ORIGINAL ARTICLE

Structural modulation of gut microbiota during alleviation of type 2 diabetes with a Chinese herbal formula

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Introduction

Type 2 diabetes (T2D), which is characterized by low-grade inflammation, insulin resistance (Shoelson, 2006) and β-cell failure (Butler et al., 2003), has become increasingly prevalent worldwide (Xu et al., 2013). The estimated proportion of diabetes among adults is 8.3% in 2010, among which T2D accounts for at least 90% (Alberti and Zimmet, 1998; Whiting et al., 2011). This proportion is projected to increase to 9.9% by 2030 (Whiting et al., 2011). Development of T2D results mostly from obesity, which has low-grade inflammation and insulin resistance (Hotamisligil, 2006). The gut microbiota may have a vital role in obesity development (Backhed et al., 2004; Collins et al., 2013; Le Chatelier et al., 2013; Zhao, 2013). For example, endotoxin produced by an opportunistic pathogen in the gut, such as Escherichia coli, induced obesity and insulin resistance when a purified form was subcutaneously infused into mice (Cani et al., 2007a). A greater abundance of opportunistic pathogens, such as Betaproteobacteria, was found in the gut of diabetic patients compared with healthy controls (Larsen et al., 2010). A more recent comparative metagenomic analysis of the fecal samples of 171 diabetic patients and 174...
healthy controls showed that diseased samples had lesser abundance of butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, but greater abundance of opportunistic pathogens, including *Clostridium bolteae* and *Desulfovibrio* sp. (Qin et al., 2012). Another study found that the early onset of high-fat-diet-induced T2D was characterized by an increased bacterial translocation from the intestine towards tissues (Amar et al., 2011). An opportunistic pathogen, *Enterobacter cloacae* B29, isolated from the gut of a morbidly obese and diabetic patient, induced obesity and insulin resistance in germ-free mice (Fei and Zhao, 2013). Taken together, these studies indicate that a dysbiotic gut microbiota may causatively contribute to obesity and diabetes development, and thus may serve as a potential new target for disease control.

To treat obesity, T2D and other metabolic diseases, several attempts have been made to target the gut microbiota (Cani et al., 2007b, 2009; Park et al., 2013). Berberine, the major pharmacologic component of a Chinese herb *Coptis chinensis* (Huang-Lian) originally used to treat bacterial diarrhea (Khin Maung et al., 1985; Rabbani et al., 1987; Tang et al., 2009), showed clinical efficacy in treating diabetes in a multicentered, randomized, double-blinded and placebo-controlled clinical trial (Zhang et al., 2008). The herb *C. chinensis* has been used in traditional Chinese medicine (TCM) for diarrhea control for nearly 2000 years. Our recent study showed that berberine prevented high-fat-diet-induced obesity and insulin resistance, enriched short-chain fatty acid-producing bacteria, reduced numbers of opportunistic pathogens and alleviated inflammation in Wistar rats (Zhang et al., 2012b).

Drugs that treat bacterial diarrhea, such as berberine, might be useful for T2D treatment because both diseases share a dysbiotic gut microbiota. A standardized berberine-containing Chinese herbal formula, Gegen Qinlian Decoction (GQD), has been used for diarrhea control for nearly 2000 years. Our recent study showed that berberine prevented high-fat-diet-induced obesity and insulin resistance, enriched short-chain fatty acid-producing bacteria, reduced numbers of opportunistic pathogens and alleviated inflammation in Wistar rats (Zhang et al., 2012b).

Moreover, the mechanism underlying GQD's impact on glycemic efficacy has barely been elucidated. A recent nuclear magnetic resonance-based plasma metabonomic study revealed that 5 weeks of GQD treatment conspicuously modulated gut microbial metabolism by degradation of choline into methylamines, together with a decrease in FBG and an expansion of islets in STZ and high-fat-diet-induced diabetic rats (Tian et al., 2013). This finding indicates that the gut microbiota might have a pivotal role in the effect GQD has on diabetic subjects. However, there is still a lack of direct evidence showing that GQD can modulate gut microbiota, particularly in humans.

In this study, we conducted a randomized, double-blinded, placebo-controlled clinical trial to evaluate the efficacy and safety of GQD in the treatment of T2D. Furthermore, we examined the structural alterations of gut microbiota in response to GQD treatment intended to alleviate T2D.

### Materials and methods

#### Study design

The study was a 12-week, randomized, double-blinded and placebo-controlled clinical trial that included a 2-week washout period. It was approved by the Ethics Committee of Guang'anmen hospital of China Academy of TCM. Participants were recruited by Guang’anmen hospital, Dong Zhi Qiao Hospital affiliated to Beijing TCM University, China–Japan Friendship Hospital or Ji Shui Tan Hospital of Beijing from August 2010 to May 2011. All participants signed informed consent forms before beginning the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The inclusion and exclusion criteria of the patients’ enrollments can be found in the Supplementary Materials and methods. Using an initial screening, including FBG test and 75-g oral glucose tolerance test, 629 recently diagnosed T2D patients who had not received prior pharmacologic treatment for T2D were recruited into the study. After a 2-week washout period and the review of a series of examinations, 403 patients were excluded for not meeting the inclusion criteria and 2 patients were excluded for other reasons. The remaining 224 patients were randomly assigned to four groups of 56 patients. Each group received one of the following treatments: high (HD), moderate (MD) or low dose (LD) GQD, or the placebo for 12 weeks. Randomization was performed centrally and was concealed and stratified in blocks of eight by the PROC PLAN process using the SAS software (SAS Institute Inc., Cary, NC, USA). After the study was completed, a total of 187 patients were included for the final analysis by the verification of data examination committee (Supplementary Figure 1).
Drug administration

The TCM formula in our study was GQD, composed of four herbs, namely: Gegen (Radix Puerariae), Huangqin (Radix Scutellariae), Huanglian (Rhizoma Coptidis) and Cancao (Honey-fried Licoriza Root) (Supplementary Figure 2a). Supplementary Table 1 lists the amount of each herb in one unit of GQD formula in each group. Herbs were all provided and quality controlled by Beijing Shuangqiaoanjing Chinese herb manufacturer (Supplementary Materials and methods). The TCM intervention and placebo were given as decoction; these were prepared by Beijing Jiulong Pharmaceutical Factory according to a standard production process (Supplementary Materials and methods). Each unit of GQD formula or placebo yielded 300 ml of decoction. Each patient orally took 150 ml of the decoction two times daily for 12 weeks. All of the drugs and decoctions were quality controlled throughout the trial, and the placebo decoction was prepared by the same standardized process (Supplementary Materials and methods).

Study evaluation and outcomes

The following primary efficacy outcomes were used: changes in HbA1c, FBG and 2-h postprandial blood glucose (2h-PBG) levels. Secondary efficacy outcomes included changes in serum insulin, lipids levels and body mass index. Study assessments were performed at 0, 4, 8 and 12 weeks. Measurements of FBG, 2h-PBG, body mass index, waist circumference and hip circumference were taken at 0, 4, 8 and 12 weeks. Serum HbA1c, insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol measurements were performed at weeks 0 and 12. Fecal samples were collected every 4 weeks until the end of trial for gut microbiota analysis.

Clinical and biochemical measurements

Biochemical measurements of glucose, serum lipids, HbA1c and insulin were performed in a central laboratory (Guang’anmen Hospital of China Academy of TCM, Beijing, China). Glucose, serum total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were measured by enzymatic methods (Olympus AU2700; Olympus Co. Ltd., Tokyo, Japan). HbA1c was measured by high-performance liquid chromatography using a variant hemoglobin HbA1c assay (ADAMS A1c HA-8160; Arkray Inc., Kyoto, Japan). Serum insulin was measured using a double-antibody RIA (ADVIA Centaur; Bayer Diagnostics, Leverkusen, Germany). ELISA Kits were used to measure plasma orosomucoid (Assaypro, St Charles, MO, USA), adiponectin (R&D System, Minneapolis, MN, USA), tumor necrosis factor-α (R&D System) and serum amyloid A protein (Invitrogen, Carlsbad, CA, USA) levels.

Fecal DNA extraction and pyrosequencing

Genomic DNA of each fecal sample was extracted by a InvitMag Stool DNA Kit (Invitek, Berlin, Germany) combined with bead beating as previously published (Zhang et al., 2012b). The extracted genomic DNA was used as the template to amplify the V3 region of 16S rRNA genes. PCR reactions, pyrosequencing of the PCR amplicons and quality control of raw data were performed as described previously with minor modification (Zhang et al., 2009; Wang et al., 2011).

Bioinformatics and multivariate statistics

High-quality sequence alignments were performed using NAST. Sequence clustering by CD-hit and OUT delineation by DOTUR were performed as described previously (Zhang et al., 2012a, b). The representative sequences of operational taxonomy units (OTUs) with their relative abundance were used to calculate rarefaction analysis and Shannon diversity index by QIIME (Caporaso et al., 2010). In addition, the representative sequences were inserted into a pre-established phylogenetic tree of the full-length 16S rRNA gene sequences in ARB (Ludwig et al., 2004). Then, the phylogenetic tree and the relative abundance table of representative sequences of OTUs were used for UniFrac principal coordinate analysis (PCoA) (Lozupone and Knight, 2005). The statistical significance between different groups was assessed by multivariate analysis of variance in MATLAB 2010b (The MathWorks Inc., Natick, MA, USA). Redundancy analysis was performed using CANOCO for Windows 4.5 (Microcomputer Power, Ithaca, NY, USA) according to the manufacturer’s instructions (Braak and Smilauer, 2002). Statistical significance was assessed by MCPP with 499 random permutations under the full model. Ribosomal Database Project Classifier was used to assess the amounts of different genera by taxonomic assignment of all sequences.

Real-time quantitative PCR of F. prausnitzii

Real-time quantitative PCR (q-PCR) was used to determine the amounts of total bacteria and F. prausnitzii through detection of 16S rRNA genes. A set of universal primers was used to amplify a conserved 16S rDNA sequence in all bacteria as shown before (Wang et al., 2011). A set of specific primers was used to amplify a conserved 16S rDNA sequence in F. prausnitzii and the q-PCR reaction system and the program was described before (Balamurugan et al., 2008). A plasmid containing a F. prausnitzii full-length 16S rDNA from a previous study (Shen et al., 2006) was prepared using the EZNA Plasmid Mini Kit I (OMEGA, Doraville, GA, USA) and diluted from $10^3$ to $10^9$ copies/μl to construct a standard curve for the detection of F. prausnitzii. We selected reactions with efficiencies ranging from 0.90 to 1.05 for
further analysis. Standard and quantified samples were performed in triplicate. PCR reactions were performed using iQ SYBR Green SuperMix (Bio-Rad, Richmond, CA, USA) on a MasterCycler ep Realplex 4s (Eppendorf, Hamburg, Germany).

Spearman’s correlation coefficient ($R$) and $P$-value were used to compare the amounts of $F$. prausnitzii measured by q-PCR and pyrosequencing. This coefficient was also used to evaluate the relationship between $F$. prausnitzii and clinical parameters using MATLAB 2010b.

Results

The major components of GQD decoction

There were four major categories of compounds in the GQD decoction. Flavones (baicalin, puerarin, wogonoside, daidzin, liquiritin, baicalein and wogonin), alkaloids (berberine, coptisine, palmatine and jatrorrhizine) and triterpenoid sapnins (glycyrrhizin) were detected in the decoction, among which baicalin, puerarin and berberine were the major components (Supplementary Table 2). The chemical structures of these 12 components are shown in the Supplementary Figure 2c. Carbohydrates (starch, sucrose, reducing sugar and soluble dietary fiber) were also detected. Insoluble dietary fiber was undetectable in GQD decoction (Supplementary Table 3).

GQD significantly improved glycemic control in T2D patients

In our 12-week, randomized, double-blinded, placebo-controlled clinical trial (Supplementary Figure 1), the data of 187 participants were analyzed as shown in Supplementary Table 4. The baseline variables were not significantly different among the four groups. After 12 weeks of treatment, GQD significantly improved glycemic control in T2D patients. The HD and MD groups, when compared with the placebo and LD groups, showed significant reductions in adjusted mean changes from baseline of FBG ($-1.46 \pm 0.23$ and $-1.09 \pm 0.21$ vs $-0.16 \pm 0.22$ and $-0.24 \pm 0.24$ mmol l$^{-1}$; $P<0.001$ for HD vs LD and placebo; $P<0.01$ for MD vs LD and placebo). Similarly, the HD and MD groups showed significantly reduced HbA1c ($-0.88 \pm 0.14$ and $-0.75 \pm 0.13$ vs $-0.35 \pm 0.13$ and $-0.36 \pm 0.15$%; $P<0.01$ for HD vs LD; $P<0.05$ for HD vs placebo; $P<0.05$, MD vs LD and placebo) (Figures 1a and b). A decrease in the mean change of 2h-PBG from baseline was also observed in the treated groups, although not reaching significant level. (Supplementary Figure 3). In addition, ANCOVA analysis showed that HOMA-$\beta$ was significantly improved by HD GQD treatment compared with the placebo and LD groups (Figure 1c).

Plasma orosomucoid was significantly reduced by HD GQD treatment ($P=0.023$) (Supplementary Figure 4a) and the HD group showed a significant reduction ($P=0.034$) in mean change from baseline of plasma orosomucoid compared with the LD group (Supplementary Figure 4b). No significant difference was observed in plasma adiponectin, tumor necrosis factor-$\alpha$ or serum amyloid A among the four groups (Supplementary Figures 5a–c). Finally, no drug-related serious adverse events occurred in this study.

Overall structural modulation of gut microbiota after GQD treatment

First, we used a bar-coded pyrosequencing run to analyze the structural changes of gut microbiota in the four groups before and after GQD treatment. In total, 483,304 usable raw sequences (34,753 unique sequences) and 3222 OTUs were obtained from 235 samples with an average of $2057 \pm 396$ per sample. Rarefaction and Shannon diversity curves revealed that, although no rarefaction curves plateaued with the current sequencing, most of the diversity had already been captured (Supplementary Figure 6). Weighted and unweighted UniFrac PCoA analysis revealed that gut microbiota structure of the treated groups showed a dose-dependent deviation.
from the baseline structure, with the HD group reaching significant level in multivariate analysis of variance test (Figures 2a and b and Supplementary Figures 7a and b).

To monitor the dynamic changes of gut microbiota during GQD treatment, we analyzed the fecal samples collected at weeks 0, 4, 8 and 12 in HD and placebo groups with a second pyrosequencing run. In total, we generated 680,774 usable raw sequences (37,498 unique sequences) and 4,251 OTUs from 288 samples with an average of 2,364 ± 443 per sample (one sample was excluded in later analysis because only 81 reads were obtained). Rarefaction and Shannon diversity curves revealed that most of the diversity had already been captured (Supplementary Figure 8). UniFrac PCoA and PCA showed that after 4 weeks of treatment, the gut microbiota structure of the HD group had already significantly diverged from that of its baseline and of the placebo group (Figures 3a and b; Supplementary Figure 9). At that same 4-week analysis, 2h-PBG was significantly reduced, but FBG did not reach a significant level in treated groups (Supplementary Figure 10). As the treatment

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**Figure 2** Dose-dependent alterations of the gut microbiota in T2D patients treated with different doses of GQD at weeks 0 and 12. (a) Weighted UniFrac PCoA of gut microbiota based on the OUT data from the first pyrosequencing run. (b) Clustering of gut microbiota based on mahalanobis distances calculated with multivariate analysis of variance (MANOVA). Each point represents the mean principal coordinate (PC) score of all patients in a group at one time point, and the error bar represents the s.e.m. The sample number (n) at week 0: placebo = 30, LD = 28, MD = 32 and HD = 28. The sample number (n) at week 12: placebo = 30, LD = 28, MD = 32 and HD = 28. ***P < 0.0001.

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**Figure 3** Trajectory of the gut microbiota in T2D patients treated with HD GQD and placebo at weeks 0, 4, 8 and 12. (a) Unweighted UniFrac PCoA of gut microbiota based on the OUT data from the second pyrosequencing run. (b) Clustering of gut microbiota based on mahalanobis distances calculated with multivariate analysis of variance (MANOVA). Each point represents the mean principal coordinate (PC) score of all patients in a group at one time point, and the error bar represents the s.e.m. Placebo: n = 36; HD: n = 36. ***P < 0.0001.
progressed, the gut microbiota made no additional changes (Figures 3a and b and Supplementary Figure 9), but the FBG and 2h-PBG continued to improve until the end of the study (Supplementary Figure 10).

**Key phylotypes responding to the GQD treatment in T2D patients**

By using redundancy analysis, we identified 146 key variables that responded to the GQD treatment (Figure 4). Forty-seven OTUs were increased by GQD and 99 were decreased. Among the 47 OTUs enriched by GQD, 17 OTUs showed a significant negative correlation with FBG; the OTUs belong to *Faecalibacterium* (*n* = 4), *Gemmiger* (*n* = 4), *Bifidobacterium* (*n* = 3), *Lachnospiracea_incertae_sedis* (*n* = 2) and *Escherichia* (*n* = 1). Nine OTUs showed a significant negative correlation with HbA1c; two of which were from *Faecalibacterium*, two from *Gemmiger*, one from *Bifidobacterium*, one from *Parasutterella* and one from *Escherichia* (Figure 5 and Supplementary Tables 5 and 6).

Among the 99 OTUs decreased by GQD treatment, 22 OTUs showed a significant positive correlation with FBG: the OTUs belong to *Alistipes* (*n* = 6), *Odoribacter* (*n* = 2), *Parabacteroides* (*n* = 2), *Bacteroides* (*n* = 2), *Pseudobutyrivibrio* (*n* = 2) and one OTU to each of the following genera: *Butyricimonas*, *Barnesiella*, *Oscillibacter* and *ClostridiumXlVa*. Fourteen OTUs showed a significant positive correlation with HbA1c, four of which were from *Alistipes*, two from *Odoribacter*, one from *Parasutterella* and one from *Escherichia* (Figure 5 and Supplementary Tables 5 and 6).

Additionally, taxon-based analysis at the genus level showed that the relative abundance of *Faecalibacterium, Gemmiger, Bifidobacterium* and *Lachnospiracea_incertae_sedis* was significantly higher after HD GQD treatment, whereas *Alistipes, Parabacteroides* and *Pseudobutyrivibrio* were significantly decreased (relative abundance >1% and *P*<0.01) (Supplementary Figure 11).

**Quantification of F. prausnitzii and its association with glycemic parameters**

One previous study showed that *F. prausnitzii* is more abundant in the gut of healthy people compared with T2D patients (Qin et al., 2012). In our study, pyrosequencing results indicated that *Faecalibacterium* was substantially enriched after GQD treatment. To confirm the pyrosequencing results, we quantified the relative abundance of *F. prausnitzii*, the prominent species of the *Faecalibacterium* genus, by q-PCR. All three doses of GQD treatment significantly enriched *F. prausnitzii* compared with baseline (*P* = 0.004 for the HD group, *P* = 0.024 for the MD group, *P* = 0.011 for the LD group, *P* = 0.217 for the placebo group) (Figure 6a). Particularly, the relative abundance of this bacterium was significantly higher in HD group than in the other three groups after 12 weeks of treatment (*P* = 0.0002, HD vs placebo; *P* = 0.0435, HD vs MD; *P* = 0.0120, HD vs LD), while no significant difference was observed at baseline (Figure 6b). The relative abundance of *Faecalibacterium* by q-PCR and pyrosequencing showed a high significant correlation with each other, indicating that the differences of *Faecalibacterium* among four groups found by pyrosequencing is reliable (Supplementary Figure 12). Moreover, the relative
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abundance of this bacterium negatively correlated with HbA1c, FBG and 2h-PBG, and positively correlated with HOMA-β by Spearman’s correlation coefficient (Supplementary Table 7).

### Discussion

To our knowledge, this study is the first registered clinical trial to evaluate the dose-dependent efficacy and safety of a Chinese herbal decoction following a randomized, double-blinded and placebo-controlled design. In our study, GQD treatment provided clinically meaningful and dose-dependent reductions in FBG and HbA1c compared with placebo. HOMA-β was also significantly improved by HD GQD treatment. Compared with those in T2D patients treated with berberine in a previously published clinical trial, the HD group in our study showed similar reductions of FBG and HbA1c; however, we did not observe an improvement of dyslipidemia, which had been significantly ameliorated by berberine in the other study (Zhang et al., 2008). In addition, we found a slight alleviation of inflammation, indicated by the reduction of plasma orosomucoid in the HD group. This finding was consistent with a previous nuclear magnetic resonance-based plasma metabonomic study in T2D rats treated with GQD (Tian et al., 2013). These data show that the Chinese herbal formula is effective for glycemic control, making it a promising candidate for diabetes management.

Concomitant with the improved glucose homeostasis, we observed an altered microbial composition induced by GQD. In other studies, diet (Cani et al., 2008; Turnbaugh et al., 2008; Xiao et al., 2013), drugs (Zhang et al., 2012b; Shin et al., 2013), prebiotics (Cani et al., 2007b, 2009) and probiotics (Park et al., 2013) have been used to modulate the gut microbiota in metabolic diseases, for example, the increase of Akkermansia spp. by metformin in the improvement of glucose homeostasis in high-fat-diet-induced obese mice (Shin et al., 2013). Berberine, the major pharmacologic component of a Chinese herb C. chinensis (Huang-Lian), has been shown to enrich short-chain fatty acid producers in parallel with the prevention of obesity and insulin resistance in rats (Zhang et al., 2012b). C. chinensis is also a major component of GQD. However, no gut microbiota modulation by berberine or Chinese herbs has been reported in humans. To our knowledge, this study is the first direct evidence in humans that TCMs can modulate the structure of the gut microbiota.

Consistent with the dose-dependent manner of T2D amelioration, GQD also exerted a dose-dependent modulation on the gut microbiota, suggesting a strong association between the modulation of gut microbiota and T2D alleviation. Dose-dependent modulation of gut microbiota was also reported in prebiotic studies. For example, a stepwise increase of Bifidobacteria was observed by q-PCR when nine healthy adults received successively increased dosage levels of galactooligosaccharides (Davis et al., 2010). Our study is the first example that dose-dependent modulation of the gut microbiota was concomitant with a dose-dependent alleviation of T2D in humans. Moreover, the gut microbiota alterations occurred before significant improvement of T2D symptoms, supporting the hypothesis that changes in the gut microbiota induced by GQD might contribute to improved glucose homeostasis.

**Figure 6** Relative abundance of *F. prausnitzii* as quantified by q-PCR. (a) The impact of different treatments on the relative abundance of *F. prausnitzii* before and after treatment. The sample number (n) for each group: placebo = 21, LD = 19, MD = 19 and HD = 20. (b) The differences of *F. prausnitzii* among four groups at week 12. The sample number (n) for each group at week 0: placebo = 31, LD = 25, MD = 26 and HD = 29. The sample number for each group at week 12: placebo = 26, LD = 25, MD = 24 and HD = 28. *P* < 0.05, **P** < 0.01. Placebo, LD, or MD vs HD using Mann–Whitney test. Data are represented as mean ± s.e.m.

**Figure 5** Heat map of redundancy analysis (RDA)-identified key OTUs responding to GQD treatment and Spearman’s correlation between identified OTUs and HbA1c or FBG. The color of the spots in the left panel represents the mean relative abundance (normalized and log-transformed) of the OTU in each group. The OTUs are organized according to their phylogenetic positions. The color of the spots in the right panel represents R-value of Spearman’s correlation between the OTU and HbA1c or FBG. The family and genus names of the OTUs are shown on the right. *P* < 0.05.
in T2D patients rather than a mere consequence after the symptoms have been alleviated.

Several putative beneficial genera that responded to GQD treatment were identified using redundancy analysis, including *Faecalibacterium*, *Bifidobacterium* and *Gemmiger*. *Faecalibacterium*, a butyrate-producing bacterial group (Duncan et al., 2002), which was profoundly enriched by GQD in our study, shows anti-inflammatory effects partly through reducing colonic cytokine synthesis and increasing anti-inflammatory cytokine secretion (Sokol et al., 2008). Diabetic obese patients have lower abundance of *F. prausnitzii* compared with non-diabetic obese patients, which demonstrates a negative correlation with inflammatory cytokines C-reactive protein and interleukin-6 (Furet et al., 2012). Furthermore, *F. prausnitzii* has been shown to be a functionally important phylotype because it was associated with eight urinary metabolites (Li et al., 2008). In our study, the relative abundance of *F. prausnitzii* was negatively correlated with HbA1c, FBG and 2h-PBG, and positively correlated with HOMA-β, suggesting that *F. prausnitzii* might be a pivotal phylotype associated with the improvement of T2D.

Two other genera, *Bifidobacterium* and *Gemmiger*, which are also reported to confer beneficial effects (Gossling and Moore, 1975; Sokol et al., 2008; Fukuda et al., 2011), were significantly enriched by GQD in T2D patients. A selective increase of *Bifidobacteria* induced by a prebiotic (namely oligofructose) improved gut permeability and inflammation in ob/ob mice (Cani et al., 2009). In addition, several genera were significantly inhibited by GQD, such as *Alistipes* and *Odoribacter*. Higher abundance of several taxa of the genus *Alistipes* were associated with greater frequency of abdominal pain in irritable bowel syndrome pediatric patients (Saulnier et al., 2011). One example from this genus, *Alistipes putredinis*, was isolated from inflamed and non-inflamed intestinal tissues of children with suspected acute appendicitis (Rautio et al., 2000, 2003). *Alistipes* and *Odoribacter* were significantly increased in grid floor stress-induced BALB/c mice (Bangsgaard Bendtsen et al., 2012). These results suggest that the enrichment of beneficial bacteria, particularly *Faecalibacterium* spp., and reduction of pathogen-like bacteria might be involved in the amelioration of T2D by GQD.

TCM formula, a form of polypharmacy, has been developed and advocated for use in the treatment of many diseases for over 2500 years in China. However, the complexities and unknown mechanisms of TCMs prevent the active chemical components from being identified. Our study suggests that gut microbiota might be involved in the effect of a widely used TCM formula, GQD. This opens an avenue for identifying chemical components in TCMs, which can modulate gut microbiota structure as a potential mechanism for disease alleviation. Baicalin, puerarin and berberine were identified as the three most abundant chemical components in GQD. Baicalin, which demonstrated antioxidant properties together with the glucose-lowering effects in STZ-induced diabetic rats (Waisundara et al., 2009), showed dose-dependent synergic effects with β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* and other β-lactam-resistant strains of *S. aureus* in vitro (Liu et al., 2000). Puerarin can dose-dependently increase glucose utilization in STZ-induced diabetic rats (Hsu et al., 2003). However, it showed poor absorption into the bloodstream after oral administration in vivo (Luo et al., 2011) and was mainly excreted via feces in its intact form (Zhu et al., 1979). It is possible that puerarin could have an impact on gut microbiota, but no study about this has been reported yet. Berberine showed significant glucose-lowering effects in a multicentered, randomized, double-blinded and placebo-controlled clinical trial (Zhang et al., 2008). Additionally, berberine has been reported to modulate gut microbiota during prevention of high-fat-diet-induced obesity and insulin resistance in rats (Zhang et al., 2012b), implying that berberine could be one of the major active ingredients in GQD that modulated the gut microbiota in our study. The carbohydrate components of GQD likely exerted few effects on gut microbiota because insoluble dietary fiber was undetectable and soluble dietary fiber was very low. Additionally, these components showed no dosage differences across the four decoctions. Other carbohydrates, such as starch, were easily digested and absorbed before they reached large intestine. These results indicate that modulation of gut microbiota by chemical components, such as berberine, might be involved in improving glucose homeostasis by GQD, suggesting that TCMs may serve as a new source for drug leads in gut microbiota-targeted diabetes management.

In conclusion, our study suggests that structural alterations of gut microbiota, induced by Chinese herbal formula GQD, are associated with the anti-diabetic effects of GQD. In particular, this treatment enriched the number of beneficial bacteria, such as *Faecalibacterium* spp. in the gut. Although it is still unclear whether changes of gut microbiota by GQD directly contribute to the improvement of glucose homeostasis, our clinical study provides circumstantial evidence that gut microbiota might be involved.

**Conflict of Interest**

The authors declare no conflict of interest.

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