Effects of Non-insulin Anti-hyperglycemic Agents on Gut Microbiota: A Systematic Review on Human and Animal Studies

Thao T. B. Cao^1,2, Kun-Chang Wu^1, Jye-Lin Hsu^3, Chih-Shiang Chang^1, Chiahung Chou^4,5, Chen-Yuan Lin^1,6, Yu-Min Liao^6, Pei-Chun Lin^1, Liang-Yo Yang^7,8,9 and Hsiang-Wen Lin^1,10,11*

^1 School of Pharmacy and Graduate Institute, China Medical University, Taichung City, Taiwan, ^2 Department of Clinical Pharmacy, Hanoi University of Pharmacy, Hanoi, Vietnam, ^3 Graduate Institute of Biomedical Sciences, China Medical University, Taichung City, Taiwan, ^4 Department of Health Outcomes Research and Policy, Harrison School of Pharmacy, Auburn University, Auburn, AL, United States, ^5 Department of Medical Research, China Medical University Hospital, Taichung City, Taiwan, ^6 Division of Hematology and Oncology, China Medical University Hospital, Taichung City, Taiwan, ^7 Department of Physiology, School of Medicine, College of Medicine, China Medical University, Taichung City, Taiwan, ^8 Laboratory for Neural Repair, China Medical University Hospital, Taichung City, Taiwan, ^9 Biomedical Technology Research and Development Center, China Medical University Hospital, Taichung City, Taiwan, ^10 Department of Pharmacy, China Medical University Hospital, Taichung City, Taiwan, ^11 Department of Pharmacy System, Outcomes and Policy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, United States

Background: As growing evidence links gut microbiota with the therapeutic efficacy and side effects of anti-hyperglycemic drugs, this article aims to provide a systematic review of the reciprocal interactions between anti-hyperglycemic drugs and gut microbiota taxa, which underlie the effect of the gut microbiome on diabetic control via bug-host interactions.

Method: We followed the PRISMA requirements to perform a systematic review on human vs. animal gut microbiota data in PubMed, SCOPUS, and EMBASE databases, and used Cochrane, ROBIN-I, and SYRCLE tools to assess potential bias risks. The outcomes of assessment were trends on gut microbiota taxa, diversity, and associations with metabolic control (e.g., glucose, lipid) following anti-hyperglycemic treatment.

Results: Of 2,804 citations, 64 studies (17/humans; 47/mice) were included. In human studies, seven were randomized trials using metformin or acarbose in obese, pre-diabetes, and type 2 diabetes (T2D) patients. Treatment of pre-diabetes and newly diagnosed T2D patients with metformin or acarbose was associated with decreases in genus of Bacteroides, accompanied by increases in both Bifidobacterium and Lactobacillus. Additionally, T2D patients receiving metformin showed increases in various taxa of the order Enterobacteriales and the species Akkermansia muciniphila. Of seven studies with significant differences in beta-diversity, the incremental specific taxa were associated with the improvement of glucose and lipid profiles. In mice, the effects of metformin on A. muciniphila were similar, but an inverse association with Bacteroides was reported. Animal studies on other anti-hyperglycemic drugs, however, showed substantial variations in results.
CONCLUSIONS: The changes in specific taxa and β-diversity of gut microbiota were associated with metformin and acarbose in humans while pertinent information for other anti-hyperglycemic drugs could only be obtained in rodent studies. Further human studies on anti-hyperglycemic drugs other than metformin and acarbose are needed to explore gut microbiota’s role in their therapeutic efficacies and side effects.

KEYWORDS: anti-hyperglycemic drugs, microbiome, microbiota, association, systematic review

INTRODUCTION

Gut microbiota plays a pivotal role in the pathogenesis of diabetes as significant alterations were found in the gut microbiome composition in type 2 diabetes (T2D) patients relative to healthy individuals (1). A metagenome-wide association study reported a moderate degree of dysbiosis associated with depletion in butyrate-producing bacteria, accompanied by increases in opportunistic pathogens among diabetic patients (2). These changes were echoed by a recent systematic review, which shows an inverse association of T2D with the genera Bifidobacterium, Akkermansia and butyrate-producing bacteria (e.g., Roseburia, Faecalibacterium), in conjunction with a positive association with Ruminococcus, Fusobacterium, and Blautia (1).

From a clinical perspective, these findings provide a rationale for targeting gut microbiota imbalance as a potential strategy for T2D treatment by restoring a healthy gut microbiome, including fecal microbiota transplant and probiotic supplements (3, 4). However, the efficiency and effectiveness of these treatments remain uncertain due to concerns over the invasive nature of fecal microbiota transplant and the dosage, species, and duration required for an effective probiotic treatment. Emerging evidence indicates that the therapeutic efficacy of anti-hyperglycemic drugs might, in part, be attributable to their ability to modulate the compositions of gut microbiota (1, 3, 5–9). This compositional change might lead to enrichments in bacterial species exhibiting beneficial effects to intestinal health via the production of health-promoting metabolites, such as short-chain fatty acids (SCFAs) and bile acids (8). Nevertheless, certain anti-hyperglycemic drugs were reported to cause increases in the abundance of Escherichia and Candidatus Arthromitus, which contribute to gastrointestinal side effects and weight gain, respectively (9–11).

Among various anti-hyperglycemic drugs in clinical use, metformin, acarbose, sitagliptin, and vildagliptin (5, 6) have been investigated for their reciprocal interplay with gut microbiota by assessing their effects on human and animal gut microbiota, and vice versa (1, 8, 11). From a translational perspective, animal models might help to explore the causality of complex host-microbiota interactions and possible mechanisms of action in a controlled experimental setting. However, it should be noted that differences in dietary habits, host metabolism, inflammatory states, and body anatomy contribute to great variations in gut microbiota compositions between humans and animals, and subsequently, the respective drug effects in disease control (12). A meta-analysis of published 16S rDNA sequencing data from mouse and human fecal microbiota showed that there were significant increases in Lactobacillus and Turicibacter genera in mouse gut microbiota while the genera of Streptococcus, Ruminococcus, Lachnospira, Faecalibacterium, Dialister, and Oscillospira were elevated in human gut microbiota (12). Moreover, age, mouse strains/populations, microbiota pools in laboratories, and other practical factors might have varied to a great extent among different studies (12). Previous reviews have suggested the effects of anti-hyperglycemic drugs on gut microbiota (8, 11, 13), however, the differences in results between human and animal studies have not been differentiated.

Reciprocal interplays between individual anti-hyperglycemic drugs and gut microbiota remain unexplored with respect to the contribution of specific bacterial taxa to drug’s therapeutic efficacy in disease control (i.e., the clinical question). Thus, we conducted this systematic review aiming to shed light on the associations among anti-hyperglycemic agents, changes in specific taxonomic groups of gut microbiota, and host glucose control or metabolic profiles mainly in humans, as compared to those reported in animal studies.

METHODS

Literature Search

Our literature search strategies were designed to integrate the following PICO(S) (population, intervention, comparisons, outcomes, study design) based on the prior clinical question: Population: humans (e.g., healthy people or patients who were either obese, prediabetes, diabetes) or the corresponding animal models; Intervention: non-insulin anti-hyperglycemic drugs; Comparisons: post- vs. pre-intervention, with-vs. without-treatment, or on-vs. off-treatment; Outcomes: alteration of the gut microbial composition; Study design: clinical trials, observational studies or animal experiments, as that recommended for systematic reviews (14). We systematically searched PubMed, EMBASE, and SCOPUS databases from January 1, 2000, to November 13, 2019. The keywords and searching strategies based upon the PICO(S) were “anti-hyperglycemic drugs” and “gut microbiota” related terms (Supplementary Table 1). In addition, we searched manually the reference list of the review papers for additional publications of interest.

Study Selection Criteria

We followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (15). The inclusion criteria for the published studies included: (i) any human studies or animal experiments reporting original data of gut microbiota...
after receiving anti-hyperglycemic drugs; (ii) gut microbiota data were analyzed from feces or colonic content specimens; (iii) must be written in English or Chinese. Studies were excluded if they did not provide data of individual bacterial taxa or were only available as conference abstracts or proceedings.

Selection of Studies
Initially, the abstracts and titles of potential articles were screened, followed by the evaluation of the full-text articles for eligibility. Two authors were responsible for screening and evaluating these papers independently. Disagreements were resolved by consensus between these two authors and, if necessary, discussed by additional two authors.

Data Extraction
A standardized form in a Microsoft Excel file (e.g., characteristics of studies, participants, treatments and comparisons, methods to analyze the microbiome, and measures of outcomes) was used for data extraction. Data were extracted by one author and reviewed by a second one. All disagreements were resolved by consensus and a third or fourth author when necessary.

Quality Assessment
We used the Cochrane risk-of-bias tool to assess the risk of bias in selected randomized trials (16). For quasi-experimental and observational studies, we used the Risk of Bias in Non-randomized studies of Interventions (ROBIN-I) to assess the risk of bias (17). Further, the SYRCLE's risk of bias tool for animal studies (18) was used to assess the risk of bias. The risks of bias data were extracted by four different authors and all disagreements were resolved by consensus made by the remaining authors.

Outcomes of Assessment
Other than describing the characteristics of the evaluated human or animal studies, the primary outcome was the difference in relative abundance or change patterns of individual intestinal bacterial taxa, categorized based on six common taxonomic categories [Phylum (P), Class (C), Order (O), Family (F), Genus (G), Species (S)], in associations with the use of anti-hyperglycemic agents, among those available human or animal studies, respectively. Secondary outcomes were differences in microbial diversity, changes in intestinal or serum levels of SCFAs and/or bile acids in human or animal hosts, respectively, after taking/using individual drugs, associations between specific taxa and host metabolic parameters, e.g., glucose, body weight, and lipid profile.

Data Synthesis
We classified the primary and secondary outcomes into the following categories: significant increase, significant decrease, and no significant difference between comparison groups. Changes of each taxon were synthesized from at least 2 studies for human or animal studies, respectively. Specifically, the effects of different anti-hyperglycemic drugs on specific taxon among the evaluated human or animal studies were compared. Further, the corresponding effects of each individual drug on specific taxon were compared to explore its consistency, in terms of having the same trend of alteration on the specific taxon caused by the same specific anti-hyperglycemic drug, or not. These findings also were categorized by the target research populations (e.g., obese, pre-diabetic, newly T2D, prevalent T2D), individual treated drugs or different animal models (mice or rat models with various diets or genetic knockout). For gut microbial diversity, each study might use one or more measures to assess α- (richness and evenness) or β-diversity. We considered α-diversity as “Increase” if at least one measure showed an increase and no measure showed a decrease; “Decrease” if at least one measure showed a decrease and no measure showed an increase; “No difference” if all measures showed no difference. β-diversity was assessed as “Difference” if at least one measure showed a difference; “No difference” if all measures showed no difference. In terms of associations between specific taxa and host metabolic parameters, we collected data from specific taxa that increased or decreased significantly in participants receiving non-insulin anti-hyperglycemic drugs. Only data with statistical significance were extracted for analyses.

RESULTS
The following presented results were mainly focused on human studies, which are compared to those of animal studies.

Reviewed Studies
Overall, 2,804 citations were retrieved, and the final analysis included 17 human studies (7, 19–34) and 47 mouse studies (5, 6, 35–79) from 64 papers (Figure 1). The majority of studies were published in and after 2017 and the duration of anti-hyperglycemic treatments varied from studies to studies (i.e., from few days to several months). Of 17 human studies, seven (41.2%) were randomized control trials (7, 19–21, 32–34). Thirteen studies (76.4%) enrolled either newly diagnosed or prevalent T2D patients (20, 21, 23, 24, 26–34), whereas the remaining four studies enlisted healthy participants (22, 25), obese individuals (19), and pre-diabetic patients (7) (Tables 1, 2).

Of 47 rodent studies, 30 (63.8%) studies were conducted in mice (35–41, 44–46, 48, 52, 53, 55, 56, 59–65, 68, 70, 74–79) and the others were in rats (5, 6, 42, 43, 47, 49–51, 54, 57, 58, 66, 67, 69, 71–73). Their characteristics, housing, acclimatization, and diet treatments were presented in Table 3 and Supplementary Table 2. Overall, 14 anti-hyperglycemic agents were used in these included studies, with metformin accounting for the most. There was only one human study focused on glipizide, while the other ten listed drugs (e.g., voglibose, miglitol, vildagliptin, sitagliptin, saxagliptin) were used only in rodent studies (Table 1).

Microbiome Assessment Method
Fecal specimens of all human studies were analyzed for the composition of gut microbiota. Of 47 mouse studies, 34 studies (72.3%) used fecal samples (5, 6, 35, 36, 38, 41, 42, 44–46, 49–56, 59–61, 63–73, 76, 78). 16S rRNA gene sequencing was the most common method used in human and animal studies (Table 1).
The Risk of Bias
Among human studies, three randomized studies were at the high risk of bias in performance, detection, and attrition, while four studies were unclear risks in most domains (Supplementary Figure 1). Among quasi-experimental studies, three studies were at low risk of bias in all domains, whereas two studies were at serious risk in confounding, selection of participants, and classification of interventions (Supplementary Figure 2). All 5 cross-sectional studies were at serious risk in several domains, e.g., confounding, selection of participants, classification of intervention (Supplementary Figure 3). Almost all mouse studies were unclear risk across domains, even if some were at low risk of bias in selective outcome reporting (Supplementary Figure 4).

Outcomes of Assessment
Bacterial Taxa
Importantly, the synthesized results from animal studies reported all common six taxonomic categories (P, C, O, F, G, S) of bacterial taxa but there were only three common taxonomic categories (F, G, S) of gut microbiota taxa were reported in those from human studies based on the available data (Tables 4–7). Glipizide and
### TABLE 1 | General characteristics of all included studies.

| Characteristics                              | Human studies (N = 17) | Animal studies (N = 47) |
|----------------------------------------------|------------------------|-------------------------|
|                                              | n          | %      | n          | %      |
| **Study design**                             |            |        |            |        |
| Randomized trials                            | 7          | 41.2   | –          | –      |
| Quasi-experimental studies                   | 5          | 29.4   | –          | –      |
| Cross-sectional studies                      | 5          | 29.4   | –          | –      |
| Animal experiments                           | –          | –      | 47         | 100    |
| **Treatment**                                |            |        |            |        |
| Biguanide                                     |            |        |            |        |
| Metformin                                     | 14         | 82.4   | 24         | 51.1   |
| α-glucosidase inhibitors                     |            |        |            |        |
| Acarbose                                      | 3          | 17.6   | 6          | 12.8   |
| Voglibose                                     | –          | –      | 2          | 4.3    |
| Miglitol                                      | –          | –      | 1          | 2.1    |
| GLP-1 receptor agonists                       |            |        |            |        |
| Liraglutide                                   | 1          | 5.9    | 7          | 14.9   |
| DPP-4 inhibitors                              |            |        |            |        |
| Sitagliptin                                   | –          | –      | 4          | 8.5    |
| Vildagliptin                                  | –          | –      | 2          | 4.3    |
| Saxagliptin                                   | –          | –      | 1          | 2.1    |
| Anagliptin                                    | –          | –      | 1          | 2.1    |
| SGLT-2 inhibitors                             |            |        |            |        |
| Dapagliflozin                                 | –          | –      | 2          | 4.3    |
| Canagliflozin                                 | –          | –      | 1          | 2.1    |
| Thiazolidindiones                             |            |        |            |        |
| Pioglitazone                                  | –          | –      | 1          | 2.1    |
| Rosiglitazone                                 | –          | –      | 1          | 2.1    |
| Sulfonylure                                   |            |        |            |        |
| Glipizide                                     | 1          | 5.9    | –          | –      |
| **Published year**                            |            |        |            |        |
| 2019                                          | 3          | 17.6   | 14         | 29.8   |
| 2018                                          | 7          | 41.2   | 16         | 34.0   |
| 2017                                          | 4          | 23.5   | 6          | 12.8   |
| 2016                                          | –          | –      | 5          | 10.6   |
| 2011–2015                                     | 3          | 17.6   | 6          | 12.8   |
| **Geography**                                 |            |        |            |        |
| Asia                                          | 7          | 41.2   | 33         | 70.2   |
| Europe                                        | 7          | 41.2   | 5          | 10.6   |
| North America                                 | 2          | 11.7   | 8          | 17.0   |
| South America                                 | 1          | 5.9    | 1          | 2.1    |
| **Participants**                              |            |        |            |        |
| Human                                         |            |        |            |        |
| Newly T2D                                      | 4          | 23.5   | –          | –      |
| Prevalent T2D                                 | 9          | 52.9   | –          | –      |
| Healthy                                       | 2          | 11.8   | –          | –      |
| Obese                                         | 1          | 5.9    | –          | –      |
| Pre-diabetic                                  | 1          | 5.9    | –          | –      |
| Mice/rats                                     |            |        |            |        |
| Diet or STZ or both                           | –          | –      | 29         | 61.7   |
| Gene knockout                                 | –          | –      | 12         | 25.5   |
| Diet and gene knockout                        | –          | –      | 2          | 4.3    |
| Wild type with normal diet                    | –          | –      | 6          | 12.7   |
| Other (adenine)                               | –          | –      | 1          | 2.1    |
| **Specimens**                                 |            |        |            |        |
| Feces                                         | 17         | 100    | 34         | 72.3   |
| Intestinal, colon, cecal contents             | –          | –      | 11         | 23.4   |
| Feces and intestinal, colon contents          | –          | –      | 2          | 4.3    |
| **Assessment methods**                        |            |        |            |        |
| T-RFLP                                        | 1          | 5.9    | –          | –      |
| RT-qPCR                                       | 1          | 5.9    | 6          | 12.8   |
| Metagenomic sequencing                        | 5          | 29.4   | –          | –      |

(Continued)
Bifidobacterium treated with metformin (pre-diabetic (22)), while Wang et al. (34) found the association between liraglutide treatment and the increased abundance of genus Akkermansia in T2D patients. The assessments of the effects of metformin and acarbose on the human gut microbiota composition represented the foci of 14 studies (19–31, 34) and three studies (7, 32, 33), respectively (Tables 4, 5).

Of the phylum Bacteroidetes, the genus Bacteroides decreased in two studies treated with metformin among newly diagnosed T2D patients (20, 24), and in two studies treated with acarbose among pre-diabetic and newly diagnosed T2D patients (7, 32). Additionally, one study treated with metformin (24) and one study treated with acarbose (32) in newly diagnosed T2D patients reported similar results of decreases in seven species (e.g., Bacteroides dorei, Bacteroides finegoldii). For the phylum Firmicutes, the genus Lactobacillus increased in two studies in pre-diabetic (7) and newly diagnosed T2D patients (32) receiving acarbose, and the species L. gasseri increased in one study treated with metformin (21), and with acarbose (32), respectively, among newly diagnosed T2D patients. Meanwhile, the genus Clostridium decreased in one study among healthy participants receiving metformin (22) which was also reported in newly diagnosed T2D patients treated with acarbose (32) (Table 4). Two species, i.e., C. bartlettii and C. botulinum, consistently decreased among T2D patients receiving metformin in two separate studies (21, 31). Three out of four studies showed a decrease in the genus Intestinibacter (21, 22, 30) among healthy participants and T2D patients treated with metformin (Table 5). With respect to the phylum Actinobacteria, the genus Bifidobacterium with the species B. adolescentis increased in one study with metformin (21) and another study with acarbose (32) among newly diagnosed T2D patients (Table 4), and B. longum consistently increased among T2D patients treated with acarbose in two studies (32, 33) (Table 5).

Concerning other phyla, two studies evaluated the genus Fusobacterium (phylum Fusobacteria) (20, 27) and the species Akkermansia muciniphila (phylum Verrucomicrobia) (21, 29) among T2D patients treated with metformin. Both showed increases in the abundance of these two taxa. In the phylum Proteobacteria, conflicting results were reported in two studies with respect to the family Enterobacteriaceae in healthy participants vs. T2D patients treated with metformin (25, 27). Overall, metformin might increase different taxa from the family Enterobacteriaceae and other families in the order of Enterobacteriales. Of six studies that evaluated the genus Escherichia (19–22, 25, 30) and four studies evaluating the genus Shigella (19, 20, 22, 25), the treatment of healthy participants, obese individuals, and T2D patients with metformin all led to increased abundance of these two genera. Another two studies on metformin in newly diagnosed and prevalent T2D patients (21, 31) reported consistently increases in eight species of the order Enterobacteriales, including Citrobacter koseri, Escherichia coli, Klebsiella pneumonia (family Enterobacteriaceae), Erwinia amylovora (family Erwiniaeae), Pectobacterium wasabiae, and Dickeya dadantii (family Pectobacteriaceae) (Table 5).

The effects of 13 anti-hyperglycemic drugs on the compositions of gut microbiota were conducted in different rodent models. The results of pioglitazone was inconclusive (78), while the other 12 drugs were presented in Tables 6, 7. The Firmicutes/Bacteroidetes ratios were decreased in two studies treated with metformin in high fat diet (HDF)-fed mice (37, 48).
| References, country | Participants | $N$ | Treatment and daily dose | Duration | Specimen | Analysis method | Comparison | Outcomes |
|---------------------|--------------|-----|--------------------------|----------|----------|----------------|------------|----------|
| **Randomized trials ($n = 7$)** | | | | | | | | | |
| Ejtahed et al. (19), Iran | Obese | 20/16 | M (1,000 mg) vs. placebo | 2 m | Feces | 16S rRNA | Post vs. pre | ✓ |
| Tong et al. (20), China | Newly T2D | 100/100 | Chinese medicine vs. M (750 mg) | 12 w | Feces | 16S rRNA V3-V4 region | Post vs. pre | ✓ |
| Wu et al. (21), Europe | Newly T2D | 22/18 | M (425–1,700 mg) vs. placebo | 4 m | Feces | DNA shotgun metagenomics | Post vs. pre | ✓ |
| Zhang et al. (7), China | Pre-diabetes | 40/40 | A (50–150 mg) vs. placebo | 4 w | Feces | 16S rRNA V3–V5 region | Post vs. without M | ✓ |
| Gu et al. (32), China | Newly T2D | 51/43 | A (75–450 mg) vs. G (5–15 mg) | 3 m | Feces | DNA metagenomics | Post vs. pre | ✓ |
| Su et al. (33), China | Prevalent T2D | 59/36 | A (150 mg vs. non-A) | 4 w | Feces | 16S rDNA RT-qPCR | Post vs. pre | ✓ |
| Wang et al. (34), USA | Prevalent T2D | 19/18 | L vs. M (as usual) | 18 w | Feces | 16S rRNA V4 region | L vs. M | ✓ |
| **Quasi-experimental studies ($n = 5$)** | | | | | | | | | |
| Bryrup et al. (22), Denmark | Healthy | 25 | M (500–2,000 mg) | 6 w | Feces | 16S rRNA V4 region | Post vs. pre | ✓ |
| Huang et al. (23), Sweden | Prevalent T2D | 23/7 | M (as usual) vs. non-M | 28 w | Feces | 16S rRNA gene T-RFLP | With vs. without M | ✓ |
| Sun et al. (24), China | Newly T2D | 22 | M (2,000 mg) | 3 d | Feces | DNA metagenomics | Post vs. pre | ✓ |
| Elbere et al. (25), Latvia | Healthy | 18 | M (1,700 mg) | 7 d | Feces | 16S rRNA V3 region | Post vs. pre | ✓ |
| Napolitano et al. (26), UK | Prevalent T2D | 14/14 | on-M (as usual) vs. off-M | NA | Feces | 16S rRNA V1, V2, V3 regions | On vs. off M | ✓ |
| **Cross-sectional studies ($n = 5$)** | | | | | | | | | |
| Zhang et al. (27), China | Prevalent T2D | 51/26 | M (as usual) vs. non-treatment | – | Feces | 16S rRNA V1–V2 region | With vs. without M | ✓ |
| Barengolts et al. (28), USA | Prevalent T2D | 25/16 | M (as usual) vs. non-M | – | Feces | 16S rRNA V3–V4 region | With vs. without M | ✓ |
| De La Cuesta-Zuluaga et al. (29), Columbia | Prevalent T2D | 14/14 | M (as usual) vs. non-M | – | Feces | 16S rRNA V4 region | With vs. without M | ✓ |
| Forslund et al. (30), Denmark | Prevalent T2D | 58/17 | M (as usual) vs. non-M | – | Feces | 16S rDNA shotgun metagenomics | With vs. without M | ✓ |
| Karlsson et al. (31), Sweden | Prevalent T2D | 20/33 | M (as usual) vs. non-M | – | Feces | DNA metagenomics | With vs. without M | ✓ |

A, acarbose; G, glibizide; M, metformin; L, liraglutide; T-RFLP, terminal restriction fragment length polymorphism; RT-qPCR, Real-time quantitative polymerase chain reaction; m, months; w, weeks; d, days; –, no information; NA, not available; vs., versus.
| References, country | Animals | Models | N | Treatment and daily dose | Duration | Specimens | Analysis methods | Comparison | Outcomes |
|---------------------|---------|--------|---|--------------------------|----------|-----------|-----------------|------------|----------|
| Ryan et al. (37), Ireland | Male C57BL/6 | HFD | 14/14 | M (300 mg/kg) vs. non-treatment | 12 w | Ceca | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Ji et al. (38), China | Male C57BL/6 | HFD | 5/5 | M (300 mg/kg) vs. non-treatment | 3 w | Feces | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Adeshirlanjey et al. (41), USA | Male C57BL/6 | HFD | 10/NA | M (300 mg/kg) vs. vehicle | 10 w | Feces | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Liao et al. (60), China | Male C57BL/6 | HFD | NA | A (400 mg/kg) vs. SI (4 g/kg) vs. Sa (300 mg/kg) vs. L (200 µg/kg) vs. normal saline | 4 w | Feces | 16S rDNA V3–V4 region | With vs. without (SI) | ✓ ✓ ✓ |
| Madsen et al. (65), Denmark | Male C57BL/6 | HFD | 15/15 | L (0.4 mg/kg) vs. vehicle | 28 d | Feces | 16S rDNA V3–V4 region and metagenomics | With vs. without (A, Sa, L) | ✓ ✓ ✓ |
| Wang et al. (45), Korea | Male C57BL/6 | HFD | 16/8 | M (100 mg/kg) vs. non-treatment | 10 w | Feces | 16S rRNA gene RT-qPCR | With vs. without | ✓ ✓ |
| Lee et al. (48), Korea | Male C57BL/6 | HFD | 6/6 | M (250 mg/kg) vs. non-treatment | 16 w | Ceca | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Zhou et al. (53), China | Male C57BL/6 | HFD | NA | M (100 mg/kg) vs. non-treatment | 4 w | Feces | 16S rDNA gene RT-qPCR | With vs. without | ✓ |
| Do et al. (63), Korea | Male C57BL/6 | HFD | 9/10 | Vo (1 mg/kg) vs. non-treatment | 12 w | Feces | 16S rRNA V1–V3 region | With vs. without | ✓ ✓ ✓ |
| Lee and Ko (55), Korea | Female C57BL/6 | ND, HFD | NA | M (300 mg/kg) vs. non-treatment | 10 w | Feces | 16S rRNA V1–V3 region | With vs. without | ✓ ✓ ✓ |
| Shin et al. (56), Korea | Male C57BL/6 | ND, HFD | 12/12 | M (300 mg/kg) vs. non-treatment | 6 w | Feces | 16S rRNA gene RT-qPCR | With vs. without | ✓ ✓ |
| Dong et al. (39), USA | KC | Gene knockout with HFCD | 8/8 | M (5 mg/ml in drinking water) vs. non-treatment | 2 m | Duodena, ilea, ceca | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Brandt et al. (40), Germany | Female C57BL/6 | FFCD | 6–6/6–8 | M (300 mg/kg) vs. non-treatment | 4 d | Proximal small intestine | 16S rRNA V1–V2 region | With vs. without | ✓ ✓ ✓ |
| Baxter et al. (61), USA | Male C57BL/6 | HSD, PPD | 25/5 | A (25, 400 mg/kg) vs. non-treatment | 2 w | Feces | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Kishida et al. (64), Japan | Male C57BL/6 | HF/SD | 10/10 | M 0.04% in diet vs. non-treatment | 12 w | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Olivares et al. (74), Belgium | Male C57BL/6 | WD | 9/9 | Vi (0.6 mg/mL in drinking water) vs. non-treatment | 8 w | Ceca | 16S rRNA V5–V6 region | With vs. without | ✓ ✓ ✓ |
| Zheng et al. (44), China | Male C57BL/6 | HFD/STZ | 48/8 | M (75, 200 mg/kg) vs. normal saline | 5 w | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ |

(Continued)
# TABLE 3 | Continued

| References, country | Animals | Models | N | Treatment and daily dose | Duration | Specimens | Analysis methods | Comparison | Outcomes |
|---------------------|---------|--------|---|--------------------------|----------|-----------|-----------------|------------|----------|
| Wang et al. (73), China | Male ApoE−/− | HFD ± STZ | 20/20/20 | L (0.4 mg/kg) vs. Sa (10 mg/kg) vs. non-treatment | 8 w | Feces | 16S rRNA V1–V3 region | With vs. without (L, Sa) | ✓ ✓ ✓ |
| Xue et al. (36), China | Female C57BL/6J | DHEA+HFD | 10/10 | M (1.9 g/kg) vs. normal saline | 21 d | Feces | 16S rDNA V3–V4 region | With vs. without | ✓ |
| Moreira et al. (68), Brazil | Male C57BL/6J and female ob/ob | ND, HFD, gene knockout | 48–48/48–48 | L (400 µg/kg) vs. normal saline | 15 d | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ |
| Ma et al. (48), China | Male C57BL/6 | ND | 10/9 | M (300 mg/kg) vs. normal saline | 30 d | Feces | 16S rRNA | With vs. without | ✓ ✓ |
| Xu et al. (62), China | Male ICR | ND | 5/5 | A (4 mg/kg) vs. V0 (0.008 mg/kg) vs. non-treatment | 2 w | Intestine | 16S rRNA V4 region | With vs. without | ✓ ✓ |
| Zhang et al. (35), China | BKSLeprdb (db/db) | Gene knockout | 5/5 | M (113.75 mg/kg) vs. non-treatment | 11 w | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Lee et al. (76), USA | C57BLKS/J-Leprd (db/db) | Gene knockout | 12/12 | D (60 mg/kg in diet) vs. non-treatment | 8 w | Feces | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Li et al. (75), China | Male ICR MafA-deficient | Gene knockout | 8/8 | D (1.0 mg/kg) vs. normal saline | 6 w | Intestine and feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Li et al. (78), China | Female KKAy | Gene knockout | 6/6 | P vs. distilled water | NA | Feces | 16S rDNA DGGE Cultivation | With vs. without | ✓ ✓ |
| Wang et al. (79), China | Male KKAy | Gene knockout | 7/6 | R (2 mg/kg) vs. distilled water | 8 w | Intestine | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ |
| Smith et al. (69), USA | Offsprings of female CByB6 MF1/J and male C3D2F1/J | ND | 71/72 | A (1,000 ppm with diet) vs. non-treatment | 17–25 m | Feces | 16S rRNA V4 region | With vs. without | ✓ ✓ ✓ |
| Salomäki-Myftari et al. (52), Finland | Offsprings of homozygous OE-NPY | Gene knockout | NA | M (300 mg/kg) vs. vehicle (for dams) | 18 d | Feces | 16S rRNA V4–V5 region | With vs. without | ✓ ✓ |
| Mishima et al. (77), Japan | Male C57BL/6 | Adenine induced renal failure | 8/8 | C (10 mg/kg) vs. vehicle | 2 w | Ceca | 16S rRNA V1–V2 region | With vs. without | ✓ ✓ |

**Studies in rats (n = 17)**

| References, country | Animals | Models | N | Treatment and daily dose | Duration | Specimens | Analysis methods | Comparison | Outcomes |
|---------------------|---------|--------|---|--------------------------|----------|-----------|-----------------|------------|----------|
| Bauer et al. (43), Canada | Male SD | HFD | 6/6 | M (200 mg/kg) vs. normal saline | 1 d | Lumina | 16S rRNA V3 region | With vs. without | ✓ ✓ ✓ |
| Zhang et al. (54), China | Male W | HFD | 10/10 | M (200 mg/kg) vs. vehicle | 8 w | Feces | 16S rRNA V3 region | With vs. without | ✓ ✓ ✓ |
| Pya et al. (57), Canada | Male SD | HFHSD | 20/10 | M (300 mg/kg) vs. non-treatment | 7 w | Ceca | DNA gene RT-qPCR | With vs. without | ✓ |
| Dennison et al. (72), Canada | Female SD | HFHSD | 11–13/11–13 | M (10 mg/kg) vs. non-treatment | 12 w | Feces | 16S rRNA gene RT-qPCR | With vs. without | ✓ |
| Liu et al. (47), China | Male W | HFD/STZ | 10/10 | M (200 mg/kg) vs. non-treatment | 4 w | Colon | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ |

(Continued)
| References, country | Animals | Models | Animals | Models | N | Treatment and daily dose | Duration | Specimens | Analysis methods | Comparison | Outcomes |
|-------------------|---------|--------|---------|--------|---|--------------------------|---------|-----------|-----------------|------------|----------|
| Xu M et al. (42), China | Male SD | HF/HSD/STZ | 10/10 | M (1.8 g/kg) vs. non-M | 4 w | Feces | 16S rDNA gene qPCR | With vs. without | ✓ |
| Zhang et al. (69), China | Male SD | HFD/STZ | 6/6 | L (0.4 mg/kg) vs. normal saline | NA | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Zhang et al. (6), China | Male SD | HFD/STZ | 12/6 | Vi (0.01, 0.02 g/kg) vs. vehicle | 12 w | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Yan et al. (73), China | Male SD | HFD/C/STZ | 10/10 | Si (10 mg/kg) vs. non-treatment | 12 w | Feces | 16S rRNA V1–V3 region | With vs. without | ✓ ✓ ✓ |
| Yuan et al. (67), China | Male SD | STZ | 6/6 | L (0.6 mg/kg) vs. non-L | NA | Feces | 16S rRNA V3 region | With vs. without | ✓ |
| Zhang et al. (5), China | Male ZDF | Gene knockout | 8/8/8 | A (52.27 mg/kg) vs. M (21.15 mg/kg) vs. Si (10.76 mg/kg) vs. normal saline | 4 w | Feces | 16S rRNA V3–V4 region | A vs. M A vs. Si | ✓ ✓ ✓ |
| Shin et al. (49), Korea | Male OLETF | Gene knockout | 7/7 | M (100 mg/kg) vs. water | 12 w | Feces | 16S rRNA V1–V3 region | With vs. without (A) | ✓ ✓ ✓ |
| Wang et al. (50), Korea | Male OLETF | Gene knockout | NA | M (100 mg/kg) vs. distilled water | 12 w | Feces | 16S rRNA V3 region | With vs. without | ✓ ✓ ✓ |
| Han et al. (51), Korea | Male OLETF | Gene knockout | 7/7 | M (100 mg/kg) vs. distilled water | 12 w | Feces | 16S rRNA V1–V2 region | With vs. without | ✓ ✓ |
| Zhao et al. (58), China | Male GK | Gene knockout | 6/6 | A (50 mg/kg) vs. normal saline | 8 w | Colon, feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ ✓ |
| Zhao et al. (60), China | Male W and GK | HFD, gene knockout | 16/16 | L (400 µg/kg) vs. normal saline | 12 w | Feces | 16S rRNA V3–V4 region | With vs. without | ✓ ✓ |
| Kay et al. (71), Japan | Male OLETF | Gene knockout and PS | 10/10 | An (45 mg/kg) vs. vehicle | 8 w | 16S rRNA V4 region | With vs. without | ✓ |

α, acarbose; An, aloglipin; C, canagliflozin; D, dapagliflozin; G, glipizide; Mi, miglitol; M, metformin; L, lagliflozin; P, pioglitazone; R, rosiglitazone; Si, sitagliptin; Sa, saxagliptin; Vo, voglibose; Vi, vildagliptin; HFD, high-fat diet; ND, normal-chow diet; HFD to HFD, high-fat high-sucrose diet; HFHSD, high-fat high-sucrose diet; HFD, high-fat high-sucrose diet; HFD to HFD, high-fat high-carbohydrate diet; HFD, high-fat high-calories diet; FFCD, fat-, fructose-, and cholesterol-rich diet; HSD, high-starch diet; PPD, plant polysaccharide diet; WD, Western diet; STZ, streptozocin intraperitoneal injection; DHEA, trans-dehydroandrosterone; PS, pincere serum intraperitoneal injection; IP, Intraperitoneal injection; SD rats, Sprague-Dawley rats; ZDF rats, Male Zucker diabetic fatty rats, induced by leptin receptor gene knockout; KK mice, LSL-KrasG12D/+ and p48-Cre+/- mice, induced by LSL-KrasG12D and Cre alleles knockout; OLETF rats, Otsuka Long-Evans Tokushima Fatty rats, induced by spontaneous CCK receptor knockout; OE-NPY mice, homozygous transgenic OE-NPY mice, induced by transgenic mice overexpressing Neuropeptide Y under dopamine-β-hydroxylase promoter; W rats, Wistar rats; GK rats, Goto-Kakizaki rats, induced by polygenic Wistar substrate; ob/ob mice, mice model induced by Lepb°° gene knockout; ICR MafA-deficient mice, model induced by targeted disruption of the mafa gene in ICR mice; db/db mice, model induced by mutation in the leptin receptor gene in mice; KKAY mice, mice induced by transfer the yellow obese gene (A°°) into KK mice; RT-qPCR, Real-time quantitative polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; m, months; w, weeks; d, days; NA, not available; vs., versus.
This decrease was also noted in another two studies, in which liraglutide was given to in rat models induced by diets and gene knockout (66, 69).

Responses of the phylum Bacteroidetes to different anti-hyperglycemic agents were investigated in 15 rodent studies. No difference was noted in studies treated with acarbose (5, 58). Five out of the seven studies on the genus Bacteroides after using metformin increased this genus in mice and rats, while the results in eight studies treated with metformin (5, 171) revealed increased abundance of this genus in mice and rats.

Among 15 rodent studies (5, 6, 36, 37, 41, 47, 48, 54, 56, 58, 66, 69, 70, 73, 75) on the phylum Firmicutes, the results were inconclusive among those treated with metformin (5, 36, 37, 41, 47, 48, 54, 56), acarbose (5, 58), liraglutide (66, 69, 70), and sitagliptin (5, 73). The genus Lactobacillus was the focus of 13 studies (5, 35, 41–43, 47, 53, 54, 58, 62, 66, 70, 73). Six out of the eight studies treated with metformin (5, 35, 43, 53, 54, 62) saw an increase in this genus in mice and rats, while the results in studies treated with acarbose (5, 58, 62), liraglutide (66, 70), and sitagliptin (5, 73) were inconclusive.

With respect to other phyla, there was a trend of decrease in the phylum Proteobacteria in mice treated with metformin and liraglutide, while Verrucomicrobia and Tenericutes increased after treated with metformin and liraglutide, respectively. However, results for the phyla Actinobacteria, Cyanobacteria, Elusimicrobia, and Fusobacteria were conflicting. The genus Akkermansia (phylum Verrucomicrobia) increased in eight studies treated with metformin using dietary or genetic models (35, 37, 38, 41, 44, 48, 54, 56). Three of the four studies with metformin (41, 53, 55) reported an increase in the species A. muciniphila, and another two studies reported a similar increase in this species after treating with liraglutide (65, 68).

Diversity

Ten human studies treated with metformin (19, 20, 22–25, 27–30) and two studies treated with acarbose (7, 32) have provided the results of α-diversity. The results from those metformin studies, however, were conflicting, while both acarbose studies reported a decrease in the α-diversity among pre-diabetic and T2D patients. β-diversity was assessed in ten studies treated with metformin (19–22, 24, 25, 27–29, 34), of which six studies (20–22, 24, 27, 34) revealed a significant difference after the treatment in healthy participants and T2D patients. This difference was also noted in pre-diabetic patients treated with acarbose (32), and T2D patients treated with liraglutide (34) (Table 8).

Similar results with metformin and acarbose were reported in mouse studies. The effects of metformin on α-diversity were conflicting across different models, while the α-diversity decreased consistently in three studies treated with acarbose (5, 58, 59). Moreover, the results were inconsistent among those studies treated with liraglutide, sitagliptin, vildagliptin, and saxagliptin. In terms of β-diversity, there was higher cumulative evidence of significant difference after using metformin (5, 36–39, 41, 43–50, 54–56), and similar results were consistently reported among those studies treated with acarbose (5, 58–61), liraglutide (60, 65, 66, 69, 70), sitagliptin (5, 60, 73), and

### TABLE 4 | Effects of anti-hyperglycemic drugs on specific taxa in human gut microbiota, categorized by the target research populations*.

| Specific taxa | Phylum | Nα | N/Nα | Healthy | Obese | Pre-diabetic | Newly T2D | Prevalent T2D |
|---------------|--------|----|-------|---------|-------|-------------|----------|-------------|
| G_Alistipes   | Bacteroidetes | 2  | 151/151 |         |       |             | ↑ A (32), M (20) |             |
| G_Bacteroides | Bacteroidetes | 5  | 233/233 | ↔ M (19) | ↓ A (7) |             | ↑ A (32), M (20, 24) |             |
| S_Bacteroides dorei | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| S_Bacteroides finegalli | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| S_Bacteroides intestinals | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| S_Bacteroides stercoris | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| S_Bacteroides thetaiotaomicron | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| S_Bacteroides uniformis | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| S_Bacteroides vulgatus | Bacteroidetes | 2  | 73/73 |         |       |             | ↓ A (32), M (24) |             |
| G_Bifidobacterium | Actinobacteria | 2  | 73/73 |         |       |             | ↑ A (32), M (21) |             |
| S_Bifidobacterium adolescentis | Actinobacteria | 2  | 73/73 |         |       |             | ↑ A (32), M (21) |             |
| G_Clostridium | Firmicutes | 2  | 76/76 | ↓ M (22) |       |             | ↓ A (32) |             |
| S_Clostridium leptum | Firmicutes | 2  | 71/84 |         |       |             | ↓ A (32) | ↓ M (31) |
| F_Lachnospiraceae | Firmicutes | 2  | 140/140 |         |       |             | ↑ A (7) | ↑ M (20) |
| G_Lactobacillus | Firmicutes | 3  | 111/111 | ↔ M (19) | ↑ A (7) |             | ↑ A (32) |             |
| S_Lactobacillus gasseri | Firmicutes | 2  | 73/73 |         |       |             | ↑ A (32), M (21) |             |
| G_Megasphaera | Firmicutes | 2  | 54/54 | ↑ A (7) |       |             | ↑ M (29) |             |
| S_Pseudoflavonifractor capillosus | Firmicutes | 2  | 73/73 |         |       |             | ↓ A (32), M (21) |             |
| S_Ruminococcus sp. 5_1_39BFAA | Firmicutes | 2  | 73/73 |         |       |             | ↓ A (32), M (21) |             |

*The target research populations include obese, pre-diabetic, newly Type 2 diabetes (T2D), prevalent T2D; Number of studies; Number of participants (treatment/comparison); F, family; G, genus; S, species; M, metformin; A, acarbose; ↑, significant increase; ↓, significant decrease; ↔, no significant difference.
TABLE 5 | Consistent and inconsistent effects of each anti-hyperglycemic drug on specific taxa in human gut microbiota, categorized by the target research populations.*

| Drug/specific taxa | Phylum | N* | N/N° | Healthy | Obese | Pre-diabetic | Newly T2D | Prevalent T2D |
|--------------------|--------|----|-------|---------|-------|-------------|----------|--------------|
| **METFORMIN**      |        |    |       |         |       |             |          |              |
| Consistent results |        |    |       |         |       |             |          |              |
| G_Fusobacterium    | Fusobacteria | 2  | 151/126 |       |       |             | ↑ (20, 27)|              |
| S_Akkermansia muciniphila | Verrucomicrobia | 2  | 36/36 |       |       |             | ↑ (21) | ↑ (29) |
| G_Escherichia      | Proteobacteria | 6  | 243/202 | ↑ (22, 25)| ↑ (19) |             | ↑ (20, 21) | ↑ (30) |
| G_Shiella          | Proteobacteria | 4  | 163/163 | ↑ (22, 25)| ↑ (19) |             | ↑ (20) |              |
| S_Citrobacter koseri | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Citrobacter rodentium | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Enterobacter cloacae | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Saccharomyces cerevisiae | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Erwinia amylovora | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Pectobacterium atrosepticum | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Dickeya damasonium | Proteobacteria | 2  | 42/55 |       |       |             | ↑ (21) | ↑ (31) |
| S_Clostridium bartlettii | Firmicutes | 2  | 42/55 |       |       |             | ↓ (21) | ↓ (31) |
| S_Clostridium botulinum | Firmicutes | 2  | 42/55 |       |       |             | ↓ (21) | ↓ (31) |
| Inconsistent results |        |    |       |         |       |             |          |              |
| G_Bacteroides      | Bacteroidetes | 3  | 142/142 | ↔ (19) |       |             | ↓ (20, 24) |              |
| G_Prevotella       | Bacteroidetes | 2  | 34/34 | ↔ (19) |       |             | ↑ (29) |              |
| G_Bacteroides      | Bacteroidetes | 3  | 138/138 | ↔ (25) | ↔ (19) |             | ↑ (20) |              |
| G_Prevotella       | Bacteroidetes | 4  | 125/84 | ↓ (22) | ↔ (19) |             | ↓ (21) | ↓ (30) |
| F_Enterobacteriaceae | Proteobacteria | 2  | 69/44 | ↑ (25) |       |             | ↓ (27) |              |
| **Acarbose**       |        |    |       |         |       |             |          |              |
| Consistent results |        |    |       |         |       |             |          |              |
| G_Bacteroides      | Bacteroidetes | 2  | 91/91 |       |       |             | ↓ (7) | ↓ (32) |
| G_Lactobacillus    | Firmicutes | 2  | 91/91 |       |       |             | ↑ (7) | ↑ (32) |
| S_Bifidobacterium longum | Actinobacteria | 2  | 110/110 |       |       |             | ↑ (32) | ↑ (33) |

*The target research populations include obese, pre-diabetic, newly Type 2 diabetes (T2D), prevalent T2D; °Number of studies; °°Number of participants (treatment/comparison); F, family; G, genus; S, species; ↑, significant increase; ↓, significant decrease; ↔, no significant difference.

vildagliptin (6, 74) across different mouse models. Evidence for the effects of other drugs was limited (Table 9).

**Short-Chain Fatty Acids (SCFAs)**

In human studies, changes in the levels of three main SCFAs (acetate, propionate, and butyrate) in feces and sera were reported in three studies treated with metformin (19, 21, 23) (Supplementary Table 3). Wu et al. (21) found that the levels of fecal butyrate and propionate increased in T2D male patients. However, no difference in fecal levels of these two SCFAs was noted among obese women in Ejtahed’s study (19). In contrast, fecal acetate levels decreased in obese women (19) did not change among T2D patients in Wu’s study (21). Huang et al. (23) reported that the serum levels of all three SCFAs remained unchanged after treating with metformin in T2D patients.

In mouse studies, fecal levels of SCFAs after metformin interventions were assessed in db/db mice (35) and OLETF rats (50) (Supplementary Table 3). It was found that levels of acetate and butyrate increased, but propionate levels remained unchanged. The effects of acarbose on these SCFAs levels were also assessed in dietary models (59, 61, 62). These studies showed consistent results of increased levels of butyrate in feces and ceca, but the levels of acetate and propionate varied in a diet-dependent manner.

**Bile Acids**

In human studies, three clinical trials treated with metformin (21, 24, 26) and one randomized trial with acarbose and glipizide (32) were carried out in T2D patients to assess the respective effects on the fecal and serum levels of bile acids (Supplementary Table 4). Regarding metformin, one study (24) showed increases in the fecal level of conjugated secondary bile acids, while no difference was reported in the other two studies (21, 26). Two of these three studies (21, 24) reported increases in the blood level of
TABLE 6 | Effects of anti-hyperglycemic drugs on gut microbiota in mouse models, categorized by the treated, individual anti-hyperglycemic drug.

| Specific taxon          | N  | M    | A | Mi | Vo | L | An | Sa | Si | Vi | C | D | R |
|------------------------|----|------|---|----|----|---|----|----|----|----|---|---|---|
| **Firmicutes/bacteroidetes** |    |      |   |    |    |   |    |    |    |    |   |   |   |
| P_Bacteroidetes         | 15 | ↔(3), ↓(5), ↑(2) | ↔(2) | \(\uparrow\) | \(\downarrow\) | \(\uparrow\) | \(\downarrow\) | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| C_Bacteroidia           | 3  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| F_Bacteroidaceae        | 5  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| G_Bacteroides           | 18 | ↔(2), ↓(5) | ↑ | ↑ | ↑ | ↑ | ↑ | ↑(2) | ↑ | ↑ | ↑ | ↑ | ↑ |
| S_Bacteroides acidifaciens | 2  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| G_Butyricimonas         | 3  | ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| F_Porphyromonadaceae    | 4  | ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Odonibacter           | 4  | ↔, ↓(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Parabacteroides       | 8  | ↓, ↑(4) |    |    |    |   |    |    |    |    |   |   |   |
| F_Prevotellaceae        | 5  | ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Prevotella            | 8  | ↓, ↑(3) |    |    |    |   |    |    |    |    |   |   |   |
| G_Prevotella_9          | 3  | ↓, ↑ |    |    |    |   |    |    |    |    |   |   |   |
| F_Rikenellaceae         | 3  | ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Alistipes             | 3  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| F_S24-7                 | 3  | ↓, ↑ |    |    |    |   |    |    |    |    |   |   |   |
| **Phylum Firmicutes**   |    |      |   |    |    |   |    |    |    |    |   |   |   |
| P_Firmicutes            | 15 | ↔(4), ↓(2), ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Turicibacter          | 5  | ↔, ↓, ↑ |    |    |    |   |    |    |    |    |   |   |   |
| O_Lactobacillales       | 2  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| F_Lactobacillaceae      | 7  | ↔, ↓(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Lactobacillus         | 5  | ↓, ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Lactobacillus_intestinalis | 1 | ↑ |    |    |    |   |    |    |    |    |   |   |   |
| S_Lactobacillus johnsonii | 2 | ↑ |    |    |    |   |    |    |    |    |   |   |   |
| F_Streptococcaceae      | 5  | ↓(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Streptococcus         | 4  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| C_Clostridia            | 2  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| O_Clostrioides          | 2  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| F_Clostrioides_vadinBB60_g | 2 | ↑ |    |    |    |   |    |    |    |    |   |   |   |
| G_Christensenellaceae   | 3  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| G_Christensenellaceae_R_7_g | 3 | ↔ |    |    |    |   |    |    |    |    |   |   |   |
| G_Candidatus Arthromitus | 3  | ↔    |    |    |    |   |    |    |    |    |   |   |   |
| G_Clostridium           | 3  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| G_Ruminoclostridium     | 2  | ↔    |    |    |    |   |    |    |    |    |   |   |   |
| G_Ruminoclostridium_6   | 2  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| G_Ruminoclostridium_9   | 3  | ↔    |    |    |    |   |    |    |    |    |   |   |   |
| F_Ruminococcaceae       | 6  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| G_Ruminococcaceae_UCG_005 | 1 | ↓ |    |    |    |   |    |    |    |    |   |   |   |
| G_Ruminococcus          | 5  | ↓(2), ↑(2) |    |    |    |   |    |    |    |    |   |   |   |
| G_Ruminococcus_2        | 2  | ↓, ↑ |    |    |    |   |    |    |    |    |   |   |   |
| F_Lachnospiraceae       | 5  | ↑    |    |    |    |   |    |    |    |    |   |   |   |
| G_Blaunia               | 8  | ↓, ↑ |    |    |    |   |    |    |    |    |   |   |   |
| G_Lachnospiraceae       | 3  | ↓    |    |    |    |   |    |    |    |    |   |   |   |
| G_Lachnospiraceae_nk4a136_g | 2 | ↔ |    |    |    |   |    |    |    |    |   |   |   |
| G_Marvinbryantia        | 2  | ↔    |    |    |    |   |    |    |    |    |   |   |   |

(Continued)
secondary bile acids, while the other one (26) revealed an inverse trend. Concerning changes in total and primary bile acids, their levels in feces were unchanged among these three trials, but results in blood levels were conflicting (21, 26). The random trial assessing the effects of acarbose and glipizide on bile acid levels in newly diagnosed T2D patients (32) showed that acarbose might increase the plasma and fecal levels of primary bile acids, accompanied by decreases in secondary bile acids. In contrast, no significant changes in bile acid levels were found in patients treated with glipizide.

**TABLE 6 | Continued**

| Specific taxon                        | N a | M  | A  | Mi | Vo | L  | An | Sa | Si | Vi | C  | D  | R |
|--------------------------------------|-----|----|----|----|----|----|----|----|----|----|----|----|----|
| G. Roseburia                         | 7   | ↔, ↓ | ↔ |    |    | ↔, ↓ |    |    |    |    |    |    |    |
| G. Peptococcus                       | 2   | ↓  | ↔  |    |    |    |    |    |    |    |    |    |    |
| G. Romboustia                        | 2   | ↓  |    |    |    |    |    |    |    |    |    |    |    |
| G. Anaerotruncus                     | 6   | ↔, ↓ | ↔ |    | ↓  | ↓  | ↓  | ↓  |    |    |    |    |    |
| G. Flavonifractor                    | 2   | ↓  |    |    |    |    |    |    |    |    |    |    |    |
| G. Oscillospira                      | 6   | ↓, ↑ | ↑ |    |    |    |    |    |    |    |    |    |    |
| F. Dehalobacteriaceae               | 3   | ↓, ↑ | ↑ |    |    |    |    |    |    |    |    |    |    |
| F. Erysipelotrichaceae              | 5   | ↑  | ↔, ↑ | ↓ | ↓  | ↑  |    |    |    |    |    |    |    |
| O. Erysipelotrichales               | 2   | ↑  |    |    |    |    |    |    |    |    |    |    |    |

**Phylum Actinobacteria**

| P. Actinobacteria                    | 5   | ↔, ↑ |    |    |    | ↔, ↓ |    |    |    |    |    |    |    |
| F. Bifidobacteriaceae               | 2   | ↓  | ↑  |    |    |    |    |    |    |    |    |    |    |
| G. Bifidobacterium                  | 8   | ↔(2), ↑, ↓ | ↑ | ↑  | ↑  | ↓  |    |    |    |    |    |    |    |
| S. Bifidobacterium spp.             | 2   | ↓  |    |    |    |    |    |    |    |    |    |    |    |
| G. Corynebacterium 1                | 2   | ↔  |    |    |    |    |    |    |    |    |    | ↑  |    |
| G. Enterorhabdus                    | 2   | ↔  |    |    |    |    |    |    |    |    |    |    |    |

**Phylum Cyanobacteria**

| P. Cyanobacteria                     | 2   | ↓  | ↓  |    |    |    |    |    |    |    |    |    |    |

**Phylum Elusimicrobia**

| P. Elusimicrobia                     | 2   | ↔  |    |    |    |    |    |    |    |    |    |    |    |
| G. Allobaculum                      | 10  | ↔, ↓(2), ↑(2) | ↑ |    | ↔, ↑ | ↑(2) |    |    |    |    |    |    |    |

**Phylum Fusobacteria**

| P. Fusobacteria                     | 3   | ↓, ↑ |    |    |    |    |    |    |    |    |    |    |    |

**Phylum Proteobacteria**

| P. Proteobacteria                    | 8   | ↓(3), ↑ | ↓ |    | ↔, ↓(2) | ↓↑ |    | ↑  |    |    |    |    |
| C. Alphaproteobacteria              | 2   | ↓  |    |    |    |    |    |    |    |    |    |    |    |
| G. Desulfovibrio                    | 5   | ↔, ↓ | ↓  | ↓  | ↑  | ↓  |    |    |    |    |    |    |    |
| F. Desulfovibrionaceae              | 4   | ↓  | ↓  | ↑  | ↑  |    |    |    |    |    |    |    |    |
| F. Enterobacteriaceae               | 2   | ↑  |    |    |    |    |    |    |    |    |    |    |    |
| G. Escherichia                      | 3   | ↔, ↑ |    |    |    |    |    |    |    |    |    |    |    |
| G. Helicobacter                     | 2   | ↓  |    |    |    |    |    |    |    |    |    |    |    |

**Phylum Tenericutes**

| P. Tenericutes                      | 6   | ↔, ↑ | ↔ | ↑(2) | ↔, ↑ | ↓  |    |    |    |    |    |    |    |
| C. Mollicutes                       | 2   | ↑  |    |    |    |    |    |    |    |    |    |    |    |

**Phylum Verrucomicrobia**

| P. Verrucomicrobia                  | 8   | ↔, ↓(2), ↑(5) | ↓ |    | ↑  |    |    |    |    |    |    |    |    |
| F. Verrucomicrobiaceae              | 5   | ↑(4) | ↓(2) |    |    |    |    |    |    |    |    |    |    |
| G. Akkermansia                      | 9   | ↑(8) |    |    |    |    |    |    |    |    |    |    |    |
| S. Akkermansia muciniphila          | 6   | ↔, ↑(3) | ↑(2) |    |    |    |    |    |    |    |    |    |    |

*Number of studies; M, metformin; A, acarbose; Mi, miglitol; Vo, voglibose; L, liraglutide; An, anagliptin; Sa, saxagliptin; Si, sitagliptin; Vi, vildagliptin; C, canagliflozin; D, dapagliflozin; R, rosiglitazone; P, Phylum; C, Class; O, Order; F, Family; G, Genus; S, Species; ↑, significant increase; ↓, significant decrease; ↔, no significant difference; (n), Number of studies (≥2) reported the same results.

---

[Secondary bile acids, while the other one (26) revealed an inverse trend. Concerning changes in total and primary bile acids, their levels in feces were unchanged among these three trials, but results in blood levels were conflicting (21, 26). The random trial assessing the effects of acarbose and glipizide on bile acid levels in newly diagnosed T2D patients (32) showed that acarbose might increase the plasma and fecal levels of primary bile acids, accompanied by decreases in secondary bile acids. In contrast, no significant changes in bile acid levels were found in patients treated with glipizide.]
TABLE 7 | Consistent and inconsistent effects of each anti-hyperglycemic drug on specific taxa in mouse gut microbiota, categorized by mice or rat models with three distinct animal models.

| Specific taxa | Phylum | N^a | Trend^b | Mice models | Rat models |
|---------------|--------|-----|---------|-------------|------------|
|               |        |     |         | Normal      | Dietary or STZ | Gene knockout | Normal | Dietary or STZ | Gene knockout |
|               |        |     |         |             |             |             |        |             |             |

**METFORMIN**

**Consistent results**

| Firmicutes/Bacteroidetes | Bacteroidetes | 2 | ↓ | ↓ (2) |
|--------------------------|--------------|---|----|-------|
| F_Porphyromonadaceae     | Bacteroidetes | 2 | ↑ | ↑ | ↑ |
| F_Prevotellaceae         | Bacteroidetes | 2 | ↑ | ↑ | ↑ |
| F_Rikenellaceae          | Bacteroidetes | 2 | ↑ | ↑ (2) |
| G_Butyricimonas          | Bacteroidetes | 2 | ↑ | ↑ | ↑ |
| F_Enterococcaceae        | Firmicutes   | 2 | ↓ | ↓ |
| F_Streptococcaceae      | Firmicutes   | 2 | ↓ | ↓ | ↓ |
| F_Verrucomicrobiaceae   | Verrucomicrobia | 4 | ↑ | ↑ (2) | ↑ (3) |
| G_Akkermansia            | Verrucomicrobia | 8 | ↑ | ↑ (6) | ↑ |
| S_Akkermansia spp.      | Verrucomicrobia | 2 | ↑ | ↑ | ↑ |
| F_Alcigenaceae          | Proteobacteria | 2 | ↑ | ↑ | ↑ |

**Inconsistent results**

| P_Bacteroidetes | Bacteroidetes | 8 | ↑ | ↔ | ↓ | ↓ (4) | ↔ | ↑ |
| G_Bacteroides   | Bacteroidetes | 7 | ↑ | ↑ | ↔ | ↓ | ↓ | ↓ |
| G_Odonbacter    | Bacteroidetes | 3 | ↓ | ↓ | ↔ | ← | ↓ |
| G_Parabacteroides | Bacteroidetes | 5 | ↑ | ↓ | ↓ | ↓ | ↓ |
| G_Prevotella    | Bacteroidetes | 4 | ↑ | ↑ | ↓ | ↑ (2) |
| P_Firmicutes    | Firmicutes   | 8 | o | ↔ | ↔ | ↓ | ↓ | ↓ |
| G_Allobaculum   | Firmicutes   | 5 | o | ↓ | ↓ | ↑ | ↔ |
| G_Anaerotruncus | Firmicutes   | 2 | o | ↓ | ↔ |
| G_Blaudia       | Firmicutes   | 2 | o | ↓ | ↑ |
| G_Oristensenella | Firmicutes | 2 | o | ↑ | ↔ |
| G_Coprococcus  | Firmicutes   | 3 | ↓ | ↓ | ↓ | ↑ |
| F_Dehalobacteriaceae | Firmicutes | 2 | o | ↓ | ↑ |
| G_Dehalobacterium | Firmicutes | 2 | o | ↓ | ↑ |
| G_Enterococcus  | Firmicutes   | 5 | ↓ | ↓ | ↓ (2) | ↔ | ↑ |
| F_Lactobacillaceae | Firmicutes | 2 | o | ↑ | ↓ |
| G_Lactobacillus | Firmicutes   | 8 | ↑ | ↓ | ↓ | ↓ |
| G_Lactococcus   | Firmicutes   | 2 | o | ↑ | ↓ |
| G_Oscillospira  | Firmicutes   | 2 | o | ↑ | ↓ |
| G_Roseburia     | Firmicutes   | 2 | o | ↔ | ↓ |
| G_Ruminococcus | Firmicutes   | 4 | o | ↑ | ↓ (2) | ↑ |
| G_Turbicicola  | Firmicutes   | 3 | o | ↑ | ↔ |
| G_Bifidobacterium | Actinobacteria | 4 | o | ↔ | ↔ | ↑ |
| P_Fusobacteria  | Fusobacteria | 2 | o | ↑ | ↑ |
| G_Fusobacteria  | Fusobacteria | 2 | o | ↑ | ↑ |
| O_Fusobacteriales | Fusobacteria | 2 | o | ↑ | ↓ |
| P_Proteobacteria | Proteobacteria | 4 | ↓ | ↓ | ↑ |
| G_Desulfovibrio | Proteobacteria | 2 | o | ↓ | ↔ |
| G_Escherichia   | Proteobacteria | 2 | o | ↑ | ↔ |
| S_Escherichia coli | Proteobacteria | 2 | o | ↓ | ↔ |
| G_Klebsiella    | Proteobacteria | 2 | o | ↔ | ↑ |

(Continued)
### TABLE 7 | Continued

| Specific taxa                      | Phylum        | N<sup>a</sup> | Trend<sup>b</sup> | Mice models          | Rat models          |
|------------------------------------|---------------|---------------|-------------------|----------------------|---------------------|
|                                    |               |               |                   | Normal               | Dietary or STZ      | Gene knockout      | Normal               | Dietary or STZ      | Gene knockout      |
| G_Parasutterella                   | Proteobacteria| 3             | ↑                 | ↑                   | ↔                   | ↑                   |                       |                      |                     |
| G_Proteus                          | Proteobacteria| 2             | o                 | ↑                   | ↓                   |                       |                      |                      |                     |
| G_Sutterella                       | Proteobacteria| 3             | ↑                 | ↑                   | ↔                   | ↑                   |                       |                      |                     |
| G_Trabulsiella                     | Proteobacteria| 2             | o                 | ↓                   | ↔                   |                       |                      |                      |                     |
| P_Tenericutes                      | Tenericutes   | 2             | o                 | ↑                   | ↔                   | ↑                   |                       |                      |                     |
| P_Verrucomicrobia                  | Verrucomicrobia| 7             | ↑                 | ↔                   |↑ (4)               | ↑, ↓                | ↓                     |                      |                     |
| S_Akkermansia muciniphila         | Verrucomicrobia| 4             | ↑                 | ↔                   |↑, ↑ (3)            |                      |                      |                      |                     |
| G_AF12                             | NA            | 2             | o                 | ↓                   | ↔                   | ↑                   |                       |                      |                     |

**ACARBOSE**

**Consistent result**

| F_Lactobacillaceae | Firmicutes 2 | ↓ | ↓ | ↓ |

**Inconsistent results**

| F_S24-7 | Bacteroidetes 2 | o | ↑ | ↓ |
| P_Firmicutes | Firmicutes 2 | o | ↔ | ↑ |
| F_Erysipelotrichaceae | Firmicutes 2 | o | ↔ | ↑ |
| G_Lachnospiraceae UCG-001 | Firmicutes 2 | o | ↔ | ↑ |
| G_Lactobacillus | Firmicutes 3 | o | ↔ | ↑ |
| G_Ruminococcus 2 | Firmicutes 2 | o | ↓ | ↑ |

**DAPAGLIFLOZIN**

**Inconsistent result**

| G_Enterococcus | Firmicutes 2 | o | ↑ | ↓ |

**LIRAGLUTIDE**

**Consistent results**

| Firmicutes/Bacteroidetes | 2 | ↓ | ↓ | ↓ | ↓ |
| G_Blatia | Firmicutes 2 | ↑ | ↓ | ↓ | ↓ |
| S_Akkermansia muciniphila | Verrucomicrobia 2 | ↑ | ↑ (2) | ↓ | ↓ |

**Inconsistent results**

| P_Actinobacteria | Actinobacteria 2 | o | ↓ | ↔ | ↔ |
| P_Bacteroidetes | Bacteroidetes 3 | ↑ | ↓ | ↑ (2) | ↑ |
| G_Bacteroides | Bacteroidetes 3 | o | ↓ | ↑ | ↔ |
| G_Bacteroidia | Bacteroidetes 2 | o | ↓ | ↑ | ↑ |
| G_Bacteroidales | Bacteroidetes 2 | o | ↓ | ↑ | ↑ |
| F_Porphyromonadaceae | Bacteroidetes 2 | o | ↓ | ↑ | ↑ |
| G_Prevotella_9 | Bacteroidetes 2 | o | ↓ | ↑ | ↑ |
| P_Firmicutes | Firmicutes 3 | o | ↑ | ↔, ↓ | ↔ |
| G_Allobaculum | Firmicutes 2 | o | ↑ | ↔ | ↔ |
| G_Christensenellaceae_R_7_group | Firmicutes 2 | o | ↓ | ↔, ↓ | ↔ |
| F_Lachnospiraceae | Firmicutes 2 | o | ↓ | ↔ | ↔ |
| F_Ruminococcaceae | Firmicutes 2 | o | ↔ | ↑ | ↔ |
| G_Lactobacillus | Firmicutes 2 | o | ↑ | ↓ | ↔ |
| G_Roseburia | Firmicutes 2 | o | ↓ | ↔ | ↔ |
| P_Proteobacteria | Proteobacteria 3 | ↓ | ↓ | ↓ (2) | ↓ | ↔ | ↔ |
| P_Tenericutes | Tenericutes 2 | o | ↓ | ↔, ↓ | ↔ |
| P_Verrucomicrobia | Verrucomicrobia 2 | o | ↑ | ↑ | ↔ | ↔ |

(Continued)
### TABLE 7 | Continued

| Specific taxa | Phylum     | N<sup>a</sup> | Trend<sup>b</sup> | Mice models | Rat models |
|--------------|------------|---------------|------------------|-------------|------------|
|              |            |               | Normal | Dietary or STZ | Gene knockout | Normal | Dietary or STZ | Gene knockout |
| SITAGLIPTIN  |            |               |        |              |             |        |              |             |
| **Consistent results** |            |               |        |              |             |        |              |             |
| G, Bacteroides | Bacteroidetes | 2           | ↑      | ↑            | ↑            |         |              |             |
| G, Blautia    | Firmicutes  | 2            | ↓      | ↓            | ↓            |         |              |             |
| **Inconsistent results** |            |               |        |              |             |        |              |             |
| P, Bacteroides | Bacteroidetes | 2           | o      | ↑            |              |         |              |             |
| F, Prevotellaceae | Firmicutes | 2          | o          | ↑            |              |         |              |             |
| G, Lactobacillus | Firmicutes | 2           | o      | ↓            | ↑            |         |              |             |
| G, Roseburia  | Firmicutes  | 2            | o      | ↓            | ↑            |         |              |             |
| P, Proteobacteria | Proteobacteria | 2 | o | ↑          | ↓            |         |              |             |
| P, Tenericutes | Tenericutes | 2          | o | ↔ | ↑            |             |         |              |             |

### VILDAGLIPTIN

| Specific taxa | Phylum     | N<sup>a</sup> | Trend<sup>b</sup> | Mice models | Rat models |
|--------------|------------|---------------|------------------|-------------|------------|
|              |            |               | Normal | Dietary or STZ | Gene knockout | Normal | Dietary or STZ | Gene knockout |
| **Consistent result** |            |               |        |              |             |        |              |             |
| G, Oscillibacter | Firmicutes | 2           | ↓      |              |             |         |              |             |
| **Inconsistent result** |            |               |        |              |             |        |              |             |
| G, Bacteroides | Bacteroidetes | 2           | o      | ↔ | ↑            |             |         |              |             |

<sup>a</sup> Number of studies; <sup>b</sup> Trend of alteration (reported in > 50% of studies): ↑, a trend of increase; ↓, a trend of decrease; o, inconclusive results; Alteration of specific taxa: ↑, significant increase; ↓, significant decrease; ↔, no significant difference; (n), number of papers (≥2) reported the same results.

### TABLE 8 | Effects of anti-hyperglycemic drug on diversity of human gut microbiota.

| Drugs          | Object     | α-diversity | β-diversity<sup>c</sup> | References |
|----------------|------------|-------------|--------------------------|------------|
| Metformin      | Healthy    | –           | ↓                        | ns         | (25)       |
|                | ns         | ns          |即使是             |            | (22)       |
| Obese          | ns         | ns          | ns                       | (19)       |
| Newly T2D      | ns         | ↑           |即使是             |            | (20)       |
| Prevalent T2D  | –          | –           |即使是             | (21)       |
|                | ns         | ns          | –                       | (23)       |
|                | –          | ↓           |即使是             | (27)       |
|                | –          | ns          | ns                       | (28)       |
|                | ns         | –           | ns                       | (29)       |
| Acarbose       | Pre-diabetic | ns        | ↓                        |即使是             | (34)       |
|                | Newly T2D  | –           | ↓                        |即使是             | (33)       |
| Glipizide      | Newly T2D  | ns          | ns                       | –          | (32)       |

<sup>a</sup> Richness was assessed by Chao1, ACE, and Rarefaction indices, gene count, number of OTUs, or number of species; <sup>b</sup> Evenness was assessed by Shannon, Simpson indices; <sup>c</sup> β-diversity was assessed by UniFrac (weighted, unweighted), Bray-Curtis, Jensen-Shannon, or Jaccard distances using Principal Component Analysis (PCA) and Principal Coordinates Analysis (PCoA); ↑, significant increase; ↓, significant decrease;即使是, significant difference; ns, no significant difference; –, no information.
### TABLE 9 | Effects of anti-hyperglycemia drugs on diversity in mouse gut microbiota.

| Drugs   | Objects | Models  | α-diversity | β-diversity | References |
|---------|---------|---------|-------------|-------------|------------|
| Metformin | Mice    | ND      | –           | ns          | ≠          | (46)       |
|         |         | ns      | –           | ns          | (55)       |
|         |         | –       | ns          | ns          | (40)       |
|         |         | –       | –           | ns          | (56)       |
|         |         | HFD     | –           | ↓           | ≠          | (37)       |
|         |         | –       | ns          | ≠           | (38)       |
|         |         | ns      | –           | ≠           | (41)       |
|         |         | –       | –           | ≠           | (45)       |
|         |         | ns      | ns          | ≠           | (48)       |
|         |         | ↓       | –           | ≠           | (55)       |
|         |         | –       | –           | ≠           | (56)       |
|         |         | HFD     | ns          | ns          | ≠          | (39)       |
|         |         | FFC    | –           | ns          | (40)       |
|         |         | HFD/STZ | –           | –           | ≠          | (44)       |
|         |         | DHEA/HFD | –        | –           | ≠          | (36)       |
|         |         | db/db   | ns          | ↑           | ns         | (35)       |
|         |         | OE-NPY | –           | –           | ns         | (52)       |
|         | Rats    | HFD     | ns          | ns          | ≠          | (43)       |
|         |         | HFD/STZ | ↓           | ↓           | ≠          | (54)       |
|         |         | OLETEN | ↑           | –           | ≠          | (49)       |
|         |         | –       | –           | ≠           | (53)       |
|         |         | ZDF     | –           | –           | ≠          | (5)        |
| Acarbose | Mice    | ND      | ↓           | ↓           | ≠          | (59)       |
|         |         | HSD     | –           | –           | ≠          | (61)       |
|         |         | HFD     | –           | –           | ≠          | (60)       |
|         | Rats    | GK      | –           | ↓           | ≠          | (58)       |
|         |         | ZDF     | ↓           | ↓           | ≠          | (5)        |
| Miglitol | Mice    | HFHSD   | –           | ns          | ≠          | (64)       |
|         |         | HFD     | ns          | ns          | ≠          | (65)       |
|         |         | HFD/STZ | ↑           | –           | –          | (68)       |
|         |         | ob/ob   | ↑           | –           | –          | (68)       |
|         | Rats    | HFD/STZ | ↓           | ↓           | ≠          | (69)       |
|         |         | GK      | ns          | ns          | ≠          | (66)       |
|         |         | W       | ↓           | ↓           | ≠          | (66)       |
|         |         |         |             |             |             |             |
| Liraglutide | Mice | ND      | ↓           | –           | –          | (68)       |
|         |         | HFD     | ns          | ns          | ≠          | (62)       |
|         |         | HFD/STZ | ↑           | –           | –          | (68)       |
|         |         | ob/ob   | ↑           | –           | –          | (68)       |
|         | Rats    | HFD/STZ | ↓           | ↓           | ≠          | (69)       |
|         |         | GK      | ns          | ns          | ≠          | (66)       |
|         |         | W       | ↓           | ↓           | ≠          | (66)       |
|         |         |         |             |             |             |             |
|         |         | HFD     | –           | –           | ≠          | (60)       |
| Sitagliptin | Mice | HFD     | –           | –           | ≠          | (60)       |
|         | Rats    | HFH/STZ | ↑           | ↑           | ≠          | (73)       |
|         |         | ZDF     | –           | –           | ≠          | (5)        |
| Vildagliptin | Mice | WD      | ns          | ns          | ≠          | (74)       |
|         |         | HFD/STZ | ↓           | ↓           | ≠          | (6)        |
| Saxagliptin | Mice | HFD     | –           | –           | ≠          | (60)       |
|         |         | HFD     | ns          | ↓           | ns         | (70)       |

(Continued)
As for rodent studies, one study in rats (51) revealed that the fecal level of total bile acids increased while the levels in liver tissues were decreased after metformin intervention. One study in mice (63) found that voglibose treatment was associated with increases in serum levels of primary bile acids, accompanied by decreases in serum levels of secondary bile acids (Supplementary Table 4).

**Associations With Host Metabolic Parameters**

Among pre-diabetic and T2D patients treated with metformin (20, 21) or acarbose (7, 32, 33), alterations in certain specific taxa in human gut microbiota were associated with improvement in Hba1c and fasting blood glucose values, body weights, and lipid profiles (Table 10). For instance, increments in the genera *Escherichia*, *Shigella*, *Subdoligranulum*, and *Dialister*, and the species *Bifidobacterium adolescentis*, *Bifidobacterium longum*, and *Lactobacillus gasseri* were inversely associated with Hba1c after treatment with metformin or acarbose (7, 20, 21, 32). In addition, there were inverse associations between increases in the genus *Blautia* and fasting blood glucose after treating with metformin (20).

Mouse studies treated with metformin (44, 50, 55, 57), liraglutide (65, 66, 70), and saxagliptin (70) also explored the relationship between changes in the compositions of gut microbiota and improvement in various metabolic parameters (Table 11). It was found that some related specific taxa after treating with metformin in mice (i.e., *Bacteroides* spp., *Blautia*) were different from that in humans.

**DISCUSSION**

Our study provides a comprehensive review to report human and animal data separately about reciprocal interactions between anti-hyperglycemic drugs and specific taxonomic groups of gut microbiota. While other reviews suggest the effects of anti-hyperglycemic drugs on gut microbiota without discerning findings from either human or animal studies (8, 11, 13), this systematic review attempts to fill the gap of these reviews to try to explore the associations among anti-hyperglycemic agents, specific taxonomic patterns of gut microbiota, and glucose control or metabolic profiles mainly in humans, as compared to those reported in mouse studies. Further, the fact that three-quarters of included studies were published in and after 2017 implies a growing interest in this clinical question for an up-to-date systematic review.

Of the 17 human studies selected, the majority of these studies focus on either newly diagnosed or prevalent T2D patients, and directed toward investigating the interplay of metformin, and to a lesser extent, acarbose, with gut microbiota. Our results suggest that these two drugs mediate their glucose-lowering effect, in part, by stimulating beneficial gut bacteria that could produce metabolites to promote intestinal homeostasis (3, 9). We rationalize that alterations in gut microbiota compositions might also underlie the gastrointestinal side effects known to metformin, i.e., diarrhea and fecal incontinence (10, 25, 80). In contrast, results from other anti-hyperglycemic drugs analyzed in this study showed inconsistency with respect to their effects on the compositions of gut microbiota, which might be attributable to small numbers of studies and, equally important, differences in animal models and experimental conditions used among these studies.

Further, treatment durations of anti-hyperglycemic drugs in available studies, regardless of human or animal, varied to a great extent (i.e., few days to few months). Thus, the reported drug effects on the gut microbiota structure were diverse. Indeed, the anti-hyperglycemic drugs, i.e., metformin, could affect the intestinal bacterial compositions after 1 or several days of treatment (24, 25, 40, 43), or after prolonged periods of treatments (21). For instance, Wu et al. found that gut microbiota compositions after a 2- and 4-month treatment of metformin in newly T2D patients were not identical (21). In contrast, Wang et al. did not find significant changes in the gut microbiota compositions among T2D patients after different periods of metformin or liraglutide treatment, given their baseline gut microbiota compositions were unknown (34). Thus, there are no consistent findings on gut microbiota after various treatment durations of anti-hyperglycemic drugs, and further studies are warranted to explore the treatment duration of anti-hyperglycemics required for emergence of beneficial gut bacteria.
Evidence indicates that the use of metformin or acarbose in T2D patients was associated with increases in the abundance of beneficial bacteria, including the genera *Bifidobacterium* (phylum *Actinobacteria*) and *Lactobacillus* (phylum *Firmicutes*), and the species *A. municiphila* (phylum *Verrucomicrobia*). The increase in the genus *Bifidobacterium* was positively associated with diabetes control, which is consistent with that reported in the review by Gurung et al. (1). In addition, two included studies showed an increase in two specific species of the genus *Bifidobacterium* (*B. adolescentis* and *B. longum*), which was inversely associated with HbA1C levels among newly diagnosed T2D patients (21, 32).

A number of human studies have reported positive associations between the abundance of the genus *Lactobacillus* (phylum *Firmicutes*) and improved T2D control (81, 82). For example, T2D patients treated with acarbose showed increased *L. gasseri* levels, accompanied by lower HbA1C and body weights (32). In addition, as several species in the genus *Lactobacillus* have been used as probiotics, administration of these *Lactobacillus* strains showed beneficial effects on glycemic control and lipid profiles in T2D patients (4). Moreover, almost all animal studies that tested the efficacy of several species from the genus as probiotics for T2D reported improvements of glucose parameters (1).

A previous report found decreased abundance in the mucus-degrading bacterium *A. municiphila* in patients with metabolic disorders, including obesity, impaired glucose tolerance, and diabetes, which were associated with insulin resistance, dyslipidemia, and overweight (83). Two other studies showed increased amounts of *A. municiphila* in newly diagnosed and prevalent T2D patients treated with metformin, which, however, did not provide pertinent information on metabolic parameters. The potential role of this mucus-degrading bacterium in ameliorating metabolic disorders was further confirmed by a series of animal experiments. For example, mice treated with metformin and liraglutide showed increased levels of *A. municiphila* in association with improved control of blood glucose and body weight (55, 65). More importantly, HFD-fed mice treated with *A. municiphila* exhibited similar improvements in glucose tolerance and goblet cell production and inflammatory regulations as compared to the metformin treatment group (56).

The effect of metformin and acarbose on the abundance of different species of the genus *Bacteroides* (phylum *Bacteroidetes*) is interesting. The genus *Bacteroides* seem to play a beneficial role in glucose metabolism where *B. intestinalis* and *B. vulgatus* were decreased in T2D patients, and *B. stercoris* was enriched in patients with diabetes remission (1). The same phenomenon was also noted in experimental animals (1). However, decreased abundance of some *Bacteroides* species, including *B. plebeius*, *B. dorei*, *B. vulgatus*, after using acarbose in newly diagnosed T2D patients was reported to be positively associated with body weight in one study (32). As for rodent studies, colonization of *B. fragilis* was associated with more severe glucose intolerance in HFD-fed mice (24). A recent study, which compared fecal microbiota compositions between T2D patients and non-diabetic individuals, showed that *Bacteroides* was an independent risk factor of the disease by diminishing insulin sensitivity (84).

The effects of metformin on *A. municiphila* were similar in both human and rodent studies. However, there was an inverse association of *Bacteroides* and metformin use in human and mouse studies in this review. Alterations of many other taxa in humans treated with metformin or acarbose were not the same as in mouse studies, and *vice versa*. The diverse dietary habits, metabolism or inflammatory statuses of host, body sizes and organs in these human and mouse studies might contribute to inconsistent findings of gut microbiota compositions (12, 85). Although the gut microbiota of human

---

**TABLE 10** | Association between specific taxa and human metabolic parameters.

| Parameters       | Association | Specific taxa                                                                 | Alteration | Drugs | Participants | References |
|------------------|-------------|-------------------------------------------------------------------------------|------------|-------|--------------|------------|
| HbA1C            | Negative    | G. *Escherichia*                                                             | ↑          | M     | Newly T2D    | (20)       |
|                  |             | G. *Shigella*                                                                | ↑          | M     | Newly T2D    | (21)       |
|                  |             | S. *Bifidobacterium adolescentis*                                            | ↑          | A     | Newly T2D    | (32)       |
|                  |             | S. *Lactobacillus gasseri*                                                   | ↑          | A     | Pre-diabetic | (7)        |
|                  |             | S. *Bifidobacterium longum*                                                  | ↑          | A     | Pre-diabetic | (7)        |
|                  |             | G. *Subdoligranum*                                                           | ↑          | M     | Newly T2D    | (20)       |
|                  |             | G. *Dialister*                                                               | ↑          | A     | Newly T2D    | (32)       |
| Fasting blood glucose | Negative | G. *Bifidobacterium adolescentis*                                             | ↑          | M     | Newly T2D    | (20)       |
| Body weight      | Positive    | S. *Bacteroides plebeius*                                                    | ↓          | A     | Newly T2D    | (32)       |
|                  | Negative    | S. *Bacteroides dorei*                                                       | ↑          | A     | Newly T2D    | (32)       |
|                  | Negative    | S. *Bacteroides vulgatus*                                                    | ↑          | A     | Newly T2D    | (32)       |
| HDL cholesterol  | Positive    | S. *Bifidobacterium longum*                                                  | ↑          | A     | Prevalent T2D | (33)       |
| LDL cholesterol  | Negative    | G. *Bifidobacterium adolescentis*                                             | ↑          | M     | Newly T2D    | (20)       |

G, genus; S, species; M, metformin; A, acarbose; ↑, significant increase; ↓, significant decrease.
| Parameters       | Association | Specific taxa                                              | Alteration | Drugs       | Models          | References |
|------------------|-------------|------------------------------------------------------------|------------|-------------|-----------------|------------|
| Fasting blood glucose | Positive     | S._Bacteroides spp.                                        | ↓          | M           | HFD/STZ mice    | (44)       |
|                  | Negative     | S._Akkermansia muciniphila                                 | ↑          | M           | HFD mice        | (55)       |
|                  |              | S._Bifidobacterium spp.                                    | ↓          | M           | HFD/HS rat      | (57)       |
| Body weight      | Positive     | G._Candidatus Ardenticatus                                 | ↓          | L           | HFD/STZ mice    | (70)       |
|                  |              | G._Roseburia                                              |            |             |                 |            |
|                  |              | G._Marvinbryantia                                         |            |             |                 |            |
|                  |              | S._Clostridia sp., S._Clostridales spp.                   | ↓          | L           | HFD mice        | (66)       |
|                  | Negative     | S._Akkermansia muciniphila                                 | ↑          | L           | HFD/STZ mice    | (70)       |
|                  |              | S._Bifidobacterium                                        | ↑          | L           | HFD mice        | (66)       |
| LDL cholesterol  | Positive     | G._Romboutsia                                             | ↓          | L           | HFD/GK rat      | (66)       |
|                  | Negative     | G._Prevotella                                             | ↑          | L           | HFD/GK rat      | (66)       |
| Total cholesterol| Positive     | S._Prevotella sp.                                         | ↓          | M           | OLETF rat       | (50)       |
|                  |              | S._Clostridia sp.                                         |            |             |                 |            |
|                  |              | S._Clostridales spp.                                      |            |             |                 |            |
|                  |              | S._Oscillospiraceae sp.                                   |            |             |                 |            |
|                  |              | S._Erysipelatoctoclostrum sp.                             |            |             |                 |            |
|                  | Negative     | G._Romboutsia                                             | ↑          | M           | HFD/GK rat      | (66)       |
|                  |              | S._Prevotella sp.                                         | ↑          | M           | HFD/GK rat      | (66)       |
| Triglyceride     | Positive     | G._Prevotella                                             | ↑          | L           | HFD/GK rat      | (66)       |
|                  | Negative     | G._Prevotella                                             | ↑          | L           | HFD/GK rat      | (66)       |

G, Genus; S, Species; M, metformin; Sa, saxagliptin; L, liraglutide; ↑, significantly increase; ↓, significantly decrease.

and mice are dominated by two major phyla, i.e., *Bacteroidetes* and *Firmicutes*, approximately 85% of the representative gut microbiota sequences in mice were not found in humans (86).

Further, the genera *Escherichia* and *Shigella*, belonging to the order of *Enterobacterales* in phylum *Proteobacteria*, were found to increase consistently after metformin treatment in T2D patients. Certain bacteria belonging to the phylum *Proteobacteria*, including the order *Enterobacterales*, was found to be overly present in patients with metabolic disorders and T2D, and were positively related to intestinal permeability and endotoxemia in the pathophysiology of these metabolic diseases (87, 88). Enrichment in the order *Enterobacterales*, especially *Escherichia coli*, was demonstrated to play an important role in gut inflammation in patients with inflammatory bowel disease and also in various mouse model of colitis (88). Elbere et al. (25) observed an association between the severity of gastrointestinal
side effects and increased abundance of the genera Escherichia and Shigella. Thus, the enrichment of the order Enterobacteriales might contribute to gastrointestinal side effects of metformin.

In this review, the results of β-diversity indicate significant changes in gut microbiome structure related to metformin or acarbose treatment. The findings on α-diversity among those treated with metformin were inconsistent while the richness and evenness were decreased after treating with acarbose. For healthy human subjects, the reference microbiome list and abundance evenness were decreased after treating with acarbose. For healthy human subjects, the reference microbiome list and abundance evenness were decreased after treating with acarbose. For healthy human subjects, the reference microbiome list and abundance evenness were decreased after treating with acarbose. For healthy human subjects, the reference microbiome list and abundance evenness were decreased after treating with acarbose.

While metformin and acarbose have been shown to stimulate the growth of SCFA-producing bacteria, e.g., Lactobacillus and Bifidobacterium, information on the effects of these drugs on the fecal levels of various SCFAs in humans is lacking in the literature. Thus, this review entailed data from mouse studies, which are more informative. These mouse studies showed an increase trend in fecal and cecal levels of acetate, propionate, and butyrate in response to treatments of metformin, acarbose, voglibose, dapagliflozin or canagliflozin, of which the impacts on other physiological functions, other than that in gastrointestinal track, warrant further evaluations (90). With respect to bile acids, information on the effects of anti-hyperglycemic agents on their levels is limited and often conflicting in human vs. rodent studies, which merits further investigations to understand the role of other confounding factors, such as diets, antibiotic therapy, and disease states (91).

Again, the most critical limitation is lack of consistency among human and rodent studies. In humans, differences in the health status of participants, disease type or staging, ethnicity, drug dosage, and duration of treatment might directly impact gut microbiota compositions. Furthermore, it becomes difficult to come to a conclusion due to the small number of participants as well as differences in study design in each study. The risk of bias of studies needs to be taken into considerations, in which overall bias of randomized trials was high risk and unclear risk, and two out of five quasi-experimental studies and all of observational studies were at serious risk. Also, there was a high degree of heterogeneity in rodent studies due to differences in species used and environmental factors, as alluded above. Differences in microbiota analysis methods could also be a cause of deviation.

Another major limitation is lack of human studies on anti-hyperglycemic drugs beyond metformin and acarbose. Thus, no conclusion could be reached regarding the associations between human gut microbiota and these drugs. Because results from rodent models might not always be translatable to humans, conclusions should be made with cautions.

Although findings from rodent studies included in this review suggest potential positive effects of other anti-hyperglycemic drugs besides metformin and acarbose on human gut microbiota, additional human studies on these drugs are needed to clarify the role of gut microbiota in their therapeutic efficacies.

In light of the enormous amounts of published data, this systematic review aims to provide readers a comprehensive view of this emerging area by taking an integrated approach through an all-inclusive literature search in conjunction with vigorous data extraction and validation, and assessment of risk bias. Moreover, this systematic review has tried to differentiate various aspects of the anti-hyperglycemic drug-gut microbiome-host axis, thereby filling the gap of merging all available data from human or animal studies relevant to the inter-dependence between anti-hyperglycemic drugs and the specific taxon of gut microbiota. Nevertheless, more investigations are warranted to support the positive contribution of metformin and acarbose to the health of gut microbiota (e.g., A. muciniphila, Lactobacillus, Bifidobacterium longum). In addition, given the limited information available in the literature, more studies are needed to shed light onto the roles of other anti-hyperglycemic drugs (e.g., miglitol, voglibose) in modulating human host taxa of gut microbiome.

**CONCLUSION**

This review highlights that changes in specific taxa and β-diversity of gut microbiota were associated with metformin and acarbose in humans while pertinent information for other anti-hyperglycemic drugs could be only obtained in rodent studies. These results support the possible action mechanisms of these drugs, which may have translational potential to foster new approaches for the treatment of diseases related to gut dysbiosis in the future. Mouse studies on the other anti-hyperglycemics suggested the links between these drugs and gut microbiota were inconclusive. Therefore, additional human studies are needed to explore the role of gut microbiota in their therapeutic efficacies or side effects.

**DATA AVAILABILITY STATEMENT**

The original data presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

TC, P-CL, H-WL, CC, C-SC, K-CW, J-LH, and L-YY collected, screened, and extracted the data and analyzed the results. TC, H-WL, CC, and L-YY wrote the first draft of the manuscript. All authors contributed to conception, design of the study, manuscript revision, read, and approved the submitted version.
ACKNOWLEDGMENTS

We would like to appreciate Shu-Han Yang, Fang-Chu Lin, Yu-Ching Wang, Yu-Tong Wang, Shang-Hua Wu, Po-Chen Chu, and those who were not listed but helped to search and review the literature in this project.

REFERENCES

1. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. ElBioMedicine. (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051
2. Qin J, Li Y, Cai Z, Li S, Zha J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. (2012) 490:55–60. doi: 10.1038/nature11450
3. Adeshirlarjanyan A, Gewirtz AT. Considering gut microbiota in treatment of type 2 diabetes mellitus. Gut microbes. (2020) 11:253–64. doi: 10.1080/19490970.2020.1717719
4. Tiderencel KA, Hutcheon DA, Ziegler J. Probiotics for the treatment of type 2 diabetes: a review of randomized controlled trials. Diabetes Metab Res Rev. (2020) 36:e3213. doi: 10.1002/dmr.3213
5. 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:e000717. doi: 10.1136/bmjdrc-2019-000717
6. Zhang Q, Xiao X, Li M, Yu M, Ping F, Zheng J, et al. Vildagliptin increases butyrate-producing bacteria in the gut of diabetic rats. PLoS ONE. (2017) 12:e0184735. doi: 10.1371/journal.pone.0184735
7. Zhang X, Fang Z, Zhang C, Xia H, Jie Z, Han X, et al. Effects of acarbose on the gut microbiota of prediabetic patients: a randomized, double-blind, controlled crossover trial. Diabetes Ther. (2017) 8:293–307. doi: 10.1007/s13300-017-0226-y
8. Whang A, Nagpal R, Yadav H. Bi-directional drug-microbiome interactions of anti-diabetics. ElBioMedicine. (2019) 39:591–602. doi: 10.1016/j.ebiom.2018.11.046
9. Kyriachenko Y, Falalyeyeva T, Korotkyi O, Molocheck N, Kobyliak N. Crosstalk between gut microbiota and anti-diabetic drug action. World J Diabetes. (2019) 10:154–68. doi: 10.4239/wjd.v10.i3.154
10. Stein SA, Lamos EM, Davis SN. A review of the efficacy and safety of oral antidiabetic drugs. Expert Opin Drug Saf. (2013) 12:153–75. doi: 10.1517/14740338.2013.752813
11. Montandon SA, Jornayvaz FR. Effects of antidiabetic drugs on gut microbiota composition. Genes. (2017) 8:259. doi: 10.3390/genes8100250
12. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Dis Model Mech. (2015) 8:1–16. doi: 10.1242/dmm.017400
13. Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. Curr Opin Pharmacol. (2019) 49:1–5. doi: 10.1016/j.coph.2019.03.011
14. Scells H, Zuccon G, CSIRO BK, Deacon A, Azzopardi L, Geva S. Integrating the framing of clinical questions via pico into the retrieval of medical literature for systematic reviews. In: Proceedings of the 2017 ACM on Conference on Information and Knowledge Management; 2017 November 6-10; Singapore. Association for Computing Machinery, New York, NY (2017). p. 2291–94.
15. Liberiati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. (2009) 339:b2700. doi: 10.1136/bmj.b2700
16. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The cochrane collaboration’s tool for assessing risk of bias in randomised trials. BMJ. (2011) 343:d5928. doi: 10.1136/bmj.d5928
17. Sterne JA, Hernan MA, Reeves BC, Savovic J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. (2016) 355:i4919. doi: 10.1136/bmj.i4919
18. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoiting M, Langendam MW. SYRCLE’s risk of bias tool for animal studies. BMC Med Res Methodol. (2014) 14:43. doi: 10.1186/1471-2288-14-43
19. Ehteshad HS, Tito RY, Siadat SD, Hasani-Ranjbar S, Hoseini-Tavassoli Z, Rymenans L, et al. Metformin induces weight loss associated with gut microbiota alteration in non-diabetic obese women: a randomized double-blind clinical trial. Eur J Endocrinol. (2019) 180:165–76. doi: 10.1530/EJE-18-0826
20. Tong X, Xu J, Lian F, Yu X, Zhao Y, Xu L, et al. Structural alteration of gut microbiota during the amelioration of human type 2 diabetes with hyperlipidemia by metformin and a traditional Chinese herbal formula: a multicenter, randomized, open label clinical trial. mBio. (2018) 9:e02392–17. doi: 10.1128/mBio.02392-17
21. Wu H, Esteve E, Treimalori V, Khan MT, Caesar R, Manniñás-Holm I, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med. (2017) 23:850–8. doi: 10.1038/nm.4345
22. Byrump T, Thomsen CW, Kern T, Allin KH, Brandslund I, Jørgensen NR, et al. Metformin-induced changes of the gut microbiota in healthy young men: results of a non-blinded, one-armed intervention study. Diabetologica. (2019) 62:1024–35. doi: 10.1007/s00125-019-4848-7
23. Huang F, Nilholm C, Roth B, Linnings C, Høglund P, Nyman M, et al. Anthropometric and metabolic improvements in human type 2 diabetes after introduction of an okinawan-based nordic diet are not associated with
changes in microbial diversity or SCFA concentrations. *Int J Food Sci Nutr.* (2018) 69:729–40. doi: 10.1080/09637486.2017.1408059

24. Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nat Med.* (2018) 24:1919–29. doi: 10.1038/s41591-018-0222-4

25. Elberse I, Kalina I, Silamikēls I, Konrade I, Zaharenko L, Sekace K, et al. Association of metformin administration with gut microbiome dysbiosis in healthy volunteers. *PLoS ONE.* (2018) 13:e0204317. doi: 10.1371/journal.pone.0204317

26. Napolitano A, Miller S, Nicholls AW, Baker D, Van Horn S, Thomas E, et al. Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. *PLoS ONE.* (2014) 9:e100778. doi: 10.1371/journal.pone.0100778

27. Zhang F, Wang M, Yang J, Xu Q, Liang C, Chen B, et al. Response of gut microbiota in type 2 diabetes to hypoglycemic agents. *Endocrine.* (2019) 66:485–93. doi: 10.1007/s12020-019-02041-5

28. Barenholts E, Green SJ, Eisenberg Y, Akbar A, Reddivari B, Layden BT, et al. Metformin alters gut microbiota of healthy mice: implication for its potential role in gut microbiota homeostasis. *Front Microbiol.* (2018) 9:1336. doi: 10.3389/fmicb.2018.01336

29. Liu G, Bei J, Liang L, Yu G, Li L, Li Q. Stachyose improves inflammation through modulating gut microbiota of high-fat diet/streptozotocin-induced type 2 diabetes in rats. *Mal Nutr Food Res.* (2018) 62:e1700954. doi: 10.1002/mnfr.201700954

30. Lee H, Lee Y, Kim J, An J, Lee S, Kong H, et al. Modulation of the gut microbiota by metformin improves metabolic profiles in aged obese mice. *Gut Microbes.* (2018) 9:155–65. doi: 10.1080/19490976.2017.1405209

31. Shin NR, Bose S, Wang JH, Ansari A, Lim SK, Chin YW, et al. Flos lonicera combined with metformin ameliorates hepatosteatosis and glucose intolerance in association with gut microbiota modulation. *Front Microbiol.* (2017) 8:2272. doi: 10.3389/fmicb.2017.02271

32. Wang JH, Bose S, Lim SK, Ansari A, Chin YW, Choi HS, et al. Houttuynia cordata facilitates metformin on ameliorating insulin resistance associated with gut microbiota alteration in OLETF rats. *Genes.* (2017) 8:239. doi: 10.3390/genes8100239

33. Han K, Bose S, Wang JH, Lim SK, Chin YW, Kim YM, et al. In vivo therapeutic effect of combination treatment with metformin and scutellaria baicalensis on maintaining bile acid homeostasis. *PLoS ONE.* (2017) 12:e0182467. doi: 10.1371/journal.pone.0182467

34. Salomäki-Mylari H, Vähähalo LH, Ailanen L, Pietilä S, Laiho A, Hanninen A, et al. Neuropeptide Y overexpressing female and male mice show divergent metabolic but not gut microbial responses to prenatal metformin exposure. *PLoS ONE.* (2016) 11:e0163805. doi: 10.1371/journal.pone.0163805

35. Zhou ZJ, Ren LW, Zhan P, Yang HY, Chai DD, Yu ZW. Metformin exerts glucose-lowering action in high-fat fed mice via attenuating endotoxemia and enhancing insulin sensitivity. *Acta Pharmacol Sin.* (2016) 37:1063–75. doi: 10.1038/aps.2016.21

36. Zhang X, Zhao Y, Xu J, Xue Z, Zhang M, Pang X, et al. Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. *Sci Rep.* (2015) 5:14405. doi: 10.1038/rep14405

37. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol.* (2014) 80:5935–43. doi: 10.1128/AEM.01537-14

38. Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, et al. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut.* (2014) 63:727–35. doi: 10.1136/gutjnl-2012-303839

39. Pyra KA, Saha DC, Reimer RA. Prebiotic fiber increases hepatic acetyl CoA carboxylase phosphorylation and suppresses glucose-dependent insulinotropic polypeptide secretion more effectively when used with metformin in obese rats. *J Nutr.* (2012) 142:213–20. doi: 10.3945/jn.111.147132

40. Zhao J, Li Y, Sun M, Xin I, Wang T, Wei L, et al. The Chinese herbal formula shenzhu tiaopi granule results in metabolic improvement in type 2 diabetic rats by modulating the gut microbiota. *Evid Based Complement Altern Med.* (2017) 2016:967694. doi: 10.1155/2015/6967684

41. Smith BJ, Miller RA, Ericsson AC, Harrison DC, Strong R, Schmidt TM. Changes in the gut microbiome and fermentation products concurrent
with enhanced longevity in acarbose-treated mice. BMC Microbiol. (2019) 19:130. doi: 10.1186/s12866-019-1494-7

60. Liao X, Song L, Zeng B, Liu B, Qiu Y, Qu H, et al. Alteration of gut microbiota induced by DPP-4i treatment improves glucose homeostasis. EbiolMedicine. (2019) 44:665–74. doi: 10.1016/j.ebiom.2019.03.057

61. Baxter NT, Leshniak NA, Sinani H, Schloss PD, Koropatkin NM. The glucoamylase inhibitor acarbose has a diet-dependent and reversible effect on the murine gut microbiome. mSphere. (2019) 4:e00528–18. doi: 10.1128/mSphere.00528-18

62. Xu GD, Cai L, Ni YS, Tian SY, Lu YQ, Wang LN, et al. Comparisons of effects on intestinal short-chain fatty acid concentration after exposure of two glycosidase inhibitors in mice. Bioll Pharm Bull. (2018) 41:1024–33. doi: 10.1248/bpb.b17-00978

63. Do HJ, Lee YS, Ha MJ, Cho Y, Yi H, Hwang YJ, et al. Beneficial effects of voglibose administration on body weight and lipid metabolism via gastrointestinal bile acid modification. Endocr J. (2016) 63:691–702. doi: 10.1507/endocrj.EJ15-0747

64. Kishida Y, Okubo H, Ohno H, Oki K, Yoneda M. Effect of miglitol on the inhibition via dapagliflozin improves generalized vascular dysfunc tion and progression of glucose intolerance and effect of sitagliptin: an anima l study. J Diabetes Res. (2019) 25:130. doi: 10.1186/s12866-019-0410-3

65. Wang LY, Wang S, Wang Y, Ruan KF, Feng Y. Effect of MDG-1 on oral glucose tolerance and intestinal microecological balance in diabetic mice. World Chin J Digestol. (2011) 19:2058–62. doi: 10.1169/wjcd.v19.i9.2058

66. Bytzer P, Talley NJ, Jones MP, Horowitz M. Oral hypoglycaemic drugs and gastrointestinal symptoms in diabetes mellitus. Aliment Pharmacol Ther. (2001) 15:137–42. doi: 10.1046/j.1365-2036.2001.00896.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Cao, Wu, Hsu, Chang, Chou, Lin, Liao, Yang and Lin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.