Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
www.all-imm.com

ORIGINAL ARTICLE

OPEN ACCESS

Effect of metformin on gut microbiota imbalance in patients with T2DM, and the value of probiotic supplementation

Lu Li, Yanli Chen, Zhipeng Tang, Yan You, Yang Guo, Yong Liao*

Department of Pharmacy, Affiliated Renhe Hospital of China Three Gorges University, Yichang, Hubei Province, China

Received 22 March 2024; Accepted 21 May 2024
Available online 1 July 2024

KEYWORDS
gut microbiota;
inflammatory factors;
insulin resistance;
metformin;
probiotics;
type 2 diabetes

Abstract
Purpose: To investigate the effect of metformin on gut microbiota imbalance in patients with type 2 diabetes mellitus (T2DM), and the value of probiotic supplementation.
Methods: A total of 84 newly diagnosed T2DM patients were randomly divided into probiotics group, metformin group, and control group, with 28 patients in each group. The blood glucose control, islet function, gut microbiota, and inflammatory factors were compared between three groups.
Results: After 3 months of treatment, fasting plasma glucose (FPG), 2-h postprandial plasma glucose (2-h PG), and glycosylated hemoglobin A1c (HbA1c) were evidently decreased in both probiotics and metformin groups ($P < 0.05$) and were lower than that in the control group prior to treatment. Besides, FPG, 2-h PG, and HbA1c were lower in the metformin group than that in the control group. FPG, 2-h PG, and HbA1c were further lower in the probiotic group than in the metformin group ($P < 0.05$). Fasting insulin (FINS) and islet β cell (HOMA-β) function were dramatically increased in the same group ($P < 0.05$), while insulin-resistant islet β cells (HOMA-IR) were significantly lower in the same group ($P < 0.05$); FINS and HOMA-β were significantly higher, while HOMA-IR was significantly lower ($P < 0.05$) in both groups than in the control group prior to treatment. HOMA-IR was also lower in the probiotic group than in the metformin group after treatment ($P < 0.05$); the number of *lactobacilli* and bifidobacteria increased ($P < 0.05$) in both probiotic and metformin groups than in the control group prior to treatment, and the number of Enterobacteriaceae and Enterococcus was lower in the control group prior to treatment ($P < 0.05$). In addition, the number of *lactobacilli* and bifidobacteria was higher and the number of *enterobacteria* and *enterococci* was lower in the probiotic group than that in the metformin group after treatment, and the differences were statistically significant ($P < 0.05$). Lipopolysaccharide (LPS), interleukin 6 (IL-6), and C-reactive protein (CRP) levels were lower in both probiotic and metformin groups ($P < 0.05$). The serum LPS, IL-6, and CRP levels were lower in both probiotic and metformin groups, compared to the control group prior to the treatment ($P < 0.05$).

***Corresponding author:** Yong Liao, Department of Pharmacy, Affiliated Renhe Hospital of China Three Gorges University, No. 410 Yiling Avenue, Yichang City, Hubei Province 443001, China. *Email address*: ly28368@163.com

https://doi.org/10.15586/aei.v52i4.1101

Conclusion: Metformin while treating T2DM assists in improving the imbalance of gut microbiota.

Introduction

Diabetes mellitus (DM) has been a critical global public health problem since the 20th century. It is reported in the literature that the number of DM patients is expected to reach 578 million in 2030, and continue to increase to about 700 million by 2045, of which type 2 diabetes mellitus (T2DM) patients accounts for about 90%.[1-3] At present, the pathogenesis of T2DM has been relatively clear, and insulin resistance (IR) is the core link that leads to abnormal glucose metabolism.[4] Recent studies have shown that gut microbiota disorder is an essential factor in the pathogenesis of T2DM, and abnormal blood glucose and hypoglycemic agents change the composition of gut microbiota and its metabolites, thus affecting blood glucose control. Therefore, probiotic adjuvant therapy for T2DM is beneficial to improve the distribution of gut microbiota in patients, and it exerts a positive effect to improve the compliance rate of blood glucose levels.[5-7] However, owing to diverse hypoglycemic agents commonly used in clinical practice, there are still many disputations about the effects of hypoglycemic agents on the gut microbiota of T2DM patients and the corresponding probiotic treatment regimens in different reports. Metformin is the first choice of hypoglycemic agents for treating T2DM, and previous studies have shown that metformin treatment of T2DM is beneficial to regulate gut microbiota, and it effectively increases the flora abundance of short-chain fatty acids (SCFA) produced by Bacteroides species and lessens intestinal inflammation, thereby lowering IR and reducing blood glucose levels. However, the effect of combined use with probiotics remains to be explored.[8] In this study, an in-depth study was carried out on the effect of metformin on gut microbiota imbalance in T2DM patients and the value of probiotic supplementation, described in detail.

Materials and Methods

General data

A total of 84 newly diagnosed T2DM patients at Affiliated Renhe Hospital of China were selected as samples for a prospective randomized controlled study. Inclusion criteria were as follows: (1) Patients who met the diagnostic criteria of T2DM;[9] (2) age range 18–65 years; (3) body mass index (BMI): 18.5–30.0 kg/m^2; and (4) all patients were aware of the details of this study and voluntarily signed the consent form. Exclusion criteria were as follows: (1) Patients who had been treated with hypoglycemic agents, probiotics, antibiotics, or weight-loss drugs in the past 1 month; (2) patients with inflammation, ulcers, or tumors and other gastrointestinal lesions; (3) patients who have undergone gastrointestinal surgery in the past 3 months; (4) patients with hyperthyroidism, hypothyroidism, or hypercortisolism (HCM) and other endocrine lesions; (5) patients with ketoacidosis, diabetic retinopathy, or diabetic foot and other complications; (6) patients with infectious diseases or malignant tumors; (7) patients with severe liver and kidney dysfunction; (8) patients with a history of mental illness or alcohol dependence; and (9) patients with contraindications regarding metformin or probiotics treatment.

The selected patients were equally divided via simple randomization, with 28 cases in each group, into the following three groups: probiotic group, metformin group, and control group. The probiotic group had 15 males and 13 females, aged 46–65 years, with a mean age of 55.32 $\pm$ 4.38 years; BMI was 17.1–27.8 kg/m^2, and mean BMI was 21.87 $\pm$ 2.82 kg/m^2. The metformin group had 17 males and 11 females, aged 48–65 years, with a mean age of 55.25 $\pm$ 5.23 years; BMI was 17.3 ~ 28.2 kg/m^2, and mean BMI was 22.09 $\pm$ 2.48 kg/m^2. The control group had 12 males and 16 females, aged 46–65 years, with a mean age of 55.64 $\pm$ 4.86 years; BMI was 18.1–26.3 kg/m^2, and mean BMI was 22.24 $\pm$ 2.46 kg/m^2. No significant difference was discovered in baseline data between the three groups ($P > 0.05$).

The study was approved by the Ethics Committee of Affiliated Renhe Hospital of China, Three Gorges University. All participants were informed and their consents were obtained in accordance with the Declaration of Helsinki.[10] Also, written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Study protocol

All three groups actively accomplished health education on T2DM-related knowledge. The patients were asked to quit smoking and alcohol and adjust dietary patterns. Carbohydrate accounted for 50–65% of dietary energy, and protein accounted for 15–20%, with more than one-third were high-quality proteins. Salt intake was limited, and dietary fiber and trace elements were supplemented. Moderate-intensity exercises, such as brisk walking, Tai Chi, or badminton, were performed for 5–7 times a week for 30 min daily. Resistance exercises were performed twice or thrice a week with intervals of ≥48 h if allowed. On this basis, the probiotic group was prescribed metformin tablets, 0.25 g (Disha Pharmaceutical, Weihai, Shandong, China; State Medical Permit Number: H20103615) orally, 1 tablet three times a day (tds); and Bifidobacterium quadruple viable bacteria tablets, 0.5 g (Hangzhou Grand Biologic Pharmaceutical, INC, Bejing, China; State Medical Permit Number: S20060010) orally, 3 tablets tds. The metformin group was prescribed metformin tablet orally (1 tablet tds). In both groups, the intervention course was of 3 months. The control group consisted of patients who did not take any hypoglycemic drugs or had not received any hypoglycemic therapy.

Outcome measures

1. *Effect of blood glucose control*: In all groups, fasting plasma glucose (FPG) and 2-h postprandial plasma glucose (2-h PG) were measured by Biosen C-line glucometer (EKF, Germany) prior to and after the treatment. In addition, HLC-723G8 glycosylated hemoglobin A1c (HbA1c) analyzer (Tosoh Corporation, Japan) was used to measure fasting HbA1c prior to and after the treatment.
2. *Islet function*: Fasting peripheral venous blood (3 mL) was collected from the three groups prior to and after the treatment, centrifuged at 3000 revolutions/min for 10 min, and the supernatant was stored at -20°C in two parts for future use. Fasting insulin (FINS) levels were measured using a Cobas C501 automatic biochemical analyzer (Roche, Switzerland). Then, islet ß cell (HOMA-ß) function was calculated according to the following formula:

$$\text{Islet ß cell function index (HOMA-ß)} = 20 \times \text{FINS}/(\text{FPG}-3.5).$$

   A steady-state model was established to assess insulin-resistance islet ß cells (HOMA-IR).
3. *Gut microbiota*: Fresh feces were collected from the three groups prior to and after the treatment in a closed sterile container; 0.1-g fresh feces was diluted 100-fold with normal saline; then 10^{-2}–10^{-8} dilutions at different concentrations were created according to a 10-fold gradient, and 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilution samples of bifidobacteria and *lactobacilli* were inoculated in De Man–Rogosa–Sharpe (MRS) agar medium (Thermo Fisher, MA, USA) in an anaerobic environment at 37°C for 48 h; 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilution samples of Enterobacteriaceae and Enterococcus were cultured at 37°C for 24 h. Colony analysis and counting were performed using a MicroScan WAwayalk-40 automatic microorganism analyzer (SIMENS, Germany).
4. *Inflammatory factors*: Serum samples were collected from the three groups before and after treatment, and C-reactive protein (CRP), lipopolysaccharide (LPS), and interleukin 6 (IL-6) levels were measured by enzyme-linked immunosorbent serologic assay (ELISA). The kits were purchased from Shanghai Laifeng Biotechnology Co. Ltd (**Shanghai**, China). All operative steps were completed according to the given instructions.

Statistical analysis

Enumeration data were expressed as n (%). Chi-squared (x^2) test was performed for group comparison, and measurement data were expressed in the form of (x± s). One-way analysis of variance was performed for comparison of multiple groups, and independent sample *t*-test was performed for comparison between two groups; paired sample *t*-test was performed for comparison between the same group prior to and after the treatment, and the SPSS 23.0 software was used for data analysis, with $P < 0.05$ showing statistical significance.

Results

Comparison of blood glucose control effect between three groups

After 3 months of treatment, FPG, 2-h PG, and HbA1c levels were notably decreased in both probiotic and metformin groups ($P < 0.05$), and were lower than that in the control group prior to the treatment. Besides, FPG, 2-h PG, and HbA1c were lower in both probiotic and metformin groups than that in the control group, and FPG, 2-h PG, and HbA1c were further lower in the probiotic group than in the metformin group, and the differences were statistically significant ($P < 0.05$). The results are shown in Table 1.

Comparison of islet function between three groups

After 3 months of treatment, FINS, HOMA-ß, and HOMA-IR in the control group were not conspicuously different from the values prior to treatment ($P > 0.05$). FINS and HOMA-ß in both probiotic and metformin groups were evidently higher than the values prior to treatment ($P < 0.05$); however, HOMA-IR was significantly lower in both groups compared to the values before treatment ($P < 0.05$). FINS and HOMA-ß in both groups were significantly higher than that in the control group prior to treatment ($P < 0.05$); however, HOMA-IR was dramatically lower than that in the control group prior to treatment ($P < 0.05$), and the same in the probiotic group was significantly lower than that in the metformin group ($P < 0.05$). FINS and HOMA-ß values in the probiotic group were compared to that in the metformin

Table 1 Comparison of blood glucose control effect between three groups (x±s).

		FPG (mmol/L)		2 h PG (mmol/L)		HbA1c (%)	
Group	N	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Probiotic group	28	8.07±1.28	6.53±0.88$^{*\#\triangle}$	12.10±1.84	7.95±1.16$^{*\#\triangle}$	8.39±1.34	6.34±0.82$^{*\#\triangle}$
Metformin group	28	8.19±0.91	7.11±1.01$^{*\#}$	12.35±1.39	9.20±1.35$^{*\#}$	8.48±1.09	7.04±0.87$^{*\#}$
Control group	28	8.31±1.03	7.73±0.81*	12.41±1.47	10.36±1.33*	8.28±1.10	7.69±1.10*
F		0.343	12.344	0.304	24.717	0.201	14.508
P		0.711	<0.001	0.739	<0.001	0.818	<0.001

Note: Compared to the same group prior to treatment, $^{*}P < 0.05$; compared to the control group prior to treatment, $^{\#}P < 0.05$; compared to the metformin group, $^{\triangle}P < 0.05$.

group, with differences demonstrating non-statistical significance ($P > 0.05$). The results are displayed in Table 2.

Comparison of gut microbiota between three groups

After 3 months of treatment, no significant difference was observed in the number of *lactobacilli*, bifidobacteria, *enterobacteria*, and *enterococci* in the control group, compared to the numbers prior to treatment ($P > 0.05$). The number of *lactobacilli* and bifidobacteria in probiotic and metformin groups was substantially increased ($P < 0.05$), but the number of *enterobacteria* and *enterococci* was decreased ($P < 0.05$) in both probiotic and metformin groups. The number of *lactobacilli* and bifidobacteria was higher and the number of *enterobacteria* and *enterococci* was lower in both groups, compared to the control group prior to treatment. The probiotic group had higher numbers of *lactobacilli* and bifidobacteria and lower numbers of *enterobacteria* and *enterococci* than that in the metformin group. The differences were statistically significant ($P < 0.05$). The results are shown in Table 3.

Comparison of inflammatory factors between three groups

After 3 months of treatment, no significant differences were observed in serum LPS, IL-6, and CRP levels in the control group, compared to pretreatment values ($P > 0.05$); the serum LPS, IL-6, and CRP levels in both probiotic and metformin groups were evidently lower than that prior to treatment ($P < 0.05$). The serum LPS, IL-6, and CRP levels in both groups were lower than that in the control group prior to treatment. The serum LPS, IL-6, and CRP levels in the probiotic group were lower than that in the metformin group after treatment. The differences were statistically significant ($P < 0.05$). The results are shown in Table 4.

Table 2 Comparison of islet function between three groups(x±s).

		FINS (IU/L)		HOMA-β		HOMA-IR	
Group	N	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Probiotic group	28	4.45±0.60	6.32±0.80$^{*\#}$	21.39±3.02	33.26±3.85$^{*\#}$	8.35±1.31	6.11±1.02$^{*\#\triangle}$
Metformin group	28	4.38±0.85	6.04±0.60$^{*\#}$	20.52±3.11	31.90±2.99$^{*\#}$	8.61±1.25	7.07±1.50$^{*\#}$
Control group	28	4.27±0.74	4.45±0.52	21.67±2.73	21.46±2.85	7.79±1.69	7.49±1.07
F		0.424	67.260	1.151	109.806	2.404	9.477
P		0.656	<0.001	0.321	<0.001	0.097	<0.001

Note: Compared to the same group prior to treatment, $^{*}P < 0.05$; compared to the control group prior to treatment, $^{\#}P < 0.05$; compared to the metformin group, $^{\triangle}P < 0.05$.

Table 3 Comparison of gut microbiota between three groups (x±s, lg CFU/g).

		Lactobacillus		Bifidobacterium		Enterobacteria		Enterococcus	
Group	N	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Probiotic group	28	7.27±0.75	8.73±0.76$^{*\#\triangle}$	5.91±0.54	7.35±0.60$^{*\#\triangle}$	9.00±1.18	7.66±0.86$^{*\#\triangle}$	8.30±0.74	7.11±0.83$^{*\#\triangle}$
Metformin group	28	7.11±0.70	7.75±0.84$^{*\#}$	5.79±0.39	6.33±0.65$^{*\#}$	9.26±1.06	8.23±0.98$^{*\#}$	8.25±0.89	7.64±0.72$^{*\#}$
Control group	28	7.19±0.70	7.25±0.71	5.70±0.81	5.93±0.69	9.02±1.24	8.84±1.23	8.41±0.84	8.22±0.70
F		0.349	26.639	0.848	35.782	0.434	9.104	0.275	15.255
P		0.707	<0.001	0.432	<0.001	0.650	<0.001	0.760	<0.001

Note: Compared to the same group prior to treatment, $^{*}P < 0.05$; compared to the control group, $^{\#}P < 0.05$; compared to the metformin group, $^{\triangle}P < 0.05$.

Table 4 Comparison of inflammatory factors between three groups(x±s)

		LPS (ng/mL)		IL-6 (ng/mL)		CRP (mg/L)	
Group	N	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Probiotic group	28	4.35±0.74	2.17±0.58$^{*\#\triangle}$	3.19±0.71	1.85±0.49$^{*\#\triangle}$	1.51±0.39	0.58±0.22$^{*\#\triangle}$
Metformin group	28	4.35±0.90	3.59±0.59$^{*\#}$	3.18±0.86	2.46±0.59$^{*\#}$	1.38±0.61	0.88±0.29$^{*\#}$
Control group	28	4.18±1.11	4.03±1.18	2.98±0.85	3.00±0.92	1.46±0.62	1.38±0.59
F		0.312	38.218	0.393	19.383	0.398	28.548
P		0.733	<0.001	0.600	<0.001	0.673	<0.001

Note: Compared to the same group prior to treatment, $^{*}P < 0.05$; compared to the control group, $^{\#}P < 0.05$; and compared to the metformin group, $^{\triangle}P < 0.05$.

DISCUSSION

Gut microbiota is a microecosystem that comprises more than 500 bacteria involved in maintaining physiological functions, such as intestinal mucosal barrier, immune regulation, and intestinal metabolism.[11] Previous studies have shown that the structure of gut microbiota in T2DM patients is different from that in healthy individuals, and is mainly manifested as reduced beneficial flora. Increase in opportunistic pathogens triggers chronic intestinal inflammation, thus promoting the progression of IR and T2DM. Regulating gut microbiota is an important developmental direction of T2DM treatment.[12]

Metformin is the first-line drug for the treatment of newly diagnosed T2DM patients, but its hypoglycemic mechanism has not been fully clarified. In addition, to promote directly the absorption and metabolism of glucose in the liver and skeletal muscles, and improve insulin sensitivity, metformin participates in mediating bile acid cycle. Some studies have suggested that its mechanism is related to the regulation of gut microbiota.[13-15] In the current study, the effect of metformin was analyzed in newly diagnosed T2DM patients as samples. The results showed that FPG, 2-h PG, and HbA1c levels were evidently reduced after 3 months of treatment whereas FINS and HOMA-β were significantly increased, and HOMA-IR was significantly reduced. Besides, improvement of each index was superior to that of the control group, compared to the values of the same group prior to treatment, indicating that metformin has a good effect in treating newly diagnosed T2DM. Metformin improves HOMA-β cell function, promotes insulin secretion and improves its sensitivity, reduces IR levels, and produces good hypoglycemic effect; this was consistent with the results reported in the literature.[16] Additionally, the results of this study demonstrated that the number of intestinal *lactobacilli* and bifidobacteria, as well as the number of *enterobacteria* and *enterococci*, was notably reduced in the metformin group, with significantly reduced serum LPS, IL-6, and CRP levels. A significant difference was observed between the control group without any hypoglycemic therapy and the metformin group, which implied that metformin in the treatment of T2DM regulates the distribution of gut microbiota and lessens body's chronic inflammatory status, thus lowering IR and blood glucose.

Relevant domestic and foreign studies have already discussed the effect of metformin on gut microbiota. Zhang et al. confirmed through an animal experiment that metformin, acarbose, and sitagliptin showed different effects on gut microbiota composition, and the hypoglycemic effect could be improved by selectively increasing the abundance of beneficial bacteria.[17] Mueller et al. conducted a randomized controlled study with overweight/obese patients as samples, and discovered that metformin affected composition of gut microbiota and upgraded SCFA and acetate levels.[18] Imbalance in gut microbiota and increased pathogenic bacteria lead to the disruption of intestinal mucosal barrier function. Endotoxin enters blood circulation and causes chronic inflammation, giving rise to IR and islet dysfunction. Thus, improving gut microbiota has a positive effect on controlling blood glucose. The results of the current study showed that metformin treatment of newly diagnosed T2DM increased the abundance of probiotics Lactobacillus and Bifidobacterium. It is conducive to increase the synthesis of endogenous metabolites, such as branched-chain amino acids, bile acids, and SCFA, and regulate insulin function and glucose metabolism through pathways such as G protein-coupled receptors.[19-21] Therefore, it exerts a positive effect on the regulation of gut microbiota, and increases the number of probiotics as well as improves glycemic control.

The effect of probiotics combined with metformin in the treatment of T2DM is contentious. Seicaru et al. concluded that the addition of intestinal microbiota modulators, such as probiotics or prebiotics, to metformin was beneficial to enhancing its hypoglycemic effect and reducing adverse reactions.[22] Hata et al. utilized bifidobacterium G9-1 combined with metformin to treat T2DM, and the results showed that it effectively improved gastrointestinal manifestations but had no significant effect on blood glucose control.[23]

In the current study, the probiotic group was treated with Bifidobacterium quadruple viable bacteria integrated with metformin in newly diagnosed T2DM patients. The results demonstrated that FPG, 2-h PG, and HbA1c were evidently lower than that in the metformin group following 3 months of treatment, indicating that the addition of probiotics was beneficial to improving glycemic control, which could be used as an effective treatment of T2DM. Meanwhile, this study revealed no obvious difference in FINS and HOMA-β between both probiotic and metformin groups following 3 months of treatment. However, HOMA-IR was notably lower, suggesting that the mechanism by which probiotics improved hypoglycemic effect was mainly by reducing IR, and the relationship with pancreatic β-cell function and insulin secretion was to be confirmed. Previous studies demonstrated that systemic chronic inflammatory response was a critical cause of IR and abnormal glucose metabolism. Inflammatory factors, such as IL-6, block the insulin signaling pathway and lead to IR through a variety of pathways. The imbalance of gut microbiota structure and increase in pathogenic bacteria destroy intestinal mucosal barrier function, causing endotoxins to enter the blood, leading to a systemic chronic inflammatory response. Therefore, regulating the gut microbiota structure has a positive effect on lowering chronic inflammation and IR; therefore, probiotics could be added to the treatment of T2DM.

The results of the present study showed that the use of Bifidobacterium quadruple viable bacteria combined with metformin in treating newly diagnosed T2DM was beneficial to increase the number of *lactobacilli* and bifidobacteria in gut microbiota, thus reducing the number of *enterobacteria* and *enterococci* and promoting the recovery of flora structure. Besides, it reduces intestinal mucosal injury and the levels of inflammatory factors, such as LPS, IL-6, and CRP, in blood circulation, and also lowers the chronic inflammatory status and IR of the body, thereby improving the effect of blood glucose control.

Conclusion

Metformin treatment of T2DM is beneficial by reducing gut microbiota imbalance. The supplementation of probiotic further regulates gut microbiota, reduces chronic inflammation, and improves insulin function as well as blood glucose control.

Funding

No funding was received for this study.

Competing interests

The authors stated that there was no conflict of interest to disclose.

Data availability

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon request.

Author Contributions

Lu Li and Yong Liao designed and conducted the study; Lu Li, Yanli Chen, Zhipeng Tang, Yan You, and Yang Guo supervised data collection, analysis, and interpretation; Lu Li and Yong Liao prepared the manuscript for publication and reviewed its draft. All authors had read and approved the final manuscript.

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843. https://doi.org/10.1016/j.diabres.2019.107843
2. Daly A, Hovorka R. Technology in the management of type 2 diabetes: Present status and future prospects. Diabetes Obes Metab. 2021;23(8):1722-32. https://doi.org/10.1111/dom.14418
3. Ali MK, Pearson-Stuttard J, Selvin E, Gregg EW. Interpreting global trends in type 2 diabetes complications and mortality. Diabetologia. 2022;65(1):3-13. https://doi.org/10.1007/s00125-021-05585-2
4. Ferri C, Di Biase A, Bocchetti M, Zappavigna S, Wagner S, Le Vu P, et al. MiR-423-5p prevents MALAT1-mediated proliferation and metastasis in prostate cancer. J Exp Clin Cancer Res. 2022;41(1):1-16. https://doi.org/10.1186/s13046-021-02233-w
5. Salgaço MK, Oliveira LGS, Costa GN, Bianchi F, Sivieri K. Relationship between gut microbiota, probiotics, and type 2 diabetes mellitus. Appl Microbiol Biotechnol. 2019;103(23-24):9229-38. https://doi.org/10.1007/s00253-019-10156-y
6. Zheng Y, Gou X, Zhang L, Gao H, Wei Y, Yu X, et al. Interactions between gut microbiota, host, and herbal medicines: A review of new insights into the pathogenesis and treatment of type 2 diabetes. Front Cell Infect Microbiol. 2020;10:360. https://doi.org/10.3389/fcimb.2020.00360
7. Ardeshirlarijani E, Tabatabaei-Malazy O, Mohseni S, Qorbani M, Larijani B, Baradar Jalili R. Effect of probiotics supplementation on glucose and oxidative stress in type 2 diabetes mellitus: A meta-analysis of randomized trials. Daru J Fac Pharm Tehran Univ Med Sci. 2019;27(2):827-37. https://doi.org/10.1007/s40199-019-00302-2
8. Pryor R, Martinez-Martinez D, Quintaneiro L, Cabreiro F. The role of the microbiome in drug response. Ann Rev Pharmacol Toxicol. 2020;60:417-35. https://doi.org/10.1146/annurev-pharmtox-010919-023612
9. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Med J Br Diabetic Assoc. 1998;15(7):539-53. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S
10. Ashall V, Morton D, Clutton E. A declaration of Helsinki for animals. Vet Anaesth Analg 2023;50(4):309-14. https://doi.org/10.1016/j.vaa.2023.03.005
11. WY Ma. L-PM, Yi B, Zhang M, Feng S-X, Tian L-P. Antidiabetic activity of Callicarpa nudiflora extract in type 2 diabetic rats via activation of the AMPK-ACC pathway. Trop J Pharm Res. 2019;009(011):456-66. https://doi.org/10.4103/2221-1691.270978
12. Naowaboot J, Somparn N, Saenthaweesuk S. Renoprotective effect of umbelliferone in high-fat diet/streptozotocin-induced type 2 diabetic rats. Trop J Pharm Res. 2020;010(001):11-7. https://doi.org/10.4103/2221-1691.273089
13. Mueller NT, Differding MK, Zhang M, Maruthur NM, Juraschek SP, Miller III ER, et al. Metformin affects gut microbiome composition and function and circulating short-chain fatty acids: A randomized trial. Diabetes Care. 2021;44(7):1462-71. https://doi.org/10.2337/dc20-2257
14. Karusheva Y, Koessler T, Strassburger K, Markgraf D, Mastrototaro L, Jelenik T, et al. Short-term dietary reduction of branched-chain amino acids reduces meal-induced insulin secretion and modifies microbiome composition in type 2 diabetes: A randomized controlled crossover trial. Am J Clin Nutr. 2019;110(5):1098-1107. https://doi.org/10.1093/ajcn/nqz191
15. de Siqueira Cardinelli C, Torrinhas RS, Sala P, Pudenzi MA, Angolini CFF, da Silva MM, et al. Fecal bile acid profile after Roux-en-Y gastric bypass and its association with the remission of type 2 diabetes in obese women: A preliminary study. Clin Nutr. 2019;38(6):2906-12. https://doi.org/10.1016/j.clnu.2018.12.028
16. Zhao L, Lou H, Peng Y, Chen S, Fan L, Li X. Elevated levels of circulating short-chain fatty acids and bile acids in type 2 diabetes are linked to gut barrier disruption and disordered gut microbiota. Diabetes Res Clin Prac. 2020;169:108418. https://doi.org/10.1016/j.diabres.2020.108418
17. Zhang M, Feng R, Yang M, Qian C, Wang Z, Liu W, et al. Effects of metformin, acarbose, and sitagliptin monotherapy on gut microbiota in Zucker diabetic fatty rats. BMJ Open Diabetes Res Care. 2019;7(1):e000717. https://doi.org/10.1136/bmjdrc-2019-000717
18. Mueller NT, Differding MK, Zhang M, Maruthur NM, Juraschek SP, Miller ER, 3rd, et al. Metformin affects gut microbiome composition and function and circulating short-chain fatty acids: A randomized trial. Diabetes Care. 2021;44(7):1462-71. https://doi.org/10.2337/dc20-2257
19. Karusheva Y, Koessler T, Strassburger K, Markgraf D, Mastrototaro L, Jelenik T, et al. Short-term dietary reduction of branched-chain amino acids reduces meal-induced insulin secretion and modifies microbiome composition in type 2

diabetes: A randomized controlled crossover trial. Am J Clin Nutr. 2019;110(5):1098–1107. https://doi.org/10.1093/ajcn/nqz191

20. de Siqueira Cardinelli C, Torrinhas RS, Sala P, Pudenzi MA, Fernando FAC, Marques da Silva M, et al. Fecal bile acid profile after Roux-en-Y gastric bypass and its association with the remission of type 2 diabetes in obese women: A preliminary study. Clin Nutr (Edinburgh, Scotland). 2019;38(6):2906–12. https://doi.org/10.1016/j.clnu.2018.12.028
21. Zhao L, Lou H, Peng Y, Chen S, Fan L, Li X. Elevated levels of circulating short-chain fatty acids and bile acids in type 2 diabetes are linked to gut barrier disruption and disordered gut microbiota. Diabetes Res Clin Prac. 2020;169:108418. https://doi.org/10.1016/j.diabres.2020.108418
22. Seicaru EM, Popa Ilie IR, Cătinean A, Crăciun AM, Ghervan C. Enhancing metformin effects by adding gut microbiota modulators to ameliorate the metabolic status of obese, insulin-resistant hosts. J Gastrointest Liver Dis (JGLD). 2022;31(3):344–54. https://doi.org/10.15403/jgld-4248
23. Hata S, Nakajima H, Hashimoto Y, Miyoshi T, Hosomi Y, Okamura T, et al. Effects of probiotic Bifidobacterium bifidum G9-1 on the gastrointestinal symptoms of patients with type 2 diabetes mellitus treated with metformin: An open-label, single-arm, exploratory research trial. J Diabetes Invest. 2022;13(3):489–500. https://doi.org/10.1111/jdi.13698