus johnsonii increased significantly after cinnamaldehyde intervention, especially in the gut of T1D mice, where its relative abundance increased by 10.4 times. It is the core strain (hub species) involved in the regulation of blood glucose and blood lipids in mice. Studies have shown that the number of lactic acid bacteria, bifidobacteria, Bacillus globigii/Eubacterium rectale, and Prevotella is higher in the gut of healthy children, while children with T1D have a higher number of Clostridium, Bacteroides, and Veillonella.

In addition, within 4 weeks of intake of Lactobacillus acidophilus by patients with type 2 diabetes, their insulin sensitivity was maintained, and no systemic inflammatory response was found(Li et al., 2024)[10].Another study found that continuous consumption of yogurt containing Lactobacillus acidophilus La5 and Bifidobacterium Bb12 for 6 weeks could improve the oxidative and glucose metabolism in patients with type 2 diabetes. Multiple studies have found that Lactobacillus gasseri BNR17 can affect the blood glucose concentration and body weight in mice with type 2 diabetes. After intragastric administration at a dosage of 10^{10} CFU twice a day for 12 consecutive weeks, the blood glucose concentration in mice with type 2 diabetes decreased significantly, and the level of glycated hemoglobin HbA1c (used to reflect the combined amount of hemoglobin and blood glucose in blood red blood cells) also showed a certain downward trend. Al - Salami et al. found through animal experiments that feeding rats with a composite probiotic containing Lactobacillus acidophilus, Bifidobacterium lactis, and Lactobacillus rhamnosus could reduce the blood glucose concentration in diabetic rats, but had no effect on the blood glucose level in healthy rats. The succession characteristics of functional bacterial genera in this study indicate that the intake of cinnamaldehyde improved the intestinal flora structure in mice and promoted the transformation of the enterotype from the disease state to the healthy state by enriching multiple functional strains including Lactobacillus johnsonii.

In addition, another target strains for the intervention effect of cinnamaldehyde are Parabacteroides and Lactobacillus reuteri. Among them, Parabacteroides distasonis is one of the core gut microbiota in humans. Correlation analysis shows

that its abundance is significantly negatively correlated with disease states such as obesity, non - alcoholic fatty liver disease, and diabetes, suggesting that it may play a positive regulatory role in glucose and lipid metabolism(Kochumon et al., 2024)[11].Recently, a study by Liu Shuangjiang et al. (2020) found that Parabacteroides distasonis can significantly improve the symptoms of obesity, insulin resistance, lipid metabolism disorder, and non - alcoholic fatty liver disease in diet - induced obesity (DIO) mice and ob/ob obese model mice[12]. Through in vivo and in vitro experiments, it was first discovered that Parabacteroides distasonis has a wide - range of bile acid conversion functions, being able to hydrolyze various conjugated bile acids and convert them into various secondary bile acids (such as lithocholic acid and ursodeoxycholic acid); it can also produce a large amount of succinic acid. Secondary bile acids such as lithocholic acid improve lipid metabolism disorder by activating the intestinal FXR signaling pathway; ursodeoxycholic acid can repair the integrity of the intestinal wall; succinic acid activates intestinal gluconeogenesis by acting on the key enzyme fructose - 1,6 - bisphosphatase (FBPase) in the intestinal gluconeogenesis (IGN) pathway, thereby regulating appetite, promoting liver glycogen synthesis, and improving the host's glucose metabolism disorder. Parabacteroides distasonis exerts multi - target overall regulatory effects by producing succinic acid and secondary bile acids to activate different signaling pathways. Lactobacillus reuteri has a strong adhesion ability to the intestinal mucosa, which can improve the distribution of the gut microbiota and antagonize the colonization of harmful bacteria. The Chinese Ministry of Health approved Lactobacillus reuteri as a microbial strain for human health products in 2003, and this bacterium is already an internationally recognized new probiotic lactic acid bacterium. Some studies have confirmed that daily intake of Lactobacillus reuteri increases the secretion of insulin and incretin in humans with glucose tolerance. Besides the microbial abundance, the diversity and stability of the gut microbiota are also related to the development of type 1 diabetes (T1D). This study reveals that the intake of cinnamaldehyde and excipients respectively affects the gut microbiota structure of normal mice and type 1 diabetes mellitus (T1DM) model mice, and regulates the enrichment of different genera, including the abundances of Lactobacillus johnsonii, Lactobacillus murinus, and other functional strains, thereby regulating the structure of the entire gut microbiota community. During the intervention period, it shows the characteristics of directional succession, increasing the diversity and stability of the gut microbiota structure, which is in line with the characteristics of the improvement of autoimmune diseases (Giongo, 2011). In addition, in animal models, the regulation of the gut microbiota helps to regulate insulin resistance, the post - prandial glucose response, and the co - secretion concentration of GLP - 1, and has an effect on enteromuscular nutrition. The improvement of the intestinal barrier function is further associated with a decrease in the concentration of portal - related plasma lipopolysaccharide (endotoxin) and a reduction in systemic and liver inflammation. Multiple strains such as Lactobacillus johnsonii and Lactobacillus murinus have been proven to affect the composition of the gut microbiota, change intestinal permeability, increase plasma GLP - 1 concentration, and reduce post - prandial glucose concentration. The compound probiotics stimulate the gut microbiota to produce insulin - secreting polypeptides and glucagon - like peptide - 1, which promotes the muscle absorption of glucose and the synthesis of liver glycogen in the liver, thereby reducing the blood glucose concentration in diabetic rats. In this paper, through correlation analysis, it is further confirmed that the main groups of the microbial community succession induced by cinnamaldehyde intake are significantly positively correlated with the clinical blood lipid indicators of mice.

Acknowledgments

This work was Supported by Shenzhen Science and Technology Program, (No: JCYJ20230807150802006) (No: JCYJ20210324125002006).

References

[1] Abuqwider, J., Corrado, A., Scidà, G., Lupoli, R., Costabile, G., Mauriello, G., & Bozzetto, L. (2023). Gut microbiome and blood glucose control in type 1 diabetes: a systematic review. *Frontiers in Endocrinology*, *14*, 1265696.

[2] Blok, L., Hanssen, N., Nieuwdorp, M., & Rampanelli, E. (2025). From microbes to metabolites: advances in gut Microbiome research in type 1 diabetes. *Metabolites*, *15*(2), 138.

[3] Del Chierico, F., Rapini, N., Deodati, A., Matteoli, M. C., Cianfarani, S., & Putignani, L. (2022). Pathophysiology of type 1 diabetes and gut microbiota role. *International Journal of Molecular Sciences*, *23*(23), 14650.

[4] Franzosa, E. A., McIver, L. J., Rahnavard, G., Thompson, L. R., Schirmer, M., Weingart, G., Lipson, K. S., Knight, R., Caporaso, J. G., Segata, N., & Huttenhower, C. (2018). Species-level functional profiling of metagenomes and metatranscriptomes. *Nature Methods*, *15*(11), 962-968.

[5] Kochumon, S., Malik, M. Z., Sindhu, S., Arefanian, H., Jacob, T., Bahman, F., Nizam, R., Hasan, A., Thomas, R., Al-Rashed, F., Shenouda, S., Wilson, A., Albeloushi, S., Almansour, N., Alhamar, G., Al Madhoun, A., Alzaid, F., Thanaraj, T. A., Koistinen, H. A.,…Ahmad, R. (2024). Gut dysbiosis shaped by cocoa butter-based sucrose-free HFD leads to steatohepatitis, and insulin resistance in mice. *Nutrients*, *16*(12), 1929.

[6] Li, S. M., Liu, Z. C., Zhang, Q., Su, D., Wang, P. J., Li, Y. X., Shi, W. B., & Zhang, Q. (2024). The antidiabetic potential of probiotics: a review. *Nutrients*, *16*(15), 2494.

[7] Li, W. Z., Stirling, K., Yang, J. J., & Zhang, L. (2020). Gut microbiota and diabetes: From correlation to causality and mechanism. *World Journal of Diabetes*, *11*(7), 293-308.

[8] Liu, L. L., Zhang, J. H., Cheng, Y., Zhu, M., Xiao, Z. F., Ruan, G. C., & Wei, Y. L. (2022). Gut microbiota: A new target for T2DM prevention and treatment. *Frontiers in Endocrinology*, *13*, 958218.

[9] Tamahane, V., Bhanushali, S., Shah, N., Gupta, A., Khadilkar, V., Gondhalekar, K., Khadilkar, A., & Shouche, Y. (2024). A comparative study of the gut microbiome in Indian children with type 1 diabetes and healthy controls. *Journal of Diabetes*, *16*(5), e13438.

[10] Zhao, H. L., Wu, H. Y., Duan, M. T., Liu, R. X., Zhu, Q. H., Zhang, K., & Wang, L. L. (2021). Cinnamaldehyde improves metabolic functions in streptozotocin-induced diabetic mice by regulating gut microbiota. *Drug Design, Development and Therapy*, *15*, 2339-2355.

[11] Zhao, Q. X., Wu, J. Y., Ding, Y., Pang, Y. L., & Jiang, C. T. (2023). Gut microbiota, immunity, and bile acid metabolism: decoding metabolic disease interactions. *Life Metabolism*, *2*(6), load032.

[12] Liu Shuangjiang, Liu Hongwei, Wang Kai, Liao Mingfang, Zhou Nan, Bao, L., Ma, K., Zheng, Z.Y., Wang, Y.J., Liu, C., Wang, W.Z., & Wang, J. (2020). Parabacteroides distasonis alleviates obesity and metabolic dysfunction by producing succinic acid and secondary bile acids. Science News, (2), 106.