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*Original Article*

# Berberine ameliorates type 2 diabetes via modulation of *Bifidobacterium* species, tumor necrosis factor-α, and lipopolysaccharide

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**Abstract:** Type 2 diabetes (T2D) is one of the most common metabolic diseases. T2D is considered an inflammatory disorder triggered by disordered metabolism and is associated with detection of high blood C-reactive protein (CRP), lipopolysaccharide (LPS), and tumor necrosis factor (TNF)-α levels. Recent studies show the involvement of gut microbiota especially *Bifidobacterium* in T2D. Berberine is a potent oral hypoglycemic agent but the exact mechanism underlying its anti-diabetic effect is unclear. Here, we hypothesized that these effects are related to the modulation of CRP, LPS, TNF-α, and *Bifidobacterium* species. Therefore, we aimed to study the effects of berberine on these inflammatory proteins and *Bifidobacterium* species, and correlate our findings with their status in patients with newly diagnosed T2D before and 8 weeks after treatment. Results showed significant decreases in body mass index (BMI), fasting blood glucose (FBG), glycated hemoglobin (HbAlc), fasting insulin, triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) after berberine treatment for 8 weeks compared with before treatment ($P<0.01$). Compared to before berberine treatment, CPR, TNF-α, and LPS were markedly reduced after treatment ($P<0.01$). In addition, total *Bifidobacterium*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium adolescentis*, and *Bifidobacterium infantis* levels changed significantly after berberine treatment compared with before treatment ($P<0.01$), and all except *Bifidobacterium breve* correlated significantly with TNF-α and LPS levels. Finally, all fecal *Bifidobacterium* species were not significantly correlated with CRP ($P>0.05$). In conclusion, these results suggest that berberine markedly ameliorates T2D via modulation of *Bifidobacterium* species, TNF-α, and LPS.

**Keywords:** Type 2 diabetes, *Bifidobacterium* species, C-reactive protein, lipopolysaccharide, tumor necrosis factor-α

## Introduction

Type 2 diabetes (T2D) is one of the most common metabolic diseases in which patients cells are unable to respond to the defective or insufficient levels of insulin produced, leading to high blood glucose level. The elevated glucose level has long-term harmful effects on several body organs, especially the kidneys, eyes, heart, and nerves [1]. T2D is considered an inflammatory disorder triggered by disordered metabolism and high blood CRP, LPS, and TNF-α can be detected in patients [2, 3]. The prevention and treatment of T2D have become a major global public health concern. The drugs commonly prescribed for treatment T2D include insulin, metformin, sulfonylureas, and natural products [4-6].

Previous studies have reported berberine as a safe natural product widely used in diabetes care because of its anti-oxidant, anti-inflammatory, anti-obesity, and anti-hyperglycemic properties [7-9]. Berberine, a single purified isoquinoline alkaloid, is responsible for the major pharmacological properties of *Coptischinensis* (Huang-Lian, a common herb in traditional Chinese medicine) [10]. This popular herb has been widely used in Eastern Asia to promote health over 1000 years. The dried powder of *C. chinensis* has been used in traditional Chinese medicine to prevent or treat different diseases

including intestinal infections, particularly bacterial-associated diarrhea. In 1980, the hypoglycemic effect of berberine was clinically proven in China following its use in the treatment of diarrhea in patients with diabetes. Subsequently, berberine has been used as an an-ti-hyperglycemic agent by many physicians in China [11]. Furthermore, accumulating evidence continues to support berberine as a potent oral hypoglycemic agent that is clinically effective in alleviating T2D and significantly decreases FBG, HbA1c, TC, HDL, and LDL cholesterol levels [12, 13]. However, the exact mechanism underlying its anti-diabetic effect is unclear.

The gut microbiota consists of an enormous number and diversity of microorganisms that play an important role in maintaining the health of their human hosts [14]. Numerous studies have demonstrated that gut microbiota are involved in regulating lipogenesis and are associated with the development of obesity and T2D [15]. *Bifidobacterium* species are the most predominant probiotics in gut microbiota and their health benefits include maintenance of the balance between gut microbiota and host, as well as normal host metabolism. A decline in levels of *Bifidobacterium*can lead to a microbial dysbiosis and imbalance, which promotes the development of metabolic disorders [16]. In contrast, adequate levels of *Bifidobacterium* improve glucose tolerance, restore glucose-induced insulin secretion, lower levels of endotoxemia and proinflammatory cytokines in adipose tissue, and ultimately improve metabolic disorders such as T2D [17, 18]. Another animal study showed that *Bifidobacterium adolescentis (B. adolescentis)* improved metabolic disorders and dyslipidoses [19].

A series of experiments showed that microbial dysbiosis caused by a high-fat diet leads to an inflammatory processes called “metabolic endotoxemia”. The inflammation is associated with insulin resistance and T2D. *Bifidobacteria* down-regulate LPS and TNF-α responsiveness in infancy to enhance immune functions and decreased the risk of disease [20]. In addition, these species are highly prevalent in the human gut, and can easily be added or removed from probiotic food preparations. This feature makes them ideal candidates for use as potential clinical interventions in diseases associated with gut microbiota, and more studies investigating the differences in fecal *Bifidobacterium* profile of patients with T2D and healthy individuals are needed.

Therefore, in this study, we used quantitative real-time polymerase chain reaction (qRT-PCR) to detect copy numbers of total *Bifidobacterium*, *Bifidobacterium longum (B. longum)*, *Bifidobacterium breve (B. breve)*, *B. adolescentis*, and *Bifidobacterium infantis (B. infantis)* in fecal samples of patients with T2D treated with or without berberine. Furthermore, we analyzed serum inflammatory factors including CRP, LPS, and TNF-α using enzyme-linked immunosorbentassay (ELISA) to determine the correlation between these *Bifidobacterium* species and inflammatory factors in patients with T2D, thereby providing further evidence of the effectiveness of berberine treatment.

## Subjects and methods

### *Subjects*

Thirty patients with T2D (mean age 37±14 years, 18 male and 12 female) were recruited from April 2014 to March 2015 in the Shenzhen People’s Hospital. All participants conformed to the inclusion and exclusion criteria while informed, written, and signed consent was obtained from both eligible patients and healthy participants following the explanation of the study procedure. All of the experiments were approved by the Ethics Committee of the Institute.

The common inclusion criteria for T2D patients included aged 18-65 years and not administered berberine, antibiotics, biogen, lactulose, and other drugs for a month before sampling or treatment with berberine. The major inclusion criteria for patients with T2D included a body mass index (BMI) >25 kg/m$^2$ and diagnosis of T2D according to the World Health Organization (WHO) T2D Diagnosis Standard for no more than 1 year. Patients with T2D were excluded if they had diabetic complications, digestive system and autoimmune diseases, and moderately or severely impaired liver or kidney function (alanine transaminase [ALT]/aspartate transaminase [AST] >2.5 or creatinine clearance [Ccr] <25 ml/min). Patients were also excluded if they had serious adverse reactions.

**Table 1.** The 16SrRNA-targeted genus- and species-specific PCR primers

| *Bifidobacterium* spp | | 5'-3' primer sequences | bp |
|---|---|---|---|
| *Bifidobacterium* | Forward | GCGTGCTTAACACACATGCAAGTC | 126 |
| | Reverse | CACCCGTTTCCAGGAGCTATT | |
| *B. longum* | Forward | GAGACAGAAACTTTCGAAGC | 112 |
| | Reverse | GAAGTCTGTGGTATCCAATCC | |
| *B. breve* | Forward | TTCCGCATTCGTGTTATTGA | 279 |
| | Reverse | CGAAGGCTTGCTCCCAGT | |
| *B. adolescentis* | Forward | CTCCAGTTGGATGCATGTC | 122 |
| | Reverse | CGAAGGCTTGCTCCCAGT | |
| *B. infantis* | Forward | CCATCTCTGGGATCGTCGG | 563 |
| | Reverse | TATCGGGGAGCAAGCGTGA | |

*Berberine treatment*

The berberine used in this study was purchased from Guangdong South China Pharmaceutical Group Co., Ltd., (approval number Guoyaozhunzi H44020757 and lot number 140502). In this study, 30 patients were administered 300 mg berberine three times daily, 0.5 h after each major meal for 8 weeks and were evaluated weekly for side effects until the end of the study. Adverse events were recorded throughout the study by direct questioning. Patients were interviewed before or after treatment using a questionnaire to record their parameters including height (m), weight (kg), BMI (kg/$m^2$).

*Determination of biochemical parameters and inflammatory factors*

Peripheral blood (20 ml) was collected via the elbow vein from all subjects in the treatment groups before or after treatment. Parameters evaluated include FPG, TG, TC, HDL, LDL, and CRP using the Roche COBAS INTEGRA 400PLUS automatic biochemical analyzer (Roche, Basle, Switzerland). HbA1c was measured using the HLC-723 G7 glycosylated hemoglobin analyzer (TOSHO, Japan). Fasting insulin, TNF-α, and LPS were detected using ELISA kits (R&D, Minneapolis, MN, USA and Jimianshiyan, Shanghai, China, respectively).

*Detection of fecal Bifidobacterium species*

The samples used in this analysis were thoroughly vortexed the tubes for 30 sec. The suspending liquid was centrifuged at 1000 × g for 5 min to obtain the supernatant, which was further centrifuged at 15000 × g for 10 min to collect the precipitates. Total DNA was then extracted from the precipitates using the E.Z.N.A.® Stool DNA Kit (Omega, Norcross, GA, USA), according to the manufacturer's instructions. The DNA concentration was determined using a NanoPhotometer™ (Uvikon 923, USA).

We designed 16SrRNA-targeted genus- and species-specific PCR primers for the selected fecal microbiota including total *Bifidobacterium*, *B. longum*, *B. breve*, *B. adolescentis*, and *B. infantis*. The primer sequences are shown in **Table 1** [17]. Then, PCR amplification was performed for each species, and their standard lines were established using qRT-PCR with the 7500Fast Real-time PCR System (Applied Biosystems, USA) with plasmids. The bacterial groups in all of the fecal samples were quantified using qRT-PCR.

*Statistical analysis*

The statistical analysis was performed using the statistical package for the social sciences (SPSS) version 19.0 software package. The data, which fit the normal distribution, were expressed as the mean ± standard deviation (SD). The data of the T2D group were compared before and after treatment using the Student's *t*-test. The data that did not fita normal distribution were expressed as the median (P25-P75). Differences between the T2D and control groups were assessed using the Mann-Whitney U Test. Correlations between *Bifidobacterium* species and inflammatory factors in patients with T2D were computed using the Pearson Rank correlation. $P<0.05$ was considered significantly different.

## Results

*Biochemical parameters before and after berberine treatment*

In this study, all of the recruited patients were eligible for the final analysis of biochemical parameters including the FBG, HbAlc, fasting insulin, TG, TC, HDL and LDL before or after treatment. Compared to before treatment, the FBG values were significantly decreased follow-

**Table 2.** Alterations in biochemical parameters indicating therapeutic effects of berberine

| | Before treatment | After treatment | *t* | *P*-value |
|---|---|---|---|---|
| BMI (kg/m$^2$) | 27.68±1.945 | 26.49±1.960 | 9.730 | <0.01** |
| FBG (mmol/L) | 8.754±1.102 | 7.201±1.105 | 5.618 | <0.01** |
| Fasting insulin (μU/mL) | 29.88±4.093 | 21.32±5.001 | 20.14 | <0.01** |
| HbAlc (%) | 7.350±1.031 | 6.524±0.9060 | 8.170 | <0.01** |
| HDL (mmol/L) | 1.209±0.4753 | 1.044±0.3184 | 3.629 | <0.01** |
| LDL (mmol/L) | 2.965±0.8197 | 2.651±0.8732 | 5.500 | <0.01** |
| TG (mmol/L) | 2.789±1.468 | 1.873±1.080 | 7.611 | <0.01** |
| TC (mmol/L) | 4.987±1.174 | 4.640±1.175 | 6.975 | <0.01** |

Data are means ± SEM, compared with before treatment, **$P$<0.01. BMI, body mass index; FBG, fasting blood glucose; HbAlc, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TC, total cholesterol.

ing treatment with berberine (by 1.55 mmol/L, $P$<0.01). The FBG levels of 16 of the 33 patients (53.33%) returned to normal levels (<7.0 mmol/L). Compared to before treatment, the BMI, HbAlc, fasting insulin, TG, TC, HDL, and LDL levels were significantly altered after 8 weeks of berberine treatment (**Table 2**).

*CRP, TNF-α, and LPS levels before and after berberine treatment*

Peripheral blood was collected from each study participant before and after treatment. The determined inflammatory factors included CRP, TNF-α, and LPS. After 8 weeks of treatment with berberine, a remarkable reduction was observed in CRP from 3.459±2.327 to 2.885±1.932 mmol/L ($t$=3.064, $P$<0.0, **Figure 1A**), TNF-α from 220.4±25.27 to 181.5±25.95 μg/L ($t$=22.88, $P$<0.01, **Figure 1B**), and LPS from 426.4±19.77 to 346.7±27.54 EU/l ($t$=23.27, $P$<0.01, **Figure 1C**).

*Fecal Bifidobacterium species levels before and after berberine treatment*

We determined the copy numbers of selected fecal microbiota of participants including total *Bifidobacterium*, *B. longum*, *B. breve*, *B. adolescentis*, and *B. infantis* and their mean (ranges) values before and after berberine treatmentwere 6.577 (6.301-6.946) and 6.877 (6.570-7.025), 4.613 (4.339-5.024) and 6.825 (4.509-5.308), 4.946 (4.724-5.333) and 5.139 (4.959-5.388), 5.181 (5.094-5.313) and 5.456 (5.415-5.544), and 4.337 (4.121-4.810) and 4.004 (3.775-4.178), respectively. Compared to before treatment, all the fecal *Bifidobacterium* species copy numbers significantly changed ($P$< 0.01, **Figure 2**).

*Correlation between inflammatory factors and Bifidobacterium species*

Before and after the berberine treatment, significant negative correlations were observed between the copy numbers of total *Bifidobacterium*, *B. longum*, *B. infantis* and levels of TNF-α and LPS ($P$<0.01). There were significant positive correlations between the copy numbers of *B. adolescentis* and levels of TNF-α and LPS ($P$<0.01 respectively). There were no correlations between the copy numbers of *B. breve* and levels of TNF-α and LPS ($P$>0.05). All the fecal *Bifidobacterium* species showed no significant correlations with CRP ($P$>0.05 respectively) and results are shown in **Tables 3** and **4**. To confirm the correlations between fecal *Bifidobacterium* species and levels of CRP, TNF-α, and LPS further, we analyzed the correlations between their different values (after berberine treatment subtracted from before berberine treatment) and found the results were similar to those obtained before and after berberine treatment and the result is shown in **Table 5**.

## Discussion

Berberine is the alkaloid responsible for the major pharmacological properties of *C. chinensis*. Accumulating evidence suggests that berberine is a potent oral hypoglycemic agent that is clinically effective in alleviating T2D [21, 22]. In this study, we found that compared to levels before treatment, FBG, HbAlc, fasting insulin, TG, TC, HDL, and LDL level were significantly decreased after treatment with berberine. In addition, the FBG levels of 16 patients (53.33%) reverted to normal (<7.0 mmol/L). These results further demonstrate that berberine treatment is effective in T2D. Recent investigations have shown that CRP, LPS, and TNF-α play an important role in the occurrence and development of T2D [23-25]. In this study, compared to levels

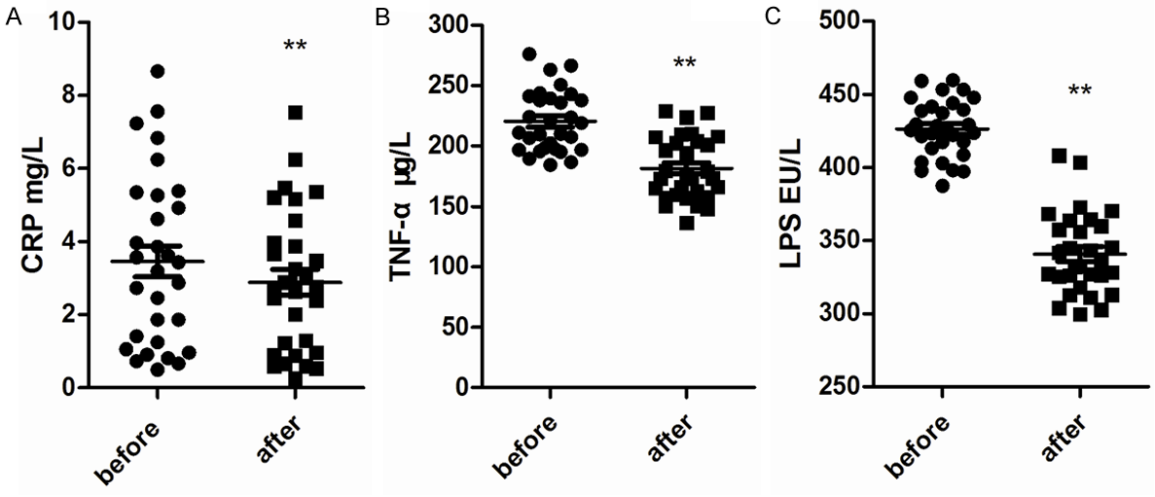


**Figure 1.** Expression levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-α, and lipopolysaccharide analyzed before and after treatment in each group. Expression levels of CRP (A), TNF-α (B), and LPS (C). Data are means ± SEM, compared with before treatment, $^{**}P<0.01$.

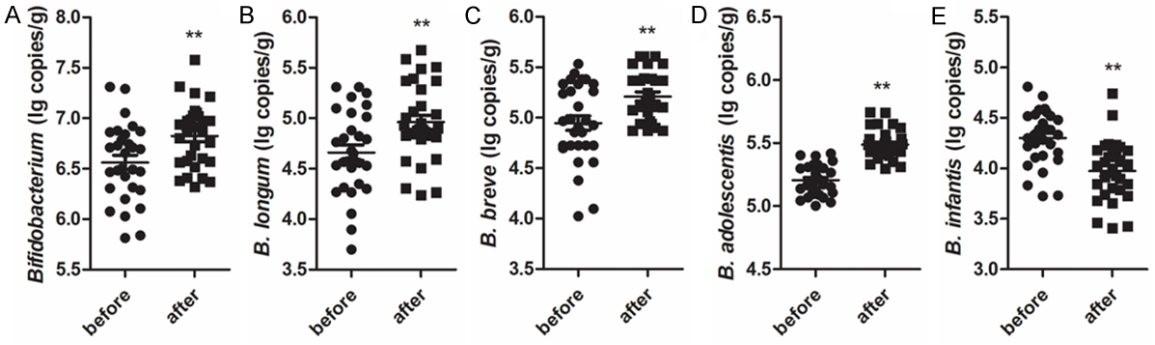


**Figure 2.** Determination of copy numbers of fecal *Bifidobacterium* species before and after berberine treatment. Copy numbers of selected fecal microbiota of participants including total *Bifidobacterium* (A), *B. longum* (B), *B. breve* (C), *B. adolescentis* (D), and *B. infantis* (E) were detected. Values are the means and ranges.

before treatment, we detected a significant reduction in the level of LPS, CRP, and TNF-α after 8 weeks of treatment with.

The antidiabetic mechanisms of berberine have been investigated in numerous studies, and suggested effects include an increase in insulin receptor expression, induction of glycolysis, and suppression of the S1P-S1P2 receptor pathway [13, 26, 27]. However, some studies have shown that the highest concentration of berberine was 0.4 ng/ml in the blood when patients with T2D were administered 400 mg berberine; however, the clinically effective concentration is more than 2.5 mg/ml in the blood. The low utilization rate of oral berberine does not explain antidiabetic mechanisms of berberine and is inconsistent with its clinical efficacy. Berberine has been used to prevent and treat intestinal infections, particularly bacterial-associated diarrhea. Therefore, we hypothesized that the efficacy of berberine in T2D may be related to actions on gut microbiota, especially the *Bifidobacterium* species. Recent studies have shown that the copy numbers of *Bifidobacterium* significant changed in patients with T2D compared with healthy people. Furthermore, *Bifidobacterium* significantly increases energy extraction and fat storage [17, 19]. In the present study, we found that compared to before treatment, the copy numbers of total *Bifidobacterium*, *B. longum*, *B. breve*, and *B. adolescentis* significant increased after treatment with berberine. The copy numbers of *B.*

**Table 3.** Correlation of *Bifidobacterium* species with levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-α, and lipopolysaccharide (LPS) before treatment

| *Bifidobacterium.* species | CRP | | TNF-a | | LPS | |
|---|---|---|---|---|---|---|
| | *r* | *P*-value | *r* | *P*-value | *r* | *P*-value |
| Total *Bifidobacterium* | 0.204 | 0.279 | -0.701 | 0.000** | 0.659 | 0.000** |
| *B. longum* | 0.168 | 0.375 | -0.650 | 0.000** | -0.604 | 0.000** |
| *B. breve* | -0.153 | 0.420 | 0.078 | 0.681 | -0.005 | 0.978 |
| *B. infantis* | 0.216 | 0.253 | -0.572 | 0.001** | -0.535 | 0.002** |
| *B. adolescentis* | -0.194 | 0.304 | 0.822 | 0.000** | 0.738 | 0.000** |

Data are means ± SEM, compared with before treatment, **$P<0.01$. *B. longum, Bifidobacterium longum; B. breve, Bifidobacterium breve; B. adolescentis, Bifidobacterium adolescentis; and B. infantis, Bifidobacterium infantis.*

**Table 4.** Correlation of *Bifidobacterium* species with C-reactive protein (CRP), tumor necrosis factor (TNF)-α, and lipopolysaccharide (LPS) after berberine treatment

| *Bifidobacterium.* species | CRP | | TNF-a | | LPS | |
|---|---|---|---|---|---|---|
| | *r* | *P*-value | *r* | *P*-value | *r* | *P*-value |
| Total *Bifidobacterium* | 0.240 | 0.201 | -0.739 | 0.000** | -0.587 | 0.001** |
| *B. longum* | 0.134 | 0.480 | -0.687 | 0.000** | -0.490 | 0.006** |
| *B. breve* | -0.085 | 0.656 | 0.068 | 0.772 | 0.006 | 0.975 |
| *B. infantis* | 0.395 | 0.031* | -0.634 | 0.000** | -0.563 | 0.001** |
| *B. adolescentis* | -0.363 | 0.049* | 0.658 | 0.000** | 0.528 | 0.003** |

Data are means ± SEM, compared with before treatment, **$P<0.01$. *B. longum, Bifidobacterium longum; B. breve, Bifidobacterium breve; B. adolescentis, Bifidobacterium adolescentis; and B. infantis, Bifidobacterium infantis.*

**Table 5.** Correlation of different values of *Bifidobacterium* species and C-reactive protein (CRP), tumor necrosis factor (TNF)-α, and lipopolysaccharide (LPS)

| *Bifidobacterium.* species | CRP | | TNF-a | | LPS | |
|---|---|---|---|---|---|---|
| | *r* | *P*-value | *r* | *P*-value | *r* | *P*-value |
| Total *Bifidobacterium* | 0.034 | 0.857 | -0.691 | 0.000** | -0.654 | 0.000** |
| *B. longum* | 0.186 | 0.325 | -0.727 | 0.000** | -0.690 | 0.000** |
| *B. breve* | 0.066 | 0.730 | 0.283 | 0.130 | 0.283 | 0.130 |
| *B. infantis* | 0.191 | 0.311 | -0.594 | 0.001** | -0.576 | 0.001** |
| *B. adolescentis* | 0.024 | 0.902 | 0.398 | 0.020** | 0.400 | 0.029** |

Data are means ± SEM, compared with before treatment, **$P<0.01$. *B. longum, Bifidobacterium longum; B. breve, Bifidobacterium breve; B. adolescentis, Bifidobacterium adolescentis; and B. infantis, Bifidobacterium infantis.*

*infantis* remarkable decreased after treatment with berberine compared to before treatment. These results indicate that the berberine treatment likely changed the copy numbers of *Bifidobacterium*, which would relieve the symptoms of patients with T2D. To confirm this observation, we analyzed the correlations between CRP, TNF-α, and LPS levels and *Bifidobacterium*.

Interestingly, we found that before treatment with berberine there were significant negative correlations between the copy numbers of total *Bifidobacterium*, *B. longum*, *B. infantis* and levels of TNF-α and LPS. There were also significant positive correlations between the copy numbers of *B. adolescentis* and levels of TNF-α and LPS. However, there were no correlations between the copy numbers of *B. breve* and levels of TNF-α and LPS. The correlations observed after berberine treatment for 8weeks were similar to those observe before treatment. Furthermore, we found that differences in TNF-α and LPS levels and *Bifidobacterium* observed before and after treatment showed similar correlations. Therefore, we concluded that one outcome of berberine treatment was a reduction in the FBG of patients with T2D. This effect was likely mediated by an adjustment in the number of *Bifidobacterium*, which further reduced the expression of TNF-α and LPS.

In conclusion, we demonstrated that berberine effectively decreased the FBG, HbAlc, fasting insulin, TG, TC, HDL, and LDL in patients with T2D. Berberine also obviously reduced the expression levels of CRP, TNF-α, and LPS. Moreover, compared to before treatment, the fecal *Bifidobacterium* species were significantly altered after berberine treatment. Furthermore, the levels of TNF-α and LPS showed a remarkable correlation with *Bifidobacterium, B. longum, B. infantis* and *B. adolescentis* but not with *B. breve*. However, the precise mechanism underlying the adjusting of the copy numbers of *Bifidobacterium* species by berberine, as well as the subsequent influenceon the expression

of TNF-α and LPS, warrants further explorations.

## Disclosure of conflict of interest

None.

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## References

[1] Naqshbandi M, Harris SB, Esler JG and Antwi-Nsiah F. Global complication rates of type 2 diabetes in Indigenous peoples: A comprehensive review. Diabetes Res Clin Pract 2008; 82: 1-17.

[2] Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K and Ganai BA. Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. World J Diabetes 2015; 6: 598-612.

[3] Kaplon-Cieslicka A, Postula M, Rosiak M, Peller M, Kondracka A, Serafin A, Trzepla E, Opolski G and Filipiak KJ. Association of adipokines and inflammatory markers with lipid control in type 2 diabetes. Pol Arch Med Wewn 2015; 125: 414-423.

[4] Rios JL, Francini F and Schinella GR. Natural Products for the Treatment of Type 2 Diabetes Mellitus. Planta Med 2015; 1: 1.

[5] Stern RJ and Murphy EJ. Metformin as Initial Oral Therapy in Type 2 Diabetes. JAMA 2015; 313: 2484-2485.

[6] Chen YH, Du L, Geng XY, Peng YL, Shen JN, Zhang YG, Liu GJ and Sun X. Effects of sulfonylureas on lipids in Type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. J Evid Based Med 2015; 4: 12157.

[7] Lan J, Zhao Y, Dong F, Yan Z, Zheng W, Fan J and Sun G. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. J Ethnopharmacol 2015; 161: 69-81.

[8] Dong H, Wang N, Zhao L and Lu F. Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. Evid Based Complement Alternat Med 2012; 591654: 15.

[9] Dong H, Zhao Y, Zhao L and Lu F. The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. Planta Med 2013; 79: 437-446.

[10] Liu B, Li W, Chang Y, Dong W and Ni L. Extraction of berberine from rhizome of Coptis chinensis Franch using supercritical fluid extraction. J Pharm Biomed Anal 2006; 41: 1056-1060.

[11] Yin J, Zhang H and Ye J. Traditional chinese medicine in treatment of metabolic syndrome. Endocr Metab Immune Disord Drug Targets 2008; 8: 99-111.

[12] Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G and Ning G. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J Clin Endocrinol Metab 2008; 93: 2559-2565.

[13] Zhang H, Wei J, Xue R, Wu JD, Zhao W, Wang ZZ, Wang SK, Zhou ZX, Song DQ, Wang YM, Pan HN, Kong WJ and Jiang JD. Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 2010; 59: 285-292.

[14] Guamer F. [Role of intestinal flora in health and disease]. Nutr Hosp 2007; 2: 14-19.

[15] Tai N, Wong FS and Wen L. The role of gut microbiota in the development of type 1, type 2 diabetes mellitus and obesity. Rev Endocr Metab Disord 2015; 16: 55-65.

[16] Brown K, DeCoffe D, Molcan E and Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 2012; 4: 1095-1119.

[17] Xu X, Hui H and Cai D. [Differences in fecal Bifidobacterium species between patients with type 2 diabetes and healthy individuals]. Nan Fang Yi Ke Da Xue Xue Bao 2012; 32: 531-533.

[18] Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V and Akbarian-Moghari A. Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci 2011; 94: 3288-3294.

[19] Xu Z, Wang R, Yang Y, Dai R, Shang J and Yu Q. [Intervention effect of probiotic bifidobacterium on type 2 diabetes mellitus rats]. Wei Sheng Yan Jiu 2014; 43: 277-281.

[20] Sjogren YM, Tomicic S, Lundberg A, Bottcher MF, Bjorksten B, Sverremark-Ekstrom E and Jenmalm MC. Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin Exp Allergy 2009; 39: 1842-1851.

[21] Pang B, Zhao LH, Zhou Q, Zhao TY, Wang H, Gu CJ and Tong XL. Application of berberine on treating type 2 diabetes mellitus. Int J Endocrinol 2015; 905749: 11.

[22] Chang W, Chen L and Hatch GM. Berberine as a therapy for type 2 diabetes and its complications: From mechanism of action to clinical studies. Biochem Cell Biol 2014; 1: 1-8.

[23] Kim KB. Effect of different training mode on Interleukin-6 (IL-6) and C-reactive protein (CRP) in type 2 diabetes mellitus (T2DM) patients. J Exerc Nutrition Biochem 2014; 18: 371-378.
[24] Jayashree B, Bibin YS, Prabhu D, Shanthirani CS, Gokulakrishnan K, Lakshmi BS, Mohan V and Balasubramanyam M. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. Mol Cell Biochem 2014; 388: 203-210.
[25] Swaroop JJ, Rajarajeswari D and Naidu JN. Association of TNF-alpha with insulin resistance in type 2 diabetes mellitus. Indian J Med Res 2012; 135: 127-130.
[26] Xia X, Yan J, Shen Y, Tang K, Yin J, Zhang Y, Yang D, Liang H, Ye J and Weng J. Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis. PLoS One 2011; 6: 0016556.
[27] Yin J, Gao Z, Liu D, Liu Z and Ye J. Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol Endocrinol Metab 2008; 294: 30.