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Dysbiosis, obesity, and inflammation: Interrelated phenomena causes or effects of metabolic syndrome?

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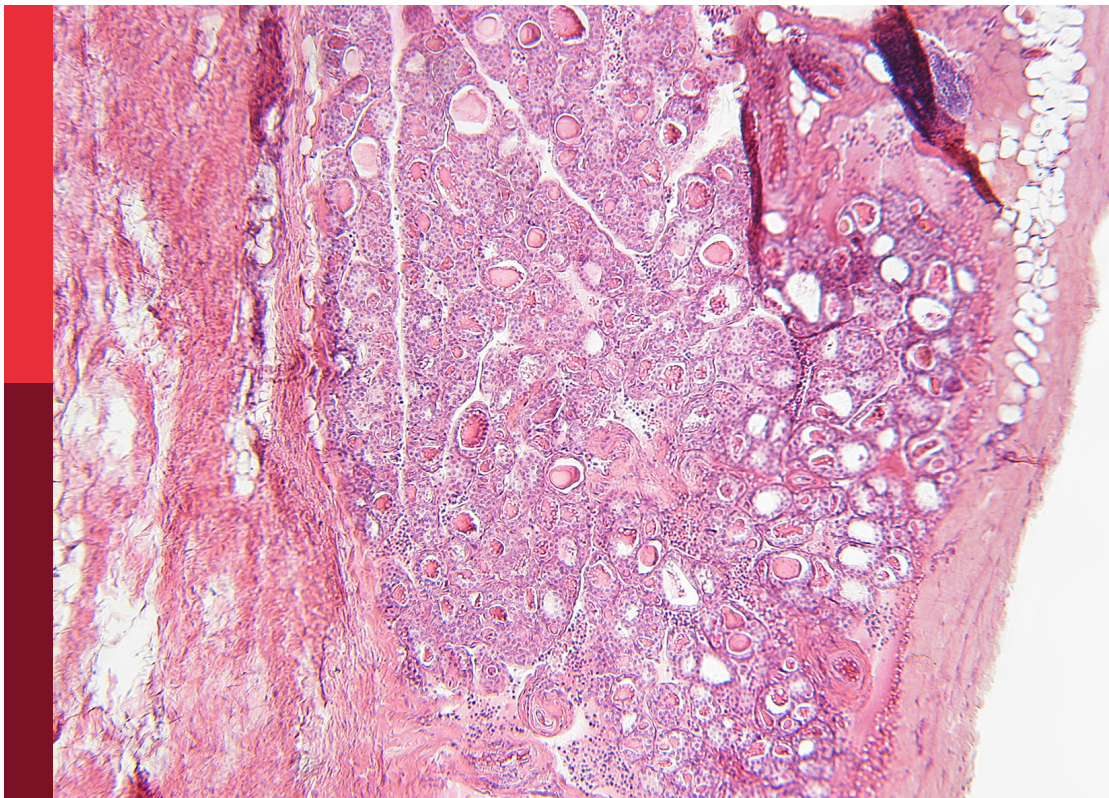

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Dysbiosis, obesity, and inflammation: Interrelated phenomena causes or effects of metabolic syndrome?

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Table of contents

05 Editorial: Dysbiosis, obesity, and inflammation: interrelated phenomena causes or effects of metabolic syndrome?
Kaiser Wani, Shakilur Rahman and Hossam Draz

09 Triglyceride-Rich Lipoproteins and Glycoprotein A and B Assessed by 1H-NMR in Metabolic-Associated Fatty Liver Disease
Juan Moreno-Vedia, Roser Rosales, Enrique Ozcariz, Dídac Llop, Maribel Lahuerta, María Benavent, Ricardo Rodríguez-Calvo, Núria Plana, Angels Pedragosa, Lluís Masana, Antoni Castro, Daiana Ibarretxe and Josefa Girona

20 Genetically Predicted Causality of 28 Gut Microbiome Families and Type 2 Diabetes Mellitus Risk
Kun Xiang, Jing-Jing Zhang, Yuan-Yuan Xu, Xing Zhong, Jing Ni and Hai-Feng Pan

27 Randomized Clinical Trial: Probiotics Alleviated Oral-Gut Microbiota Dysbiosis and Thyroid Hormone Withdrawal-Related Complications in Thyroid Cancer Patients Before Radioiodine Therapy Following Thyroidectomy
Baiqiang Lin, Fuya Zhao, Yang Liu, Xin Wu, Jing Feng, Xiangren Jin, Wei Yan, Xiao Guo, Shang Shi, Zhiyong Li, Lujia Liu, Hongye Chen, Haoran Wang, Shuang Wang, Yu Lu and Yunwei Wei

42 Composition of the Gut Microbiota in Attention Deficit Hyperactivity Disorder: A Systematic Review and Meta-Analysis
Ning Wang, Xuping Gao, Zifeng Zhang and Li Yang

61 Serum Bilirubin Level Is Increased in Metabolically Healthy Obesity
Jing Fu, Qiu Wang, Lin Zhang, Jia Liu and Guang Wang

67 Effects of Oral Glucose-Lowering Agents on Gut Microbiota and Microbial Metabolites
Dongmei Wang, Jieying Liu, Liyuan Zhou, Qian Zhang, Ming Li and Xinhua Xiao

83 Gut microbiota is associated with differential metabolic characteristics: A study on a defined cohort of Africans and Chinese
Paul Nizigiyimana, Boya Xu, Lerong Liu, Liping Luo, Tingting Liu, Meng Jiang, Zehao Liu, Changjun Li, Xianghang Luo and Minxiang Lei

98 Gut microbiota is correlated with gastrointestinal adverse events of metformin in patients with type 2 diabetes
Yuxin Huang, Xudan Lou, Cuiping Jiang, Xueying Ji, Xiaoming Tao, Jiao Sun and Zhijun Bao

112 Evidence for proton-pump inhibitor (PPI)-associated dysbiosis in metabolically unhealthy obesity
Melissa A. Burmeister, Tara E. Smith, Timothy K. Fincher and Abby J. Weldon

119 The gut-retina axis: a new perspective in the prevention and treatment of diabetic retinopathy
Haiyan Zhang and Ya Mo

129 Palmitoylethanolamide counteracts high-fat diet-induced gut dysfunction by reprogramming microbiota composition and affecting tryptophan metabolism
Claudio Pirozzi, Lorena Coretti, Nicola Opallo, Maria Bove, Chiara Annunziata, Federica Comella, Luigia Turco, Adriano Lama, Luigia Trabace, Rosaria Meli, Francesca Lembo and Giuseppina Mattace Raso

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Editorial: Dysbiosis, obesity, and inflammation: interrelated phenomena causes or effects of metabolic syndrome?

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Editorial on the Research Topic
Dysbiosis, obesity, and inflammation: interrelated phenomena causes or effects of metabolic syndrome?

Introduction

The gut microbiome, a diverse community of microorganisms in the gastrointestinal tract, plays a crucial role in metabolism and is associated with various metabolic disorders. Extensive evidence suggests that the composition of the gut microbiome is associated with various metabolic disorders (1, 2). However, there is no consensus on the causal link between gut microbiome and type 2 diabetes mellitus (T2DM), and the specific taxonomic groups responsible for T2DM remain unclear. Dysbiosis, referring to an imbalance or disruption in the gut microbiota composition, leads to increased levels of lipopolysaccharide (LPS), a bacterial toxin, in the body (3). LPS is a potent endotoxin present in the outer membrane of Gram-negative bacteria. It is a key player in inducing inflammation by activating the toll-like receptor 4 (TLR4) pathway, leading to the release of pro-inflammatory cytokines and chemokines that initiate and propagate the inflammatory response (4). Recent studies have highlighted the critical role of LPS-induced inflammation in various diseases, including sepsis, inflammatory bowel disease, and chronic inflammatory conditions (5).

Summary of articles published

In this Research Topic, we focused on the interactions between dysbiosis, obesity, inflammation, and metabolic parameters. The most common microvascular complication caused by inflammatory stress associated with metabolic disorders like diabetes mellitus is diabetic retinopathy (DR). Recent studies suggest that the gut microbiota is crucial to the

development of DR and is implicated in its pathophysiological processes (6, 7). On the one hand, several studies have shown how the gut bacteria contribute to retinal neurodegeneration (8) while on the other hand, some studies show that altered gut flora in these patients may contribute to or aggravate DR (9). A review published under this study topic by Zhang and Mo tries to emphasize the significant connection between DR and gut microbiota and the importance of gut dysbiosis in the emergence and progression of DR. Furthermore, it explores the concept of the gut-retina axis and the conditions of the gut-retina axis crosstalk, along with the process involved in modulating DR by the intestinal microbiota.

Metabolic disorders like obesity are associated with risks of developing gastrointestinal (GI) disease (10). High fat diet-induced obesity in mice promotes dysbiosis, causing a shift towards bacteria-derived metabolites which contributes to the onset and progression of GI disorders (11). Moreover, there are two categories of obesity: metabolically healthy and metabolically unhealthy. In contrast to metabolically healthy counterparts, obese individuals who are metabolically unhealthy display hallmark symptoms of the metabolic syndrome (e.g., hypertension, dyslipidemia, hyperglycemia, abdominal obesity) (12). Obesity is often also accompanied by gastroesophageal reflux disease (GERD) (13). Due to their widespread availability, proton-pump inhibitors (PPIs) are most frequently used to treat heartburn and other related symptoms associated with GERD (14). A review article published in this Research Topic by Burmeister et al. discuss how a poor diet, along with both short-term and long-term PPI usage, negatively impacts the GI microbiota to cause dysbiosis. Furthermore, this article also covers the advantage of taking probiotics to mitigate PPI-induced dysbiosis and metabolically unhealthy obesity (MUO).

High plasma triglyceride levels and chronic inflammation are important factors in metabolic-associated fatty liver disease (15, 16). Elevated triglyceride levels contribute to the accumulation of fat in the liver, while chronic inflammation exacerbates liver damage and promotes the transformation of fatty liver to more severe stages of the disease (17). Moreno-Vedia et al. in a study published under this topic used nuclear magnetic resonance (1H-NMR) to examine triglyceride-rich lipoprotein (TLR) and glycoprotein profiles in 280 patients with metabolic disease. TRL concentrations were associated with glycoproteins and liver function. Follow-up revealed new cases of fatty liver associated with baseline TRL particle numbers and glycoprotein levels. Higher TRL levels were observed in patients with hepatic steatosis, and baseline TRL particles and glycoproteins were associated with the development of metabolic-associated fatty liver disease (MAFLD). The findings suggest that TLR measurements could serve as predictive biomarkers for hepatic disease.

An article by Wang et al., published under this Research Topic, reviewed the effects of oral glucose-lowering drugs on gut microbiota and microbial metabolites. Increasing evidence suggests that oral glucose-lowering drugs modulate the gut microbiome and alter GI metabolites. Antidiabetic medication such as metformin and sulfonylurea modify the intestinal flora in T2DM in clinical research and experimental animal studies (18–20). This review also highlights the future perspective of these drugs, such as combination therapies including pre- and pro-biotics intervention in T2DM. Another study under this Research Topic explored the associations between gut microbiota and glucose metabolism in a cohort of African and Chinese healthy individuals (Nizigiyimana et al.). Microbiota diversity, richness, and composition were higher in the African group and lower in the Chinese group. The phylum *Bacteroidetes* was significantly more abundant in the Chinese group. In contrast, the phylum *Verrucomicrobia* was significantly more prevalent in the African group. Gut microbiota also correlated with parameters of glucose metabolism. The data suggest that there is an interaction between gut microbiota, and glucometabolic pathways.

Probiotic administration significantly reduces faecal and plasma concentrations of LPS in patients by reducing LPS producing bacteria and related synthesis pathways (21). Probiotics, such as *Lactobacillus* and *Bifidobacterium*, protect the gut barrier by enhancing the expression of tight junction proteins and reducing inflammation (22, 23). This concept was utilized in a randomized clinical trial by Lin et al., published under this topic, where probiotics were administered to assess their effects on alleviating postoperative complications from thyroid hormone withdrawal (THW) in thyroid cancer patients. Probiotics showed promising results in reducing complications, including lack of energy, constipation, weight gain, and dry mouth, and improving lipid indicators. They also restored gut and oral microbial diversity by increasing beneficial bacteria and reducing harmful ones. This study thus highlighted the potential of probiotics in managing THW-related complications through microbiota modulation.

Palmitoylethanolamide (PEA) is an endogenous lipid mediator that exerts anti-inflammatory effects by targeting various pathways involved in inflammation. It interacts with peroxisome proliferator-activated receptors (PPARs), leading to the downregulation of pro-inflammatory genes and the upregulation of anti-inflammatory genes (24). Additionally, PEA can inhibit immune cell recruitment promoting the synthesis of serotonin and other anti-inflammatory compounds, which collectively contribute to its anti-inflammatory properties (25). In this study topic, an article published by Pirozzi et al. also showed that PEA reduced intestinal immune cell recruitment, inflammatory response triggered by high fat diet, and corticotropin releasing hormone levels. It suggested that PEA also altered tryptophan metabolism and promotes serotonin synthesis through increased butyrate-producing bacteria, such as *Bifidobacterium, Oscillospiraceae and Turicibacter sanguinis*.

Bilirubin, a byproduct of heme metabolism, has various metabolic advantages (26–28). The link between bilirubin and metabolically healthy obesity (MHO), however, is not frequently documented. The article published here by Fu et al. elucidates the associations between serum bilirubin levels and metabolic parameters in different obesity phenotypes. For this, amongst 1,042 participants, 541 were obese patients and 501 were healthy control subjects. The obese patients were further divided into MHO group and metabolically unhealthy obesity (MUHO) group according to the levels of fasting plasma glucose (FBG), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and blood pressure (BP). It was observed that compared with MUHO group, MHO group had favorable BP, glucose and lipid

profiles, apart from increased total bilirubin (TBil) and direct bilirubin (DBil) levels, and decreased hsCRP and HOMA-IR levels. Multivariate regression analysis shows that HOMA-IR is independently correlated with TBil and DBil levels.

Conclusion

This editorial focuses on findings published in the Research Topic "*Dysbiosis, Obesity, and Inflammation: Interrelated Phenomena Causes or Effects of Metabolic Syndrome?*". Recent evidence supports the significant role of gut microbiota in diabetic retinopathy and other metabolic complications. Additionally, it discusses the therapeutic potential of probiotics and endogenous lipid mediator, palmitoylethanolamide, in reducing inflammation and managing metabolic diseases. Moreover, it explores the associations between elevated triglyceride levels, chronic inflammation, and metabolic-associated fatty liver disease. Overall, this Research Topic provides valuable insights into the gut microbiota's impact on metabolic health and potential interventions for metabolic disorders.

Author contributions

KW: Conceptualization, Writing – original draft, Writing – review & editing. SR: Writing – original draft, Writing – review & editing. HD: Writing – original draft, Writing – review & editing.

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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.

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References

1. Zhang L, Liu Y, Sun Y, Zhang X. Combined physical exercise and diet: regulation of gut microbiota to prevent and treat of metabolic disease: A review. *Nutrients* (2022) 14:4774. doi: 10.3390/nu14224774

2. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, et al. Gut microbiota, obesity and diabetes. *Postgrad Med J* (2016) 92:286–300. doi: 10.1136/postgradmedj-2015-133285

3. Anto L, Blesso CN. Interplay between diet, the gut microbiome, and atherosclerosis: Role of dysbiosis and microbial metabolites on inflammation and disordered lipid metabolism. *J Nutr Biochem* (2022) 105:108991. doi: 10.1016/j.jnutbio.2022.108991

4. Mohammad S, Thiemermann C. Role of metabolic endotoxemia in systemic inflammation and potential interventions. *Front Immunol* (2021) 11:594150. doi: 10.3389/fimmu.2020.594150

5. Moseley CE, Webster RG, Aldridge JR. Peroxisome proliferator-activated receptor and lipopolysaccharide signaling in sepsis-induced inflammation and injury. *Am J Respir Cell Mol Biol* (2021) 64:577–88. doi: 10.1111/j.1750-2659.2010.00155.x

6. Rowan S, Taylor A. The role of microbiota in retinal disease. *Adv Exp Med Biol* (2018) 1074:429–35. doi: 10.1007/978-3-319-75402-4_53

7. Liu K, Zou J, Fan H, Hu H, You Z. Causal effects of gut microbiota on diabetic retinopathy: A Mendelian randomization study. *Front Immunol* (2022) 13:930318. doi: 10.3389/fimmu.2022.930318

8. Dong L, Zhang Z, Liu X, Wang Q, Hong Y, Li X, et al. RNA sequencing reveals BMP4 as a basis for the dual-target treatment of diabetic retinopathy. *J Mol Med (Berl)* (2021) 99:225–40. doi: 10.1007/s00109-020-01995-8

9. Das T, Jayasudha R, Chakravarthy S, Prashanthi GS, Bhargava A, Tyagi M, et al. Alterations in the gut bacterial microbiome in people with type 2 diabetes mellitus and diabetic retinopathy. *Sci Rep* (2021) 11:2738. doi: 10.1038/s41598-021-82538-0

10. Emerenziani S, Guarino MPL, Trillo Asensio LM, Altomare A, Ribolsi M, Balestrieri P, et al. Role of overweight and obesity in gastrointestinal disease. *Nutrients* (2019) 12:111. doi: 10.3390/nu12010111

11. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cell* (2016) 167:915–32. doi: 10.1016/j.cell.2016.10.027

12. Iacobini C, Pugliese G, Blasetti Fantauzzi C, Federici M, Menini S. Metabolically healthy versus metabolically unhealthy obesity. *Metabolism* (2019) 92:51–60. doi: 10.1016/j.metabol.2018.11.009

13. El-Serag HB, Graham DY, Satia JA, Rabeneck L. Obesity is an independent risk factor for GERD symptoms and erosive esophagitis. *Am J Gastroenterol* (2005) 100:1243–50. doi: 10.1111/j.1572-0241.2005.41703.x

14. Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Camargo CA Jr. Body-mass index and symptoms of gastroesophageal reflux in women. *N Engl J Med* (2006) 354:2340–8. doi: 10.1056/NEJMoa054391

15. Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. *Mol Metab* (2021) 50:101238. doi: 10.1016/j.molmet.2021.101238

16. Tilg H, Effenberger M. From NAFLD to MAFLD: when pathophysiology succeeds. *Nat Rev Gastroenterol Hepatol* (2020) 17:387–8. doi: 10.1038/s41575-020-0316-6

17. Peiseler M, Schwabe R, Hampe J, Kubes P, Heikenwaelder M, Tacke F. Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease–novel insights into cellular communication circuits. *J Hepatol* (2022) 77 (4):1136–60. doi: 10.1016/j

18. 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

19. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Manneras-Holm L, 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

20. Huo T, Xiong Z, Lu X, Cai S. Metabonomic study of biochemical changes in urinary of type 2 diabetes mellitus patients after the treatment of sulfonylurea antidiabetic drugs based on ultra-performance liquid chromatography/mass spectrometry. *BioMed Chromatogr* (2015) 29:115–22. doi: 10.1002/bmc.3247

21. Hutchinson AN, Tingo L, Brummer RJ. The potential effects of probiotics and omega-3 fatty acids on chronic low-grade inflammation. *Nutrients* (2020) 12. doi: 10.3390/nu12082402

22. Rao RK, Samak G. Protection and restitution of gut barrier by probiotics: nutritional and clinical implications. *Curr Nutr Food Sci* (2013) 9:99–107. doi: 10.2174/1573401311309020004

23. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* (2011) 141:769–76. doi: 10.3945/jn.110.135657

24. Skaper SD, Facci L, Fusco M, Della Valle MF, Zusso M, Costa B. Palmitoylethanolamide, a naturally occurring disease-modifying agent in neuropathic pain. *Inflammopharmacology* (2021) 29:35–47. doi: 10.1007/s10787-013-0191-7

25. Keppel Hesselink JM, Kopsky DJ, Paladini A. Palmitoylethanolamide, a neutraceutical, in nerve compression syndromes: efficacy and safety in sciatic pain and carpal tunnel syndrome. *J Pain Res* (2020) 13:715–27. doi: 10.2147/JPR.S93106

26. Hinds TD Jr., Stec DE. Bilirubin, a cardiometabolic signaling molecule. *Hypertension* (2018) 72:788–95. doi: 10.1161/HYPERTENSIONAHA.118.11130

27. Jung CH, Lee MJ, Kang YM, Hwang JY, Jang JE, Leem J, et al. Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: a 4 year retrospective longitudinal study. *Metabolism* (2014) 63:87–93. doi: 10.1016/j.metabol.2013.09.011

28. Tian J, Zhong R, Liu C, Tang Y, Gong J, Chang J, et al. Association between bilirubin and risk of Non-Alcoholic Fatty Liver Disease based on a prospective cohort study. *Sci Rep* (2016) 6:31006. doi: 10.1038/srep31006

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Triglyceride-Rich Lipoproteins and Glycoprotein A and B Assessed by 1H-NMR in Metabolic-Associated Fatty Liver Disease

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High plasma triglyceride (TG) levels and chronic inflammation are important factors related to metabolic-associated fatty liver disease in patients at cardiovascular risk. Using nuclear magnetic resonance (^{1}H-NMR), we aimed to study the triglyceride-rich lipoprotein (TRL) and acute-phase glycoprotein profiles of a cohort of patients with metabolic disease and their relationship with fatty liver. Plasma samples of 280 patients (type 2 diabetes, 81.1%; obesity, 63.3%; and metabolic syndrome, 91.8%) from the University Hospital Lipid Unit were collected for the measurement of small, medium and large TRL particle numbers and sizes and glycoprotein profiles (Glyc-A and Glyc-B) by ^{1}H-NMR. Liver function parameters, including the fatty liver index (FLI) and fibrosis-4 (FIB-4) score, were assessed. Hepatic echography assessment was performed in 100 patients, and they were followed up for 10 years. TRL particle concentrations showed a strong positive association with Glyc-A and Glyc-B (ρ=0.895 and ρ=0.654, p<0.001, respectively) and with the liver function-related proteins ALT ρ=0.293, p<0.001), AST (ρ=0.318, p<0.001) and GGT (ρ=0.284, p<0.001). Likewise, TRL concentrations showed a positive association with FLI (ρ=0.425, p<0.001) but not with FIB-4. During the follow-up period of 10 years, 18 new cases of steatosis were observed among 64 patients who were disease-free at baseline. Baseline TRL particle numbers and glycoprotein levels were associated with the new development of metabolic-associated fatty liver disease (MAFLD) (AUC=0.692, p=0.018 and AUC=0.669, p=0.037, respectively). Overall, our results indicated that TRL number and acute-phase glycoproteins measured by ^{1}H-NMR could be potential biomarkers of the development of hepatic steatosis in patients at metabolic risk.

Keywords: triglyceride-rich lipoproteins, glycoproteins, NMR, metabolic-associated fatty liver disease, cardiovascular risk

INTRODUCTION

Triglycerides (TGs) are important cardiovascular risk factors (1). These lipids are transported mainly by triglyceride-rich lipoproteins (TRLs), a group of circulating lipoproteins that include chylomicrons (only present postprandially or in pathological conditions), VLDL and their remnants, and IDL. Therefore, TRLs are a group of lipoproteins with a high TG content. Increases in TG levels and, thus, in TRL particle numbers, are associated with cardiovascular risk and metabolic disorders (2, 3).

Hypertriglyceridemia is a common component of metabolic alterations such as type 2 diabetes (T2DM), metabolic syndrome and obesity. Under these conditions, the development of insulin resistance leads to hepatic TG accumulation due to an enhanced FA uptake into the liver and an increased *de novo* lipogenesis, enhancing TG availability. The imbalance between VLDL oversynthesis and the secretion of larger $VLDL_1$ particles in the abovementioned metabolic diseases leads to both ectopic hepatic fat accumulation and higher plasma TG concentrations, leading to fatty liver disease (3, 4). These metabolic alterations are also associated with a low degree of chronic systemic inflammation along with local liver inflammation secondary in part to lipotoxicity. These highly prevalent conditions lead to metabolic-associated fatty liver diseases (MAFLDs), cirrhosis or even liver cancer, among others (5, 6).

Recently, as previously discussed (7), MAFLD has become a major cause of chronic liver disease worldwide, and it has become a challenge to public health, with an estimated prevalence of 25% worldwide and 23% in Europe (8). It is defined as the accumulation of fat in the liver in the presence of metabolic dysfunction and can range from simple steatosis, with or without mild nonspecific inflammation, to steatohepatitis characterized by the presence of inflammation and hepatocyte damage that can eventually lead to progressive fibrosis and cirrhosis (9, 10). Considering the vital role of the liver in lipid metabolism (including the uptake and secretion of plasma lipoproteins) (11) and its central role in the inflammatory cascade, hepatic alterations could be expected as a consequence of a liver overloaded with fat. Moreover, all components of metabolic syndrome (Met-S) correlate with liver fat content, with insulin resistance being one of the main pathogenic factors of hepatic fat accumulation. Such metabolic comorbidities are known to generate multiple signals in an inflammatory environment/status that can contribute to liver damage (12, 13).

New biomarkers, such as plasma acute-phase glycoproteins, which can be detected by ^{1}H-NMR as glycoproteins A and B, have emerged as promising tools to detect inflammatory patterns. Human plasma acute-phase glycoproteins are synthesized by the liver and induced by inflammatory cytokines such as IL-1, IL-6 or tumor necrosis factor, representing a systemic inflammatory response (14). They are almost all N-linked glycoproteins containing oligosaccharide chains attached to asparagine residues (15). ^{1}H-NMR detects the signal produced by the acetyl groups (-COCH3) of N-acetylglucosamine and N-acetylgalactosamine (Glyc-A) and N-acetylneuraminic acid (Glyc-B). Glyc-A and B are composite biomarkers that integrate the protein levels and glycation states of several of the most abundant acute phase proteins in the serum, including alpha-1-acid glycoprotein (AGP), alpha-1-antitrypsin (AAT), alpha-1-antichymotrypsin (AACT), haptoglobin, and transferrin. This makes them a more stable measure of inflammatory status with less intraindividual variability than other biomarkers, such as hsCRP (16, 17).

Glycoproteins A and B have been reported to be elevated in subclinical chronic inflammatory states such as obesity, diabetes, chronic infections or autoimmune diseases and are known to be strong biomarkers of cardiovascular diseases (18–20). The detection of glycation patterns, as well as the determination of acute-phase glycoprotein serum concentrations, has provided new insights into the field of liver disease, with special interest in their possible applications to distinguish the presence of inflammation during the course of the disease (21–24). In this field, ^{1}H-NRM has emerged as a promising strategy to measure plasma levels of glycoprotein-related signals and patterns (16).

In the present study, we aimed to describe the type of TRL particle fraction and the acute phase protein profile, both determined by ^{1}H-NMR in a cohort of dysmetabolic patients and their association with hepatic damage. Considering the inflammatory status related to metabolic syndrome and hepatic steatosis, we investigated the possible role of glycoproteins A and B in the detection of hepatic disease and their possible predictive capacities over a 10-year follow-up.

MATERIALS AND METHODS

Design and Study Subjects

We performed a baseline cross-sectional and a retrospective/prospective study at the 10-year follow-up.

At baseline, we included 280 patients who were willing to participate that were attending the Lipid Unit of our University Hospital due to lipid metabolism disturbances and associated disorders such as obesity, T2DM and Met-S. Obesity, T2DM and Met-S were diagnosed according to standard clinical criteria. Subjects with chronic lung or renal diseases or cancer were excluded. Patients on lipid-lowering drugs underwent a 6-week wash-out period (8 weeks if they were on fibrates). Anamnesis, anthropometric, and physical examination data were recorded. Liver ultrasound (i.e., greyscale abdominal ultrasound evaluation of the liver) was performed at baseline for 100 patients to evaluate the presence of hepatic steatosis. Hepatic steatosis was defined by an increased echogenicity of the hepatic parenchyma, which provides a brighter image than the kidney's cortex (25).

For the prospective study, we studied the association between the baseline data and liver ultrasound echography data obtained after 10 years of follow-up for 64 patients who were free of hepatic steatosis at baseline.

This study was approved by the Ethical and Clinical Investigation Committee of the Pere Virgili Institute for Health Research (IISPV) and fulfilled the principles of the Helsinki Declaration. A written consent form was signed by all participants.

Non-Invasive Fatty Liver Disease Indexes

The fatty liver index (FLI) was calculated based on the body mass index (BMI), waist circumference, triglycerides and γ-glutamyltransferase (GGT) using the following formula:

$$FLI = \frac{(e^{0.953\times log\ e(triglycerides)+0.139\times BMI+0.718\times log\ e(GGT)+0.053\times waistcircumferecence-15.745)}}{1+(e^{0.953\times log\ e(triglycerides)+0.139\times BMI+0.718\times log\ e(GGT)+0.053\times waistcircumferecence-15.745)}}\times 100$$

as described previously (26). The fibrosis 4 score (FIB4) was calculated based on age (years), AST and ALT levels (U/L), and platelet counts (10^9/L) using the following formula (27):

$$FIB4 = \frac{Age \times AST}{Platelet\ \ count \times \sqrt{ALT}}$$

Clinical and Standard Biochemical Analysis

Anamnesis and anthropometric data, including sex, age, clinical history and medication, were recorded and included in our database. BMI was calculated from the weight and height measurements (kg/m^2). A blood sample was obtained from each patient after overnight fasting. Aliquots were prepared for immediate storage at -80°C in the BioBank at our centre prior to use. Standard biochemical parameters, including lipids, apolipoproteins, blood glucose, hsCRP, and transaminases, were measured using colorimetric, enzymatic and immunoturbidimetric assays (Spinreact, SA, Spain; Horiba, SA, Spain), which were adapted to the Cobas Mira Plus Autoanalyser (Roche Diagnostics, Spain).

TRL Particle Analysis by ^{1}H-NMR

The TRL particle number and size were assessed by the Liposcale test®, which is a new generation 2D-^{1}H-NMR test developed with the collaboration of our group (28). In brief, 200 µl of serum was diluted with 50 µl of deuterated water and 300 µl of 50 mM phosphate buffer solution (PBS) at pH 7.4. ^{1}H-NMR spectra were recorded at 305.95 K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600.20 MHz (14.1 T). The particle size (Z) and particle number concentration (P) of three subtypes of TRLs (including large, medium, small and total TRLs) were analysed. Particle concentrations and diffusion coefficients were obtained from the measured distinct methyl groups of the 2D ^{1}H-NMR spectra after the deconvolution analysis of the signals of the NMR pulse. The methyl signal was surface fitted with the Lorentzian functions associated with each lipoprotein subtype. The area of each Lorentzian function reflected the lipid concentration of each subtype, and the size of each subtype was calculated from the diffusion coefficient. The particle number of each TRL subtype was calculated by dividing the lipid volume by the particle volume of a given class. Lipid volumes were determined using common conversion factors to convert the concentration units into volume units. The variation coefficients for particle number were between 2% and 4%. The variation coefficients for particle size were lower than 0.3%.

Glycoprotein Analysis by ^{1}H-NMR

The same processing prior to NMR analysis was performed for plasma glycoprotein analysis, following previously reported procedures (18). Briefly, the region of the ^{1}H-NMR spectrum where the glycoproteins resonate (2.15–1.90 ppm) was analysed using several functions according to the chemical shift: Glyc-A and Glyc-B. For each function, we determined the total area and transformed it to concentration according to the number of sugar–protein bonds. The area, height, position, and bandwidth and their ratios were also calculated. The concentrations of Glyc-A and Glyc-B provided the amount of acetyl groups of protein bond N-acetylglucosamine, N-acetylgalactosamine (Glyc-A), and N-acetylneuraminic acid (Glyc-B), the predominant sialic acid found (16).

Statistical Analysis

The normality of continuous variables was determined by the Kolmogorov–Smirnov test. Data are presented as the medians and 25th and 75th percentiles (IQR) for continuous variables not normally distributed or the mean and standard deviation (SD) when normally distributed. Categorical variables are expressed as percentages unless otherwise indicated. Differences between groups were evaluated by t-tests or Mann-Whitney U tests. Associations between the variables were analysed by Spearman's test, partial correlations and univariate regression analysis. Multivariate linear regression analysis was used to analyze the association of TRL-P with glycoproteins. The model included glycoproteins, age, BMI, hsCRP, ALT, AST, GGT, systolic BP, glucose, total cholesterol and sex as covariates. Linear multivariate models were performed in order to study the association between hepatic steatosis with biochemical data, hepatic indexes, TRL lipidomics and inflammation parameters. These associations were adjusted for known confounders to avoid spurious associations. Random forest classification models (RF) were performed based on conditional inference trees to evaluate the importance of each variable in the decision method, which was represented in terms of the mean decrease Gini plot. All statistical analyses were performed using SPSS software (IBM SPSS Statistics, version 27.0.1.0, Madrid, Spain) and R Studio (version 3.6). Statistical tests $p < 0.05$ were defined as significant.

RESULTS

Participant's Characteristics

Our study included 280 patients, with a median age of 61 (52-66) years, of whom 48.9% were female. Type 2 diabetes was present in 81.1%, obesity in 63.3% and Met-S in 91.8% of the participants. **Table 1** summarizes the clinical, anthropometric, and biochemical characteristics of the patients grouped by sex. The women were older than the men (p=0.023). The men had a higher waist circumference, diastolic BP and glucose levels than the women (p<0.05). The plasma lipid profile showed significant differences, with TGs being higher in men (p<0.001) and LDL-C and HDL-C being higher in women (p<0.001). The fatty liver index (FLI, p=0.026) and liver function-related transaminases (p<0.001) were found to be higher in men than in women. All parameters of the TRL lipidomics were higher in men

TABLE 1 | Clinical, anthropometric, and biochemical characteristics of the study population grouped by sex.

	Female n=137	Male n=143	p-value
Clinical data			
Age, years	62 (55-67)	58 (50-65)	**0.023**
BMI, kg/m^2	32.6 (28.7-37.5)	31.1 (29.1-34.8)	0.178
Waist circumference, cm	103 (96-115)	107.5 (102-113)	**0.024**
Systolic BP, mmHg	140 (130-150)	138 (130-152)	0.882
Diastolic BP, mmHg	80 (72-85)	82 (76-89)	**0.029**
Obesity, %	61.3	65.2	0.496
Type 2 diabetes, %	79.6	82.5	0.528
Metabolic syndrome, %	92.0	91.5	0.898
Hypertension, %	61.8	59.3	0.674
Biochemical data			
Total cholesterol, mmol/L	5.85 (5.12-6,98)	5.54 (4.75-6.95)	0.177
Triglycerides, mmol/L	1.69 (1.25-2.62)	2.26 (1.48-4.27)	**<0.001**
LDL-C, mmol/L	3.79 ± 1.02	3.31 ± 1.22	**<0.001**
HDL-C, mmol/L	1.26 (1.05-1.38)	0.98 (0.87-1.16)	**<0.001**
Apo B-100, mg/dL	121 (101-145)	115 (97-141)	0.201
Apo A-I, mg/dL	129 (111-149)	115 (101-129)	**<0.001**
Glucose, mg/dL	133 (107-161)	145 (117-178)	**0.016**
HbA_{1c}, %	6.3 (5.6-7.45)	6.4 (5.7-7.3)	0.748
AST, U/L	22 (18-28)	26 (21-32)	**<0.001**
ALT, U/L	17 (12-24)	23 (16-35)	**<0.001**
GGT, U/L	23 (16-38)	31 (20-51)	**<0.001**
Hepatic indexes			
FLI, %	80.6 (50.9-95.4)	87.3 (72.9-96)	**0.026**
FIB-4	1.66 (1.31-1.96)	1.53 (1.25-1.98)	0.421
TRL lipidomics			
Total TRL-P, nmol/L	61.2 (44.3-98.3)	83.2 (52.1-122.9)	**0.004**
Large TRL-P, nmol/L	1.39 (1.04-2.08)	1.86 (1.24-2.78)	**0.001**
Medium TRL-P, nmol/L	7.89 (5.49-12.9)	10.6 (6.34-16.9)	**0.004**
Small TRL-P, nmol/L	52.1 (38.0-82.8)	70.6 (44.1-99.6)	**0.004**
TRL-Z (nm)	42.31 (42.23-42.38)	42.35(42.25-42.44)	**0.007**
Inflammation parameters			
Glyc-A, µmol/L	892.1 (769.3-1093.9)	957.4 (795.9-1149.2)	0.149
Glyc-B, µmol/L	367.4 (334.0-401.4)	364 (323.6-419.8)	0.975
hsCRP, mg/L	2.59 (1.57-3.96)	2.05 (1.17-3.44)	**0.017**

Data are the means ± SD for normally distributed variables, medians (IQR) for nonparametric data or n (%). BMI, body mass index; systolic BP, systolic blood pressure; diastolic BP, diastolic blood pressure; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; Apo B-100, apolipoprotein B100; Apo A-I, apolipoprotein A1; HbA_{1c}, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, fatty liver index; FIB-4, fibrosis-4 score; TRL, triglyceride-rich lipoprotein; hsCRP, high-sensitivity C-reactive protein. p values are for group comparisons. Statistical analysis: $\chi 2$ for categorical data; t-tests or Mann-Whitney U tests were used for continuous variables.
Bold values indicate p < 0.05.

(p<0.05). The women had a higher hsCRP than the men (p=0.017).

Association of TRL-P With Plasma Glycoproteins and Hepatic Biomarkers

Positive correlations were found between total TRL-P and the NMR-measured glycoproteins Glyc-A and Glyc-B (ρ=0.895 and ρ=0.654, p<0.001, respectively). These positive associations were observed between the different TRL subclass particles measured, including large, medium and small particles (**Figure 1**). No correlation was found between total (ρ=0.094, p=0.192) or subclass TRL particles and hsCRP (**Figure 1**).

To further explore the relationship between TRL-P and ^{1}H-NMR-measured glycoproteins, a multivariate linear regression analysis that included the variables age, BMI, hsCRP, ALT, AST, GGT, systolic BP, glucose, total cholesterol, sex, Glyc-A and Glyc-B was generated (**Supplementary Table 1**). The significant association between TRL-P and glycoproteins remained robust after adjustment for all covariates (p<0.001).

Table 2 shows the univariate associations of plasma TG, LDL-C, non-HDL-C, Apo B-100, TRL-P and glycoproteins with the hepatic indexes and profile. TRL-P was significantly positively associated with AST, ALT, GGT and FLI (p<0.05). Additionally, the ^{1}H-NMR-measured glycoproteins were significantly positively associated with FLI, ALT and GGT (p<0.05). After adjusting for sex, age and BMI, all TRL and glycoprotein correlations remained significant, while all hsCRP correlations, except for GGT, were lost. Likewise, Glyc-A showed a strong positive association with plasma TGs, non-HDL-C and Apo B-100 (ρ=0.804, ρ=0.479 and ρ=0.390, p<0.001 respectively), whereas no significant association was found with LDL-C. Similar associations were also observed with Glyc-B (ρ=0.583, p<0.001 for TGs; ρ=0.239, p<0.001 for non-HDL-C, and ρ=0.186, p=0.002 for Apo B-100).

TRL and Glycoprotein Profile in Ultrasound-Confirmed Hepatic Steatosis

The presence of hepatic steatosis was assessed by ultrasound echography in 100 patients in our cohort. Hepatic steatosis was

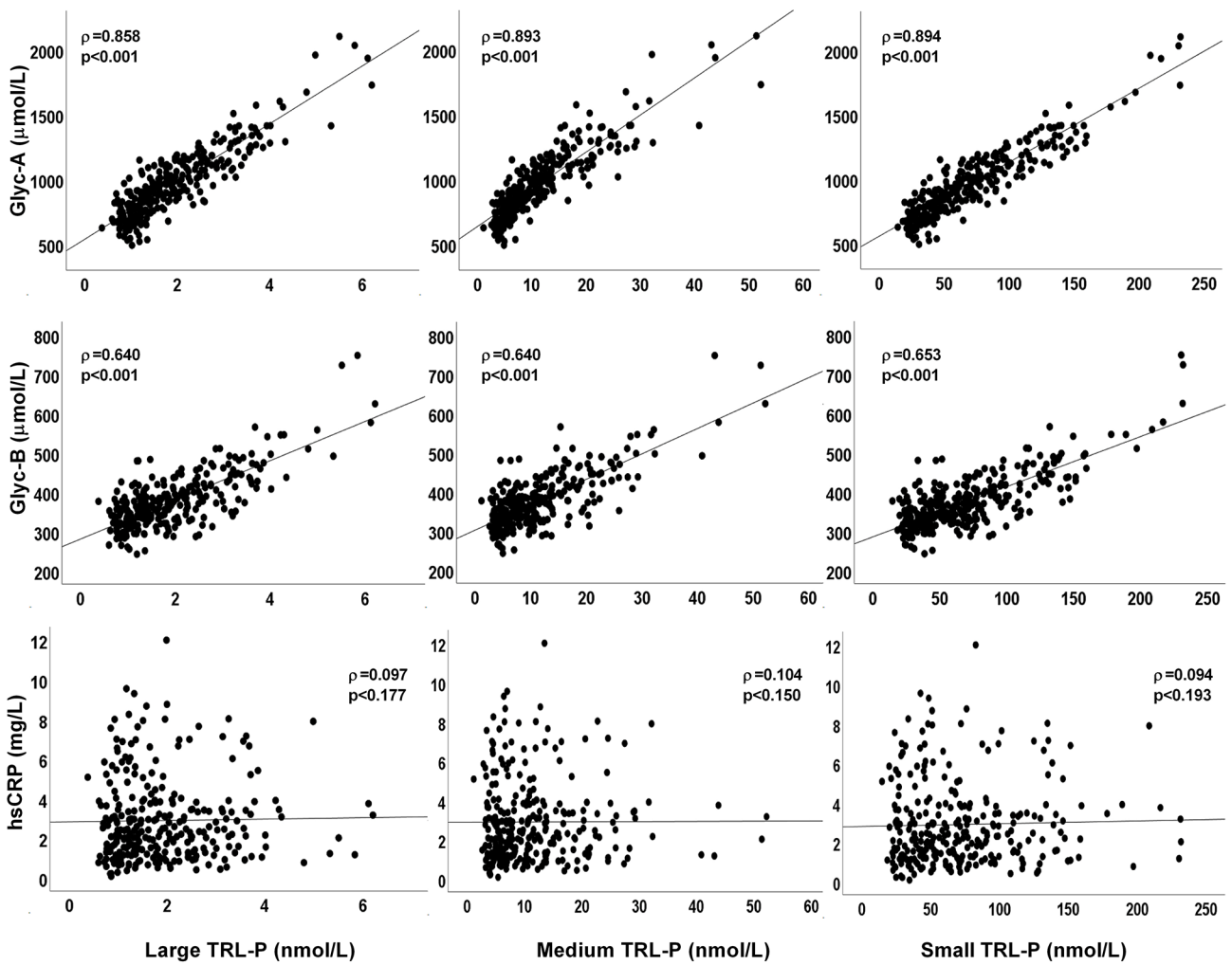

FIGURE 1 | Plots of plasma TRL-P concentrations (large, medium and small particles) with glycoproteins Glyc-A, Glyc-B, and with hsCRP. ρ from Rho coefficients from the Spearman correlation analysis.

TABLE 2 | Univariate associations of lipid-associated plasma parameters, TRL-P and acute phase proteins with standard fatty liver markers.

Variable	FLI		FIB-4		AST		ALT		GGT	
	ρ (rho)	p value	ρ (rho)	p value	ρ (rho)	p value	ρ (rho)	p value	ρ (rho)	p value
Triglycerides	0.492	<0.001*	-0.159	0.008	0.317	<0.001*	0.369	<0.001*	0.334	<0.001*
LDL-C	-0.084	0.171	0.097	0.104	-0.106	0.077	-0.179	0.003	0.016	0.789
Non-HDL-C	0.174	0.004*	0.022	0.710	0.131	0.029*	0.071	0.237	0.188	0.002*
Apo B-100	0.125	0.041*	0.034	0.570	0.057	0.344	0.001	0.993	0.166	0.006*
Large TRL-P	0.441	<0.001*	-0.126	0.035	0.300	<0.001*	0.343	<0.001*	0.287	<0.001*
Medium TRL-P	0.425	<0.001*	-0.083	0.169	0.316	<0.001*	0.326	<0.001*	0.288	<0.001*
Small TRL-P	0.422	<0.001*	-0.087	0.147	0.287	<0.001*	0.315	<0.001*	0.283	<0.001*
Total TRL-P	0.425	<0.001*	-0.088	0.144	0.293	<0.001*	0.318	<0.001*	0.284	<0.001*
Glyc-A	0.423	<0.001*	-0.070	0.244	0.239	<0.001*	0.246	<0.001*	0.277	<0.001*
Glyc-B	0.427	<0.001*	-0.140	0.019	0.115	0.056	0.184	0.002*	0.285	<0.001*
hsCRP	0.317	<0.001	-0.033	0.644	0.097	0.178	0.130	0.069	0.330	<0.001*

*Spearman correlation coefficients (rho) and significance (P-values). *Remained significance after adjustment for sex, age and BMI.*

present in 34% of the subjects studied. A higher prevalence of Type 2 Diabetes was observed among the patients who presented hepatic steatosis (p=0,034). Steatosis-free patients showed significantly lower levels of fasting plasma glucose (p=0.04), plasma TG and TRL particles (p<0.01), as well as lower levels of AST, ALT and FLI (p<0.05). We observed a nonsignificant trend towards higher NMR-glycoprotein concentrations in the group with hepatic steatosis, as confirmed by ultrasound. After adjusting by sex, age and BMI, the variables that remained significantly associated with hepatic steatosis were triglycerides, TRL-P subclasses, AST, ALT, GGT and FLI (**Table 3**). In addition, the mean decrease Gini plot from the random forest analysis showed that TRL particles, Glyc-A and B, and transaminases were determinant in order to classify patients with or without ultrasound-confirmed hepatic steatosis (**Figure 2**).

No differences were observed in the associations found between NMR-glycoproteins and TRL-P when the hepatic steatosis studied patients were stratified by glucose levels (glucose ≤ 126 mg/dL and glucose > 126 mg/dL) (**Supplementary Table 2**). We did not find significant associations between glycoprotein levels and the presence or not of hepatic steatosis when patients were sorted by glucose levels (**Supplementary Table 3**).

Glycoprotein Profile and TRL-P in the Prospective Study for the Development of Ultrasound-Confirmed Hepatic Steatosis

After a 10-year follow-up, 18 (28.13%) new cases of hepatic steatosis were confirmed among 64 patients who were disease-free at baseline. **Table 4** summarizes the baseline levels of clinical and standard hepatic biomarkers in both groups (disease-free and ultrasound-confirmed steatosis after 10 years). Both groups were age and sex balanced. The mean age of the study subjects was 63 (53-69) years, and 61.2% were women. As shown in **Table 4**, baseline plasma TG, AST, ALT, GGT and FLI were significantly higher in the group that developed ultrasound-confirmed steatosis (p<0.05). No differences between groups were seen for FIB-4 or hsCRP.

Interestingly, baseline levels of the NMR-measured TRL particles and glycoproteins were higher in the group of 18 patients who developed hepatic steatosis after 10 years (p ≤ 0.05) (**Figure 3**). After adjusting by sex, age and BMI, triglycerides, TRL-P subclasses, AST, ALT, GGT and FLI remained significantly associated with hepatic steatosis. The mean decrease Gini plot from the random forest analysis showed that TRL particles, Glyc-A and B and transaminases were determinant in the classification of patients with hepatic steatosis (**Figure 4**).

TABLE 3 | Clinical and biochemical parameters of 100 ultrasound-studied patients sorted by the presence (YES) or absence (NO) of hepatic steatosis.

	ULTRASOUND-CONFIRMED HEPATIC STEATOSIS			
	NO (n=66)	YES (n=34)	P value Univariate	P value* Multivariate
Age, years	63 (53-69)	62 (56-66)	0.716	–
Sex, male (%)	38.8	67.6	**0.006**	**-**
BMI, kg/m^2	29.78 (27,8-33,33)	31.14 (28,87-35,5)	**0,035**	**-**
Type 2 Diabetes, %	65.7	85.3	**0.037**	**-**
Insulin therapy, %	20.6	11.8	0.332	–
Oral antidiabetic therapy, %	43.3	61.8	0.079	–
Statins therapy, %	49.2	61.8	0.234	–
Hypotensors therapy, %	53.7	58.8	0.627	–
Triglycerides, mmol/L	1.86 (1.28-3.16)	2.85 (2.03-4.59)	**0.009**	**0.012**
LDL-C, mmol/L	3.85 ± 1.31	3.63 ± 1.16	0.397	0.397
Non-HDL-C, mmol/L	4.74 ± 1.34	4.83 ± 1.20	0.740	0.660
Apo B-100, mg/dL	129.23 ± 34.19	132.15 ± 30.12	0.675	0.677
Glucose, mg/dL	121 (101-157)	138 (119-155)	**0.041**	0.094
HbA_{1c}, %	5.90 (5.30-6.90)	6.40 (5.70-7.05)	0.249	0.457
Total TRL-P (nmol/L)	65.81 (45.74-107.8)	96.7 (71.03-149.71)	**0.007**	**0.006**
Large TRL-P (nmol/L)	1.48 (1.02-2.34)	2.33 (1,62-3.27)	**0.004**	**0.003**
Medium TRL-P (nmol/L)	8.29 (5.66-14.5)	13.26 (9.5-22.29)	**0.006**	**0.004**
Small TRL-P (nmol/L)	56.96 (39.02-88.19)	81.14 (61.52-124.26)	**0.008**	**0.008**
AST, U/L	22 (20-26)	25 (22-37)	**0.041**	**0.019**
ALT, U/L	17 (12-25)	23.5 (15-40)	**0.010**	**0.002**
GGT, U/L	22 (15-38)	27 (20-46)	0.069	**0.002**
FLI, %	72.05 (41.27-92.98)	86.18 (77.49-96.02)	**0.002**	**<0.001**
FIB-4	1.73 ± 0.48	1.7 ± 0.39	0.748	0.685
Glyc-A, µmol/L	885.1 (769.5-1126.4)	1021.4 (913.3-1212.1)	0.078	0.082
Glyc-B, µmol/L	361.6 (327.6-412.2)	370.5 (338.9-427.8)	0.395	0.547
hsCRP	2.17 (1.19-3.33)	2.31 (1.42-3.09)	0.801	0.809

Data are the means ± SD for normally distributed variables, medians (IQR) for nonparametric data or n (%). BMI, body mass index; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol; Apo B-100, apolipoprotein B100; HbA_{1c}, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, Fatty Liver Index; FIB-4, Fibrosis-4 score; TRL, triglyceride rich lipoprotein; hsCRP, high-sensitivity C-reactive protein. p values are for group comparisons. Statistical univariate analysis: $\chi 2$ for categorical data; t-tests or Mann-Whitney U tests were used for the continuous variables. Statistical linear multivariate analysis controlled by age, sex and BMI. p values are for group comparisons. Bold values indicate p < 0.05.*

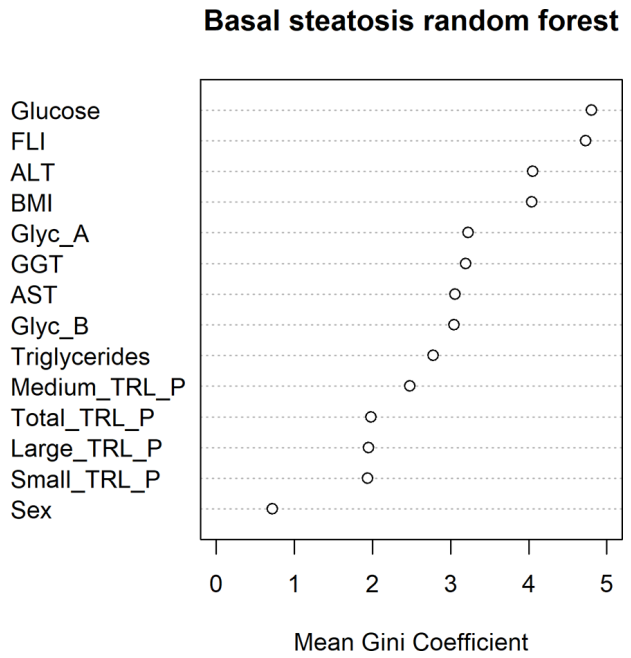

FIGURE 2 | Random forest mean Gini Coefficient for each variable in order to study their importance in the classification of ultrasound-confirmed hepatic steatosis.

DISCUSSION

In the present study, we cross-sectionally characterized the TRL subclass particle number, sorted by size, and the plasma glycoprotein profile measured by ^{1}H-NMR in 280 patients at metabolic risk. We detected remarkably significant positive associations between all TRL particle subclasses and Glyc-A and B concentrations. These findings remained robust after correction by different covariates. Likewise, we found positive associations of TRL and Glyc-A and B with hepatic injury-related markers. These correlations were weaker with the liver fibrosis index FIB-4. Liver ultrasound was performed at baseline in 100 patients to evaluate the presence of hepatic steatosis, and we detected higher levels of TRL particles in patients with steatosis. Moreover, all baseline TRL subclasses and Glyc-A and B were higher, although well within the normal range, in those subjects developing fatty liver during follow-up. Interestingly, hsCRP concentrations were not associated with TRL particles or liver alteration biomarkers.

^{1}H-NMR allows the detection of changes in the number and size of lipoprotein subclasses early either in nutritional and pharmacological intervention studies (29, 30). In this study, we measured the particle number of three VLDL subclasses by ^{1}H-NMR to assess relative differences among larger vs smaller VLDL. Moreover, we studied the association of these different VLDL particle subclasses with inflammatory markers synthesized in the liver (Glyc-A and B), hsCRP and standard clinical indexes and imaging data of liver steatosis.

TABLE 4 | Baseline clinical and biochemical parameters of ultrasound-studied patients after a 10-year follow-up, grouped as disease-free (NO) or ultrasound-confirmed hepatic steatosis (YES).

	ULTRASOUND-CONFIRMED HEPATIC STEATOSIS			
	NO (n=46)	YES (n=18)	P value Univariate	P value* Multivariate
Age, years	64 (56-69)	57 (50-69)	0.209	–
Sex, male (%)	34.8	52.6	0.182	–
BMI, kg/m^2	29.72 (27.7-33.42)	30.29 (28.69-33)	0.599	–
Type 2 Diabetes, %	65.2	68.4	0.804	–
Incident Type 2 Diabetes, %	0	25	0.576	–
Incident CVD, %	17.4	5.9	0.247	–
Insulin therapy, %	21.1	10.5	0.289	–
Oral antidiabetic therapy, %	47.8	36.8	0.418	–
Statins therapy, %	52.2	42.1	0.460	–
Hypotensors therapy, %	54.3	52.6	0.900	–
Triglycerides, mmol/L	1.73 (1.21-2.93)	2.49 (1.79-4.27)	**0.029**	**0.007**
LDL-C, mmol/L	3.83 ± 1.19	3.95 ± 1.65	0.742	0.526
Non-HDL-C, mmol/L	4.58 ± 1.22	5.21 ± 1.57	0.086	0.360
Apo B-100	126.36 ± 30.43	140.94 ± 40.51	0.124	0.563
Glucose, mg/dL	117 (96-157)	132 (104-179)	0.175	**0.045**
HbA_{1c}, %	6 (5.45-6.90)	5.50 (5.20-7.10)	0.472	0.782
ALT, U/L	18.5 (14-28)	25 (19-43)	**0.004**	**<0.001**
GGT, U/L	18.5 (14-28)	46 (23-173)	**<0.001**	**<0.001**
FLI, %	68.1 (40.5-86.7)	82.97 (63.1-96.2)	**0.019**	**<0.001**
FIB-4	1.71 ± 0.41	1.78 ± 1.13	0.595	0.587
hsCRP	2.18 ± 1.09	2.42 ± 1	0.416	0.200

Data are the means ± SD for normally distributed variables, medians (IQR) for nonparametric data or n (%). BMI, body mass index; Incident CVD, incident cardiovascular disease; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol; Apo B-100, apolipoprotein B100; HbA_{1c}, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, Fatty Liver Index; FIB-4, Fibrosis-4 score; hsCRP, high-sensitivity C-reactive protein. p values are for group comparisons. Statistical univariate analysis: $\chi 2$ for categorical data; t-tests or Mann-Whitney U tests were used for continuous variables. Statistical linear multivariate analysis controlled by age, sex and BMI. p values are for group comparisons.*

Bold values indicate $p < 0.05$.

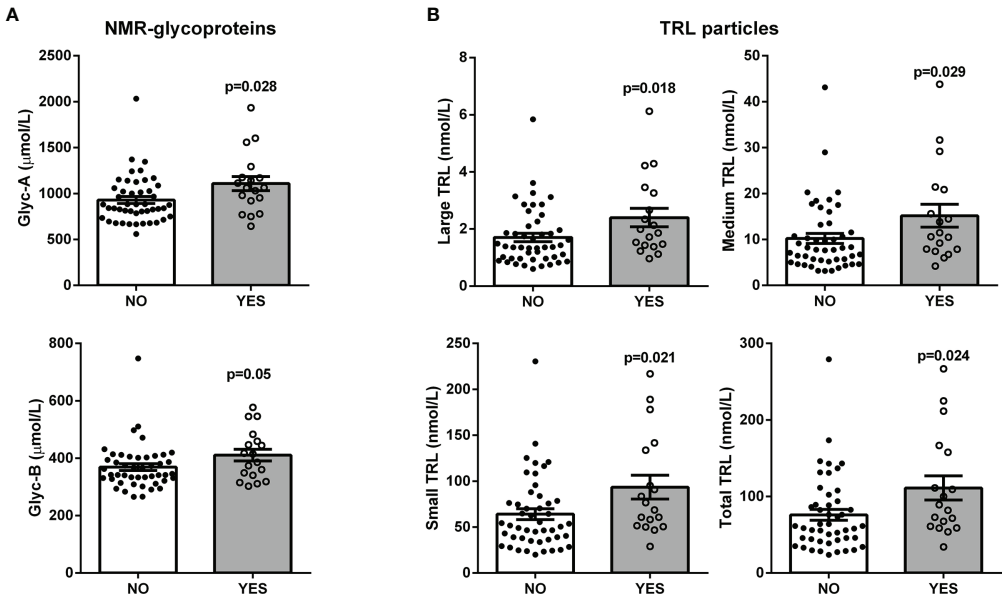

FIGURE 3 | Scatter plot with bar graphs of the plasma baseline levels of the NMR-measured glycoproteins **(A)** and TRL particles **(B)** in patients with ultrasound-confirmed hepatic steatosis after a 10-year follow-up. Each dot represents a patient: black dots for disease-free patients and white dots for patients with steatosis; bars represent mean values; p-values from Mann-Whitney U tests. Linear multivariate analysis controlled by age, sex and BMI: Glyc-A, p = 0.062; Glyc-B = 0.289; Large TRL-P, p = 0.001; Medium TRL-P, p = 0.004; Small TRL-P, p = 0.003; Total TRL-P, p = 0.003.

In our study, we found a strong and positive association between all TRL particle subclasses and ^{1}H-NMR-measured glycoproteins Glyc-A and Glyc-B. Age, BMI and sex are known factors that influence triglyceride concentrations (31).

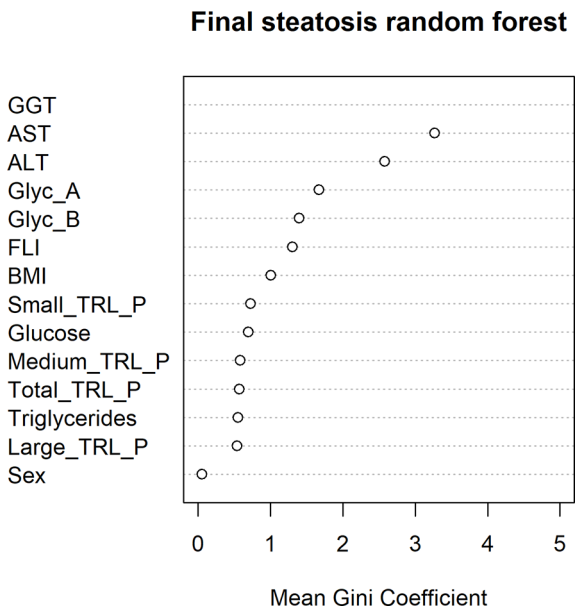

FIGURE 4 | Random forest mean Gini Coefficient for each variable in order to study their importance in the classification of ultrasound-confirmed hepatic steatosis after a 10 years' follow-up.

In our study, the associations found between TRL and glycoproteins remained positive when controlling for these covariates but also remained robust when adjusting by different covariates found to be related in the multivariate regression analysis. Recent studies have shown the implications of these glycoprotein markers in inflammatory and autoimmune diseases and in conditions such as rheumatoid arthritis, polycystic ovary syndrome and HIV infection, among others (18–20). Glyc-A has been consistently associated with systemic inflammation and atherosclerotic cardiovascular disease (17, 32–34), as well as with T2DM (35). Nevertheless, our group has previously reported the implications of the other glycoprotein marker Glyc-B in HIV and systemic inflammation (16, 19). Interestingly, in the present study, we found that all of the parameters measured (Glyc-A and Glyc-B) showed strong associations with TRL-P. This observation highlights the association of triglycerides with inflammation. Although T2DM-associated hypertriglyceridemia is due to the hypersecretion of large TRLs, in our hands, all particle subgroups, characterized by NMR, were equally associated with inflammation parameters. Interestingly, we detected higher baseline concentrations of these markers in 18 patients who developed steatosis over a 10-year follow-up study, suggesting that subclinical alterations could be present many years before its clinical manifestation.

Additionally, in our cohort of patients at metabolic risk, we found that TRL particles and NMR-glycoproteins had a positive association with hepatic dysfunction markers, including the fatty liver index (FLI) and ALT, AST and GGT, serum markers that have been used extensively to generate multiple scores and indexes for the non-invasive assessment of fatty liver disease

(26, 27, 36–40). However, their potential for distinguishing or staging more severe conditions, such as steatohepatitis and/or chronic liver disease, is limited. Interestingly, the association between TRL, Glyc-A and B and the clinical indexes of fatty liver disease was weaker or even null for FIB-4. FIB-4 is considered to be a fibrosis marker rather than an inflammation marker, which could explain this result (41).

We showed that patients diagnosed by ultrasound echography with hepatic steatosis had higher serum concentrations of TRL particles. These data agree with previously published data, were triglycerides levels and VLDL particle number were associated with hepatic steatosis (4). Furthermore, although NMR-glycoproteins did not show a significant association with hepatic steatosis, random forest analysis shows them as important variables to discriminate patients with hepatic steatosis.

As already stated, glycoproteins are known to be associated with diabetes (42). We analysed the associations between glycoproteins and TRL and hepatic steatosis stratifying by glucose levels, finding no differences regardless of glucose levels.

Plasma glycoproteins measured by ^{1}H-NMR belong to the family of acute phase proteins (14, 43) released under systemic inflammatory conditions. Under these states, plasma proteins show an increase in their glycosylated forms by increasing their oligosaccharide ramifications and monosaccharide residues (42, 44). In our study, we showed that the measurement of these glycoproteins by NMR provides a wider view of the systemic inflammatory status than the measurement of a single marker, hsCRP. Hence, the detection of these glycoforms, which are mainly produced by the liver, could reflect the consequence of (1) a liver overloaded with fat (ectopic fat accumulation), usually associated with lipotoxic fatty molecules that cannot be counterbalanced by (2) the overproduction of VLDL particles, as happens in hepatic steatosis (3, 4, 45). In accordance, given the implications of inflammation in the progression of MAFLD and the chronic inflammatory status present in metabolic syndrome and related conditions (including obesity, insulin resistance and T2DM), the role of these glycoproteins could be of potential interest in the detection of such an inflammatory status in fatty liver disease (16, 19). In addition, MAFLD has been reported to be associated with increased CVD morbidity and mortality, making its detection even more clinically important (46).

For the prospective part of this study, we evaluated new-onset liver steatosis in 64 patients without fatty liver at baseline by ultrasound. We compared baseline levels of TRL-P and glycoproteins between those who had developed steatosis and those who remained disease-free after 10 years. First, all baseline TRL-P concentrations were higher in those who developed steatosis, and their baseline glycoprotein levels were higher. Multivariate models also confirmed the associations between TRL particles and hepatic steatosis and established the glycoprotein parameters as determinant classifiers.

Some limitations of our study must be pointed out. First, our findings are based on associations and correlations, limiting the explanation of the possible causal molecular mechanisms. For the detection of hepatic steatosis, we used standard biochemical markers and clinical indexes; in addition, ultrasonography has the limitation that it can only detect steatosis with >2.5%–20% liver fat content and, therefore, a relevant number of patients with steatosis starting at 5% of liver fat content can be missed (47–49). Unfortunately, we did not have access to liver biopsies of our patients, the gold-standard technique for fatty liver disease diagnosis. Furthermore, the sample size of our follow-up study is limited; however, the follow-up period of 10 years strengthens our findings. The main strength of our study is that we used ^{1}H-NMR lipoprotein and glycoprotein profiling, providing a wide view of different plasma parameters, which can be interpreted as a molecular signature of the metabolic and inflammatory status of our patients. Indeed, the "Glyc" NMR-measured signals determine the plasma levels of various acute-phase glycoproteins, giving information about the overall inflammatory state rather than relying on the measurement of a single reactant, such as C-reactive protein (hsCRP).

In conclusion, the characterization of TRL subgroup particles and glycoprotein A and B concentrations by ^{1}H-NMR of patients at metabolic risk provides information on the inflammatory status that accompanies metabolic syndrome as well as its relationship with alterations in the liver. We found no differences in the distribution of VLDL particle subclasses, according to size, in this group of patients. In addition, we show evidence supporting that the measurement of baseline TRL particles and plasma glycoproteins could have predictive value for the development of MAFLD and its complications. This study could provide new insights into the use of NMR spectroscopy for the development of new lipidic and glycoprotein-related biomarkers for hepatic disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical and Clinical Investigation Committee of the Pere Virgili Institute for Health Research (IISPV) and fulfilled the principles of the Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM-V: study design, data analysis and interpretation, drafting of the manuscript, review of the results, review of the manuscript. RR, EO, DL, ML, MB, RR-C, NP, AP and AC: data analysis, review of the results. DI: study design, review of the results, and review of the manuscript. JG: study design, data analysis and interpretation, drafting of the manuscript, review of the results,

review of the manuscript. LM: study design, data analysis and interpretation, review of the results, review of the manuscript, overall study oversight and guarantor of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.775677/full#supplementary-material

REFERENCES

1. Holmes MV, Millwood IY, Kartsonaki C, Hill MR, Bennett DA, Boxall R, et al. Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *J Am Coll Cardiol* (2018) 71:620–32. doi: 10.1016/j.jacc.2017.12.006
2. Nordestgaard BG. Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. *Circ Res* (2016) 118:547–63. doi: 10.1161/CIRCRESAHA.115.306249
3. Adiels M, Olofsson SO, Taskinen MR, Borén J. Overproduction of Very Low-Density Lipoproteins Is the Hallmark of the Dyslipidemia in the Metabolic Syndrome. *Arterioscler Thromb Vasc Biol* (2008) 28:1225–36. doi: 10.1161/ATVBAHA.107.160192
4. Vergès B, Adiels M, Boren J, Barrett PH, Watts GF, Chan D, et al. Interrelationships Between the Kinetics of VLDL Subspecies and Hdl Catabolism in Abdominal Obesity: A Multicenter Tracer Kinetic Study. *J Clin Endocrinol Metab* (2014) 99:4281–90. doi: 10.1210/jc.2014-2365
5. Dongiovanni P, Stender S, Pietrelli A, Mancina RM, Cespiati A, Petta S, et al. Causal Relationship of Hepatic Fat With Liver Damage and Insulin Resistance in Nonalcoholic Fatty Liver. *J Intern Med* (2018) 283:356–70. doi: 10.1111/joim.12719
6. Wiebe N, Stenvinkel P, Tonelli M. Associations of Chronic Inflammation, Insulin Resistance, and Severe Obesity With Mortality, Myocardial Infarction, Cancer, and Chronic Pulmonary Disease. *JAMA Netw Open* (2019) 2:e1910456. doi: 10.1001/jamanetworkopen.2019.10456
7. Eslam M, Sanyal AJ, George J, Sanyal A, Neuschwander-Tetri B, Tiribelli C, et al. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* (2020) 158:1999–2014.e1. doi: 10.1053/j.gastro.2019.11.312
8. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global Epidemiology of Nonalcoholic Fatty Liver Disease—Meta-Analytic Assessment of Prevalence, Incidence, and Outcomes. *Hepatology* (2016) 64:73–84. doi: 10.1002/hep.28431
9. Rinella ME. Nonalcoholic Fatty Liver Disease a Systematic Review. *JAMA - J Am Med Assoc* (2015) 313:2263–73. doi: 10.1001/jama.2015.5370
10. Marchesini G, Day CP, Dufour JF, Canbay A, Nobili V, Ratziu V, et al. EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease. *J Hepatol* (2016) 64:1388–402. doi: 10.1016/j.jhep.2015.11.004
11. Alves-Bezerra M, Cohen DE. Triglyceride Metabolism in the Liver. *Compr Physiol* (2018) 8:1–22. doi: 10.1002/cphy.c170012
12. Catrysse L, van Loo G. Inflammation and the Metabolic Syndrome: The Tissue-Specific Functions of NF-κb. *Trends Cell Biol* (2017) 27:417–29. doi: 10.1016/j.tcb.2017.01.006
13. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, et al. The Metabolic Syndrome as a Predictor of Nonalcoholic Fatty Liver Disease. *Ann Intern Med* (2005) 143:722–8. doi: 10.7326/0003-4819-143-10-200511150-00009
14. Gabay C, Kushner I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. *N Engl J Med* (1999) 340:448–54. doi: 10.1056/NEJM199902113400607
15. Ohtsubo K, Marth JD. Glycosylation in Cellular Mechanisms of Health and Disease. *Cell* (2006) 126:855–67. doi: 10.1016/j.cell.2006.08.019
16. Fuertes-Martín R, Correig X, Vallvé J-C, Amigó N. Title: Human Serum/Plasma Glycoprotein Analysis by 1H-NMR, An Emerging Method of Inflammatory Assessment. *J Clin Med* (2020) 9:354. doi: 10.3390/jcm9020354
17. Connelly MA, Otvos JD, Shalaurova I, Playford MP, Mehta NN. GlycA, A Novel Biomarker of Systemic Inflammation and Cardiovascular Disease Risk. *J Transl Med* (2017) 15:219. doi: 10.1186/s12967-017-1321-6
18. Fuertes-Martín R, Taverner D, Vallvé JC, Paredes S, Masana L, Correig Blanchar X, et al. Characterization of 1H NMR Plasma Glycoproteins as a New Strategy to Identify Inflammatory Patterns in Rheumatoid Arthritis. *J Proteome Res* (2018) 17:3730–9. doi: 10.1021/acs.jproteome.8b00411
19. Malo A-I, Rull A, Girona J, Domingo P, Fuertes-Martín R, Amigó N, et al. Glycoprotein Profile Assessed by 1H-NMR as a Global Inflammation Marker in Patients With HIV Infection. A Prospective Study. *J Clin Med* (2020) 9:1344. doi: 10.3390/jcm9051344
20. Fuertes-Martín R, Moncayo S, Insenser M, Martínez-García MÁ, Luque-Ramírez M, Grau NA, et al. Glycoprotein A and B Height-to-Width Ratios as Obesity-Independent Novel Biomarkers of Low-Grade Chronic Inflammation in Women With Polycystic Ovary Syndrome (PCOS). *J Proteome Res* (2019) 18:4038–45. doi: 10.1021/acs.jproteome.9b00528
21. Blomme B, Fitzpatrick E, Quaglia A, Bruyne R, Dhawan A, Vlierberghe H. Serum Protein N-Glycosylation in Paediatric Non-Alcoholic Fatty Liver Disease. *Pediatr Obes* (2012) 7:165–73. doi: 10.1111/j.2047-6310.2011.00024.x
22. Blomme B, Francque S, Trépo E, Libbrecht L, Vanderschaeghe D, Verrijken A, et al. N-Glycan Based Biomarker Distinguishing Non-Alcoholic Steatohepatitis From Steatosis Independently of Fibrosis. *Dig Liver Dis* (2012) 44:315–22. doi: 10.1016/j.dld.2011.10.015
23. Yamasaki Y, Nouso K, Miyahara K, Wada N, Dohi C, Morimoto Y, et al. Use of Non-Invasive Serum Glycan Markers to Distinguish Non-Alcoholic Steatohepatitis From Simple Steatosis. *J Gastroenterol Hepatol* (2015) 30:528–34. doi: 10.1111/jgh.12726
24. Oswald DM, Jones MB, Cobb BA. Modulation of Hepatocyte Sialylation Drives Spontaneous Fatty Liver Disease and Inflammation. *Glycobiology* (2020) 30:346–59. doi: 10.1093/glycob/cwz096
25. Karanjia RN, Crossey MME, Cox IJ, Fye HKS, Njie R, Goldin RD, et al. Hepatic Steatosis and Fibrosis: Non-Invasive Assessment. *World J Gastroenterol* (2016) 22:9880–97. doi: 10.3748/wjg.v22.i45.9880
26. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: A Simple and Accurate Predictor of Hepatic Steatosis in the General Population. *BMC Gastroenterol* (2006) 6:33. doi: 10.1186/1471-230X-6-33
27. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a Simple Noninvasive Index to Predict Significant Fibrosis in Patients With HIV/HCV Coinfection. *Hepatology* (2006) 43:1317–25. doi: 10.1002/hep.21178
28. Mallol R, Amigó N, Rodríguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: A Novel Advanced Lipoprotein Test Based on 2D Diffusion-Ordered 1H NMR Spectroscopy. *J Lipid Res* (2015) 56:737–46. doi: 10.1194/jlr.D050120
29. Tuccinardi D, Farr OM, Upadhyay J, Oussaada SM, Mathew H, Paschou SA, et al. Lorcaserin Treatment Decreases Body Weight and Reduces Cardiometabolic Risk Factors in Obese Adults: A Six-Month, Randomized, Placebo-Controlled, Double-Blind Clinical Trial. *Diabetes Obes Metab* (2019) 21:1487–92. doi: 10.1111/DOM.13655

30. Tuccinardi D, Farr OM, Upadhyay J, Oussaada SM, Klapa MI, Candela M, et al. Mechanisms Underlying the Cardiometabolic Protective Effect of Walnut Consumption in Obese People: A Cross-Over, Randomized, Double-Blind, Controlled Inpatient Physiology Study. *Diabetes Obes Metab* (2019) 21:2086–95. doi: 10.1111/DOM.13773
31. Freedman DS, Otvos JD, Jeyarajah EJ, Shalaurova I, Cupples LA, Parise H, et al. Sex and Age Differences in Lipoprotein Subclasses Measured by Nuclear Magnetic Resonance Spectroscopy: The Framingham Study. *Clin Chem* (2004) 50:1189–200. doi: 10.1373/clinchem.2004.032763
32. Ballout RA, Remaley AT. GlycA: A New Biomarker for Systemic Inflammation and Cardiovascular Disease (CVD) Risk Assessment. *J Lab Precis Med* (2020) 5:17–7. doi: 10.21037/jlpm.2020.03.03
33. Gruppen EG, Riphagen IJ, Connelly MA, Otvos JD, Bakker SJL, Dullaart RPF. GlycA, A Pro-Inflammatory Glycoprotein Biomarker, and Incident Cardiovascular Disease: Relationship With C-Reactive Protein and Renal Function. *PloS One* (2015) 10:e0139057. doi: 10.1371/journal.pone.0139057
34. Mokkala K, Houttu N, Koivuniemi E, Sørensen N, Nielsen HB, Laitinen K. GlycA, a Novel Marker for Low Grade Inflammation, Reflects Gut Microbiome Diversity and Is More Accurate Than High Sensitive CRP in Reflecting Metabolomic Profile. *Metabolomics* (2020) 16:76. doi: 10.1007/s11306-020-01695-x
35. Akinkuolie AO, Pradhan AD, Buring JE, Ridker PM, Mora S. Novel Protein Glycan Side-Chain Biomarker and Risk of Incident Type 2 Diabetes. *Arterioscler Thromb Vasc Biol* (2015) 35:1544. doi: 10.1161/ATVBAHA.115.305635
36. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal Serum Aminotransferase Concentration and Risk of Mortality From Liver Diseases: Prospective Cohort Study. *Br Med J* (2004) 328:983–6. doi: 10.1136/bmj.38050.593634.63
37. Wai C. A Simple Noninvasive Index can Predict Both Significant Fibrosis and Cirrhosis in Patients With Chronic Hepatitis C. *Hepatology* (2003) 38:518–26. doi: 10.1053/jhep.2003.50346
38. Rossi E, Adams L, Prins A, Bulsara M, de Boer B, Garas G, et al. Validation of the FibroTest Biochemical Markers Score in Assessing Liver Fibrosis in Hepatitis C Patients. *Clin Chem* (2003) 49:450–4. doi: 10.1373/49.3.450
39. Forns X, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, et al. Identification of Chronic Hepatitis C Patients Without Hepatic Fibrosis by a Simple Predictive Model. *Hepatology* (2002) 36:986–92. doi: 10.1053/jhep.2002.36128
40. Rockey DC, Bissell DM. Noninvasive Measures of Liver Fibrosis. *Hepatology* (2006) 43:S113–20. doi: 10.1002/hep.21046
41. Mauro S, Scamporrino A, Filippello A, Pino A, Scicali R, Malaguarnera R, et al. Clinical and Molecular Biomarkers for Diagnosis and Staging of NAFLD. *Int J Mol Sci* (2021) 22:11905. doi: 10.3390/IJMS222111905
42. Connelly MA, Gruppen EG, Wolak-Dinsmore J, Matyus SP, Riphagen IJ, Shalaurova I, et al. GlycA, A Marker of Acute Phase Glycoproteins, and the Risk of Incident Type 2 Diabetes Mellitus: PREVEND Study. *Clin Chim Acta* (2016) 452:10–7. doi: 10.1016/j.cca.2015.11.001
43. Otvos JD, Shalaurova I, Wolak-Dinsmore J, Connelly MA, Mackey RH, Stein JH, et al. GlycA: A Composite Nuclear Magnetic Resonance Biomarker of Systemic Inflammation. *Clin Chem* (2015) 61:714–23. doi: 10.1373/clinchem.2014.232918
44. Connelly MA, Gruppen EG, Otvos JD, Dullaart RPF. Inflammatory Glycoproteins in Cardiometabolic Disorders, Autoimmune Diseases and Cancer. *Clin Chim Acta* (2016) 459:177–86. doi: 10.1016/j.cca.2016.06.012
45. Adiels M, Taskinen MR, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, et al. Overproduction of Large VLDL Particles Is Driven by Increased Liver Fat Content in Man. *Diabetologia* (2006) 49:755–65. doi: 10.1007/s00125-005-0125-z
46. Mantovani A, Csermely A, Petracca G, Beatrice G, Corey KE, Simon TG, et al. Non-Alcoholic Fatty Liver Disease and Risk of Fatal and Non-Fatal Cardiovascular Events: An Updated Systematic Review and Meta-Analysis. *Lancet Gastroenterol Hepatol* (2021) 6:903–13. doi: 10.1016/S2468-1253(21)00308-3
47. Mehta SR, Thomas EL, Bell JD, Johnston DG, Taylor-Robinson SD. Non-Invasive Means of Measuring Hepatic Fat Content. *World J Gastroenterol* (2008) 14:3476–83. doi: 10.3748/wjg.14.3476
48. Bril F, Ortiz-Lopez C, Lomonaco R, Orsak B, Freckleton M, Chintapalli K, et al. Clinical Value of Liver Ultrasound for the Diagnosis of Nonalcoholic Fatty Liver Disease in Overweight and Obese Patients. *Liver Int* (2015) 35:2139–46. doi: 10.1111/liv.12840
49. Paige JS, Bernstein GS, Heba E, Costa EAC, Fereirra M, Wolfson T, et al. A Pilot Comparative Study of Quantitative Ultrasound, Conventional Ultrasound, and MRI for Predicting Histology-Determined Steatosis Grade in Adult Nonalcoholic Fatty Liver Disease. *Am J Roentgenol* (2017) 208:W168–77. doi: 10.2214/AJR.16.16726

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ORIGINAL RESEARCH
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Genetically Predicted Causality of 28 Gut Microbiome Families and Type 2 Diabetes Mellitus Risk

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Mounting evidence indicates that gut microbiome may be involved in the pathogenesis of type 2 diabetes mellitus (T2DM). However, there is no consensus on whether there is a causal link between gut microbiome and T2DM risk. In the present study, the Mendelian randomization (MR) analysis was performed to investigate whether gut microbiome was causally linked to T2DM risk. The single nucleotide polymorphisms (SNPs) that were significantly related to exposure from published available genome-wide association study (GWAS) were selected as instrumental variables (IVs). The robust methods including inverse variance weighting (IVW), MR Egger, and weighted median were conducted to infer the causal links. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) and MR-Egger regression were used to test whether there was horizontal pleiotropy and identify outlier SNPs. The estimates of IVW suggested that *Streptococcaceae* (odds ratio (OR) = 1.17, 95% confidence interval (CI), 1.04–1.31, $p = 0.009$) was associated with higher risk of T2DM in European population. In Asian population, the MR IVW estimates revealed that there was a causal link between *Acidaminococcaceae* and T2DM risk (OR = 1.17, 95% CI, 1.04–1.31, $p = 0.008$). There was no evidence of notable heterogeneity and horizontal pleiotropy. However, after false discovery rate (FDR) correction, the causal link between gut microbiome and T2DM was absent (FDR, $p > 0.05$). In summary, using genetic instruments, this study does not find evidence of association between the 28 gut microbiome families and T2DM risk. However, *Streptococcaceae* and *Acidaminococcaceae* may have a borderline positive correlation with T2DM risk.

Keywords: causality, gut microbiome, mechanism, Mendelian randomization, type 2 diabetes mellitus

1 INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance and β-cell dysfunction, which occurs mostly in middle-aged or elderly individuals (1). As a major component of the global disease burden, the prevalence of T2DM is increasing (2). It is estimated that by 2040, there will be 642 million adults worldwide with diabetes, and most of which are

T2DM (3). The development of T2DM is mainly triggered by genetic factors and unhealthy lifestyle. Obesity is the primary predictor of T2DM which is responsible for more than half of diabetes cases (4). T2DM is a lifelong disease, and there is no cure. Mounting evidence suggests that gut microbiome may be involved in the pathogenesis of T2DM (5, 6).

The gut microbiome is a complex microbial community composed of a variety of bacteria living in the intestine. Recently, there has been considerable interest in the roles of the gut microbiome in regulating host physiological activities. Studies demonstrated that the gut microbiome possessed the properties that drove the development and maturation of the immune system (7) and maintained the homeostasis (8). Also, previous evidence showed that gut microbiome contributed to the development of numerous diseases by regulating cell differentiation (9), affecting the release of cytokines (10), and regulating drug absorption and metabolism (11). Concerning T2DM, gut microbiome dysbiosis is a clinical manifestation of the chronic disease (12, 13). The reason for the involvement of gut microbiome in the pathogenesis of T2DM may be that gut microbiome dysbiosis results in increased membrane transport of sugars and decreased branched-chain amino acid transport and butyrate biosynthesis, which lead to an unbalanced oxidative stress response (14). However, no consensus is reached on whether there is a causal link between the gut microbiome composition and T2DM risk.

Mendelian randomization (MR) is a commonly used approach to uncover the causal link between exposure and outcome (15), in which the genetic variations that are significantly related to exposure serve as instrumental variables (IVs). Being different from traditional observational studies, MR approach can minimize the influence of the confounding factors and reverse causation on the outcome (16). In the present study, the large-scale genome-wide association study (GWAS) summary-level data were used to perform two-sample MR analysis to infer the causality of gut microbiome composition and T2DM risk.

2 MATERIALS AND METHODS

2.1 Data Sources and Instrumental Variable Selection

The single nucleotide polymorphisms (SNPs) that served as IVs were from the latest GWAS, involving 18,473 subjects, which explore the influence of host genetics on the gut microbiome composition (17). The corresponding summary-level genetic data of T2DM risk were derived from a large GWAS involving 77,418 T2DM cases and 356,122 healthy controls of East Asian individuals (18). For the analysis of the European ancestry, the data of T2DM were obtained from a meta-analysis of GWAS with 62,892 T2DM cases and 596,424 controls (19). The corresponding information of SNPs was abstracted, including effect allele, other allele, effect size, standard error, and *p*-value.

The steps for selecting optimal IVs were as follows. First, SNPs with a *p*-value less than the locus-wide significance level (1×10^{-5}) were selected. Second, the genetic variations would be excluded if the minor allele frequency (MAF) is less than 0.01 (20). Third, in order to avoid the impact of linkage disequilibrium (LD) between the variables of interest on the results, the clumping process was performed, in which $R^2 < 0.001$ and clumping distance = 10,000 kb. Fourth, the corresponding data of the above selected SNPs were extracted from the outcome GWAS. When selected SNPs were absent from the outcome GWAS, the proxy SNPs with high LD ($r^2 > 0.90$) would be chosen to substitute the variants of interest. Fifth, in harmonizing process, the palindromic SNPs were excluded to ensure the effects of SNPs on exposure correspond to the same allele as the effects on the outcome. These stringent selected steps were conducive to ensure the authenticity of the results.

These selected IVs must meet the following three core assumptions. Firstly, IVs are significantly correlated with exposure which means that the variants of interest can predict exposure effectively. In the present study, *F* statistic was performed to confirm whether the estimates were affected by weak IVs. *F* statistic is expressed as $R^2(n\text{-}k\text{-}1)/k(1\text{-}R^2)$ where R^2 represents the estimated variance of exposure explained by the selected IVs, k is the number of IVs, and n refers to the sample size. Secondly, the IVs have to be independent of the outcome, namely the IVs can only affect outcome through exposure. Herein, MR-Egger regression and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) were used to confirm whether there was horizontal pleiotropy between IVs and outcome. Thirdly, the IVs must be independent of the confounding factors associated with exposure or outcome.

2.2 Statistical Analysis

The GWAS summary-level data were merged to infer the causal link between gut microbiome composition and T2DM risk. In the present study, the robust methods including inverse variance weighting (IVW), MR Egger, and weighted median were conducted to infer the causal links. IVW is a traditional method that merges the Wald ratio estimates of each IV in a meta-analysis manner (21). IVW equates to implement a weighted linear regression of the associations of the IVs with the outcome on the IVs with the exposure and intercept is constrained to zero (16). In the absence of horizontal pleiotropy, IVW enables to obtain unbiased estimates (22). MR Egger takes into account the pleiotropic effects, and the causal estimates represent the dose-response relationship between the genotype and outcome (23). When the Instrument Strength Independent of Direct Effect (InSIDE) hypothesis holds, MR Egger can get consistent causal effect estimates. Weighted median method allows some genetic variants are invalid, but only if at least half of them are valid instruments (24).

The MR-Egger regression and MR-PRESSO were used to confirm whether there was horizontal pleiotropy. MR-Egger regression has the property that confirms the pleiotropy between genetic instruments and outcome, and *p*-value greater than 0.05 was regarded as no horizontal pleiotropy. However, MR-Egger regression has lower precision and statistical power. MR-PRESSO can detect horizontal pleiotropy and identify pleiotropic outliers (25). If there was horizontal pleiotropy, the

analyses were repeated after removing these pleiotropic SNPs. Heterogeneity between genetic instruments was quantified by Cochran *Q* statistic. Leave-one-out sensitivity analysis was used to test whether the overall estimates were affected by strongly influencing SNPs. In addition, the Benjamini-Hochberg method was used to correct the false-discovery rate (FDR) for multiple tests. The statistical analyses were conducted by TwoSampleMR (26) and MRPRESSO (25) packages in R (version 4.0.3).

3 RESULTS

3.1 The Selection of Instrumental Variables

Initially, SNPs that were significantly related to the 28 gut microbiome families were selected. When excluding SNPs that with LD and were absent in the outcome GWAS, the remained variables of interest were selected as potential IVs. The detailed information of the selected IVs is shown in **Supplementary Tables 1, 2**.

3.2 The Estimates of Gut Microbiome With T2DM

3.2.1 European

The estimates of IVW indicated that genetically predicted *Streptococcaceae* (odds ratio (OR) = 1.17, 95% confidence interval (CI), 1.04–1.31, p = 0.009) was positively related to T2DM risk (**Table 1**; **Figure 1**). However, MR Egger and weighted median found no evidence of the association between exposure and outcome. The *Q* statistic showed that there was no notable heterogeneity (p = 0.270). MR-Egger regression and MR-PRESSO analysis further suggested no horizontal pleiotropy (p = 0.492 and p = 0.331, respectively). The results of MR-PRESSO analyses found evidence for significant horizontal pleiotropy between the IVs of *Christensenellaceae* (p = 0.004), *Enterobacteriaceae* (p = 0.041), *Methanobacteriaceae* (p = 0.046), *Peptostreptococcaceae* (p = 0.043), and *Verrucomicrobiaceae* (p = 0.020) and outcome. The causal effect estimates were recalculated after removing the outlier SNPs, and the results did not change substantially, except for *Methanobacteriaceae* (OR = 0.93, 95% CI, 0.88–0.99, p = 0.029). Leave-one-out sensitivity analysis showed that there were two strongly influencing SNPs (rs17791387, rs186073) in the IVs of *Desulfovibrionaceae* and one strongly influencing SNP (rs11123059) in the IVs of *Methanobacteriaceae* (**Supplementary Figure 1**). After removing the strongly influencing SNPs, the results changed significantly (*Desulfovibrionaceae*: OR = 1.18, 95% CI, 1.07–1.30, p = 0.001; *Methanobacteriaceae*: OR = 0.93, 95% CI, 0.88–0.99, p = 0.029). The detailed results are shown in **Supplementary Table 3**.

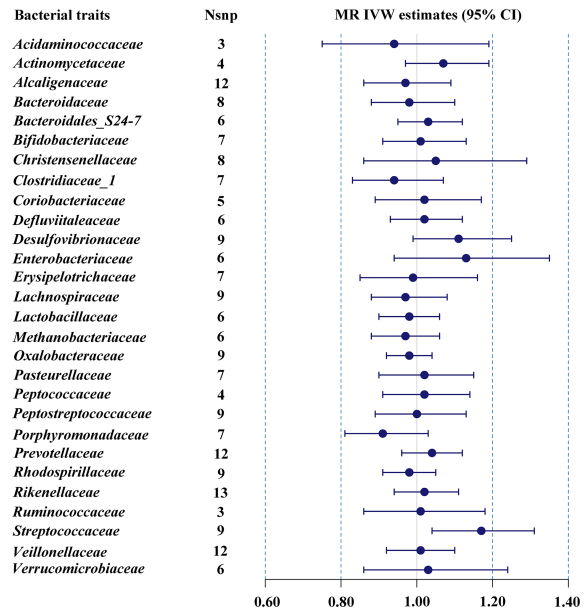

FIGURE 1 | MR IVW estimates of genetic instruments for gut microbiome and T2DM among individuals of European descent.

3.2.2 Asian

The results of IVW indicated that *Acidaminococcaceae* (OR = 1.17, 95% CI, 1.04–1.31, p = 0.008) was the risk factor for T2DM (**Table 1**; **Figure 2**). There was no evidence of notable heterogeneity (p = 0.751) across instrument SNP effects. MR-Egger regression showed that there was no horizontal pleiotropy between the variants of interest and outcome (p = 0.593). However, there were not enough SNPs for MR-PRESSO analysis. The results of MR-PRESSO suggested that there was significant horizontal pleiotropy of *Bacteroidaceae* (p = 0.014) and rs234027 was a pleiotropic SNP. In addition, MR-PRESSO detected two outliers (rs6060237 and rs7199026) in the analysis of *Desulfovibrionaceae*. After removal of these outliers, MR estimates remained null. In the sensitivity analysis, strongly influencing SNPs were identified in the IVs

TABLE 1 | MR estimates of IVs for gut microbiome and T2DM.

Ethnicity	Bacterial traits	Nsnp	Methods	Beta	SE	OR (95% CI)	*p*-value	FDR *p*-value
European	*Streptococcaceae*	9	IVW	0.15	0.06	1.17 (1.04–1.31)	0.009	0.962
			MR Egger	–0.02	0.24	0.98 (0.61–1.58)	0.948	0.965
			Weighted median	0.14	0.08	1.15 (0.99–1.34)	0.071	0.663
Asian	*Acidaminococcaceae*	3	IVW	0.16	0.06	1.17 (1.04–1.31)	0.008	0.224
			MR Egger	0.25	0.14	1.28 (0.98–1.67)	0.322	0.939
			Weighted median	0.15	0.08	1.16 (0.99–1.35)	0.051	0.607

MR, Mendelian randomization; SNP, single nucleotide polymorphism; IVW, inverse variance weighted; IVs, instrumental variables; T2DM, type 2 diabetes mellitus; FDR, false-discovery rate; OR, odds ratio.

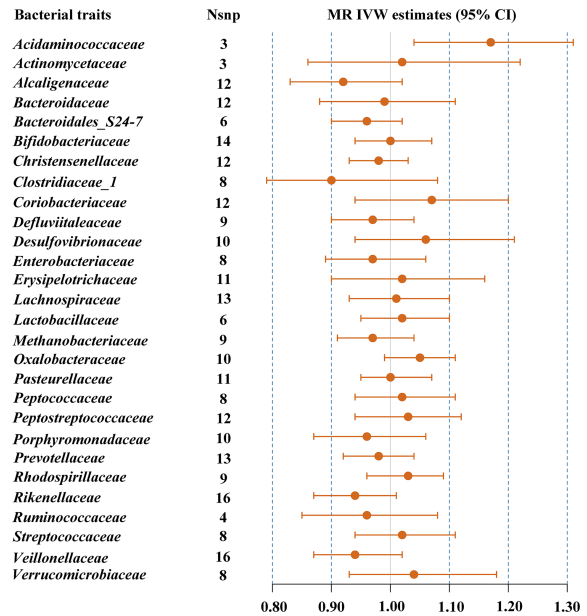

FIGURE 2 | MR IVW estimates of genetic instruments for gut microbiome and T2DM among individuals of Asian descent.

of *Acidaminococcaceae* (rs6589457), *Alcaligenaceae* (rs7638039), *Clostridiaceae_1* (rs11752225 and rs6934446), *Oxalobacteraceae* (rs36018452), *Rikenellaceae* (rs2290844, rs4264350 and rs7832304), and *Verrucomicrobiaceae* (rs9349825) (**Supplementary Figure 2**). Most results changed significantly after removing strongly influencing SNPs (*Acidaminococcaceae*: OR = 1.09, 95% CI, 0.89–1.34, $p = 0.399$; *Alcaligenaceae*: OR = 0.89, 95% CI, 0.81–0.98, $p = 0.018$; *Clostridiaceae_1*: OR = 0.83, 95% CI, 0.74–0.93, $p = 0.001$; *Oxalobacteraceae*: OR = 1.07, 95% CI, 1.01–1.13, $p = 0.024$; *Rikenellaceae*: OR = 0.90, 95% CI, 0.84–0.96, $p = 0.002$; *Verrucomicrobiaceae*: OR = 1.10, 95% CI, 1.00–1.21, $p = 0.042$). However, after FDR correction, the causal effect between gut microbiome and T2DM was absent (FDR $p > 0.05$). The detailed results are shown in **Supplementary Table 4**.

3.2.3 Further Analyses

The inverse MR was conducted to explore whether there was causal link between T2DM and gut microbiome. SNPs ($p < 5 \times 10^{-8}$) significantly associated with T2DM risk were used as IVs (**Supplementary Tables 5, 6**). For European, the IVW estimates showed that T2DM was related to a decrease in the abundance of *Bacteroidaceae* (OR = 0.97, 95% CI, 0.94–0.99, $P = 0.042$) and *Oxalobacteraceae* (OR = 0.94, 95% CI, 0.88–0.99, $p = 0.030$). The results of MR-Egger regression and MR-PRESSO suggested that there was no significant horizontal pleiotropy. However, the results changed substantially after FDR correction. For Asian, the results of IVW indicated that T2DM was associated with reduced *Oxalobacteraceae* (OR = 0.94, 95% CI, 0.88–0.99, $p = 0.036$). There was no significant horizontal pleiotropy between IVs and outcome, but no evidence for genetic correlations of T2DM with gut microbiome after FDR correction. Exact values are listed in **Supplementary Tables 7, 8**.

4 DISCUSSION

In the present study, the published available GWAS summary-level data were used to perform two-sample MR. The results revealed that genetically predicted level of some gut microbiome families was causally related to T2DM risk. However, there was no evidence for genetic correlation of the abundance of gut microbiome with T2DM after FDR correction.

The gut microbiome is a complex colony of microorganisms living in the gastrointestinal tract of the host. The gut microbiome has a critical physiological role in metabolism and mounting evidence demonstrates that gut microbiome compositions are involved in numerous metabolic disorders. A study with 292 Danish individuals demonstrated that compared with subjects with high intestinal flora richness, subjects with low intestinal flora richness were characterized by obesity, insulin resistance and dyslipidaemia (27). Cotillard et al. reported that subjects with reduced gut microbial gene richness showed significant metabolic disturbance and low-grade inflammation, which were characteristics of T2DM (28). A metagenome-wide association study (MGWAS) indicated that T2DM patients were accompanied by moderate degree of gut microbiome dysbiosis and the gut microbial markers might help classify T2DM (29). In addition, compared with participants without diabetes, patients with T2DM had a lower richness of gut microbiome (30). The mathematical model of the metagenomic profiles established based on the gut microbiome could identify T2DM with high accuracy (31). Applying this model to women with impaired glucose tolerance, it could identify women with diabetes-like metabolism. A study indicated that insulin resistance was closely related to gut microbial variations and gut microbiome could be used to develop precise medical strategies to prevent and delay T2DM (32). Recently, a separate-sample MR suggested that *Anaerostipes* was a protective factor in the development of T2DM (33). A study showed that constructed microbiome risk score was consistently associated with T2DM and future glucose increment and was related to a variety of blood metabolites derived from gut microbiome (34). Maskarinec et al. demonstrated that T2DM was related to the abundance of some intestinal floras and gut microbiome might cause chronic systemic inflammation and T2DM through bacterial translocation (35). A study showed that microbial-derived or microbial-modified metabolites in serum could predict the risk of T2DM (36). However, the available evidence was inconsistent and there was no consensus on whether there was causal link between gut microbiome and the occurrence of T2DM. In addition, the taxonomic groups of gut microbiome that are responsible for T2DM were unclear.

There has been considerable interest in the potential molecular mechanisms of gut microbiome in the onset and progression of T2DM. Gut microbiome composition was involved in the pathogenesis of T2DM by regulating inflammation, modulating energy homeostasis, interacting with diet, affecting intestinal permeability, insulin sensitivity, glucose, and lipid metabolism

(37). Gut microbiome and microbial products induced the production of interleukin (IL)-10 which improved glucose metabolism and prevented aging-related insulin resistance (38, 39). Gut microbiome composition improved IL-22 production and Treg cell differentiation, suggesting that they had the properties that restored insulin sensitivity and alleviated the symptoms of T2DM (40, 41). Increased intestinal permeability is one of the clinical signs of T2DM. *Akkermansia muciniphila* activated AMPK in the epithelium to improve the tight junctions of the intestine and thereby reduced the intestinal permeability. In addition, in the adipose tissue, *Akkermansia muciniphila* increased the levels of 2-acylglycerol, 2-palmitoylglycerol, and 2-oleoyl glycerol which increased fatty acid oxidation and fat cell differentiation (42). A study indicated that berberine had the property of improving insulin resistance by decreasing the relative abundance of gut microbiome, including *Streptococcaceae* (43). In addition, human milk insulin was negatively associated with *Streptococcaceae*, indicating that it might be related to the occurrence and development of diabetes (44). These available evidences indicate that gut microbiome composition may be involved in the course of T2DM and affect disease symptoms.

Since the implementation of the MR approach reduced the interference of confounding factors and the reverse causality of the results, the present study might be more convincing than observational studies. However, some limitations should be mentioned. First, given the absence of the data of basic demographic information and clinical manifestations, further subgroup analysis could not be carried out. Second, the current understanding of the gut microbiome limited our study. We lacked sufficient clues to infer the molecular mechanisms of gut microbiome and T2DM due to the absence of epidemiological studies on gut microbiome and metabolic disorders. Third, SNPs obtained based on genome-wide statistical significance threshold (5×10^{-8}) were too limited for further study, therefore only the SNPs that met the locus-wide significance level (1×10^{-5}) were selected. These restrictions limited the generalizability of the results and the accuracy of the study might have been compromised.

In summary, the study is leveraging MR to find that there is no evidence of the association between the 28 gut microbiome families and T2DM risk. However, in view of the biological plausibility, further studies are needed to explore the relationship between gut microbiome and the risk of T2DM, which is conducive to exploring the pathogenesis of diabetes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The article does not contain any studies with human participants or animals performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization: H-FP and JN. Writing—original draft and writing—review and editing: KX and J-JZ. Data curation: Y-YX and XZ. All authors discussed the results and contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.780133/full#supplementary-material

Supplementary Figure 1 | Sensitivity analysis of the genetic risk of T2DM with gut microbiome in European descent (**A**: *Desulfovibrionaceae*; **B**: *Methanobacteriaceae*).

Supplementary Figure 2 | Sensitivity analysis of the genetic risk of T2DM with gut microbiome in Asian descent (**A**: *Acidaminococcaceae*; **B**: *Alcaligenaceae*; **C**: *Clostridiaceae_1*; **D**: *Oxalobacteraceae*; **E**: *Rikenellaceae*; **F**: *Verrucomicrobiaceae*).

REFERENCES

1. Hung WW, Peng P, Tsai YC, Jhou PS, Chang CC, Hsieh CC, et al. Gut Microbiota Compositions and Metabolic Functions in Type 2 Diabetes Differ With Glycemic Durability to Metformin Monotherapy. *Diabetes Res Clin Pract* (2021) 174:108731. doi: 10.1016/j.diabres.2021.108731
2. Sun D, Zhou T, Heianza Y, Li X, Fan M, Fonseca VA, et al. Type 2 Diabetes and Hypertension. *Circ Res* (2019) 124:930–7. doi: 10.1161/CIRCRESAHA.118.314487
3. Zheng Y, Ley SH, Hu FB. Global Aetiology and Epidemiology of Type 2 Diabetes Mellitus and its Complications. *Nat Rev Endocrinol* (2018) 14:88–98. doi: 10.1038/nrendo.2017.151
4. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, et al. Diet, Lifestyle, and the Risk of Type 2 Diabetes Mellitus in Women. *N Engl J Med* (2001) 345:790–7. doi: 10.1056/NEJMoa010492
5. Hsieh MC, Tsai WH, Jheng YP, Su SL, Wang SY, Lin CC, et al. The Beneficial Effects of Lactobacillus Reuteri ADR-1 or ADR-3 Consumption on Type 2 Diabetes Mellitus: A Randomized, Double-Blinded, Placebo-Controlled Trial. *Sci Rep* (2018) 8:16791. doi: 10.1038/s41598-018-35014-1
6. Sedighi M, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, et al. Comparison of Gut Microbiota in Adult Patients With Type 2 Diabetes and Healthy Individuals. *Microb Pathog* (2017) 111:362–9. doi: 10.1016/j.micpath.2017.08.038
7. Shi N, Li N, Duan X, Niu H. Interaction Between the Gut Microbiome and Mucosal Immune System. *Mil Med Res* (2017) 4:14. doi: 10.1186/s40779-017-0122-9
8. Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, et al. Mining the Human Gut Microbiota for Immunomodulatory Organisms. *Cell* (2017) 168:928–43.e11. doi: 10.1016/j.cell.2017.01.022

9. Jubair WK, Hendrickson JD, Severs EL, Schulz HM, Adhikari S, Ir D, et al. Modulation of Inflammatory Arthritis in Mice by Gut Microbiota Through Mucosal Inflammation and Autoantibody Generation. *Arthritis Rheumatol* (2018) 70:1220–33. doi: 10.1002/art.40490
10. Chappert P. Role of SFB in Autoimmune Arthritis: An Example of Regulation of Autoreactive T Cell Sensitivity in the Gut. *Gut Microbes* (2014) 5:259–64. doi: 10.4161/gmic.28134
11. Scher JU, Nayak RR, Ubeda C, Turnbaugh PJ, Abramson SB. Pharmacomicrobiomics in Inflammatory Arthritis: Gut Microbiome as Modulator of Therapeutic Response. *Nat Rev Rheumatol* (2020) 16:282–92. doi: 10.1038/s41584-020-0395-3
12. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, 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
13. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, et al. Disentangling Type 2 Diabetes and Metformin Treatment Signatures in the Human Gut Microbiota. *Nature* (2015) 528:262–6. doi: 10.1038/nature15766
14. Luca M, Di Mauro M, Di Mauro M, Luca A. Gut Microbiota in Alzheimer's Disease, Depression, and Type 2 Diabetes Mellitus: The Role of Oxidative Stress. *Oxid Med Cell Longev* (2019) 2019:4730539. doi: 10.1155/2019/4730539
15. Davey Smith G, Hemani G. Mendelian Randomization: Genetic Anchors for Causal Inference in Epidemiological Studies. *Hum Mol Genet* (2014) 23:R89–98. doi: 10.1093/hmg/ddu328
16. Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, et al. Recent Developments in Mendelian Randomization Studies. *Curr Epidemiol Rep* (2017) 4:330–45. doi: 10.1007/s40471-017-0128-6
17. Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, et al. Large-Scale Association Analyses Identify Host Factors Influencing Human Gut Microbiome Composition. *Nat Genet* (2021) 53:156–65. doi: 10.1038/s41588-020-00763-1
18. Spracklen CN, Horikoshi M, Kim YJ, Lin K, Bragg F, Moon S, et al. Identification of Type 2 Diabetes Loci in 433,540 East Asian Individuals. *Nature* (2020) 582:240–5. doi: 10.1038/s41586-020-2263-3
19. Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, et al. Genome-Wide Association Analyses Identify 143 Risk Variants and Putative Regulatory Mechanisms for Type 2 Diabetes. *Nat Commun* (2018) 9:2941. doi: 10.1038/s41467-018-04951-w
20. Wu F, Huang Y, Hu J, Shao Z. Mendelian Randomization Study of Inflammatory Bowel Disease and Bone Mineral Density. *BMC Med* (2020) 18:312. doi: 10.1186/s12916-020-01778-5
21. Burgess S, Butterworth A, Thompson SG. Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data. *Genet Epidemiol* (2013) 37:658–65. doi: 10.1002/gepi.21758
22. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using Published Data in Mendelian Randomization: A Blueprint for Efficient Identification of Causal Risk Factors. *Eur J Epidemiol* (2015) 30:543–52. doi: 10.1007/s10654-015-0011-z
23. Bowden J, Davey Smith G, Burgess S. Mendelian Randomization With Invalid Instruments: Effect Estimation and Bias Detection Through Egger Regression. *Int J Epidemiol* (2015) 44:512–25. doi: 10.1093/ije/dyv080
24. Nowak C, Ärnlöv J. A Mendelian Randomization Study of the Effects of Blood Lipids on Breast Cancer Risk. *Nat Commun* (2018) 9:3957. doi: 10.1038/s41467-018-06467-9
25. Verbanck M, Chen CY, Neale B, Do R. Detection of Widespread Horizontal Pleiotropy in Causal Relationships Inferred From Mendelian Randomization Between Complex Traits and Diseases. *Nat Genet* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7
26. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base Platform Supports Systematic Causal Inference Across the Human Phenome. *Elife* (2018) 7:e34408. doi: 10.7554/eLife.34408
27. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of Human Gut Microbiome Correlates With Metabolic Markers. *Nature* (2013) 500:541–6. doi: 10.1038/nature12506
28. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, et al. Dietary Intervention Impact on Gut Microbial Gene Richness. *Nature* (2013) 500:585–8. doi: 10.1038/nature12480
29. Qin J, Li Y, Cai Z, Li S, Zhu 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
30. Chen Z, Radjabzadeh D, Chen L, Kurilshikov A, Kavousi M, Ahmadizar F, et al. Association of Insulin Resistance and Type 2 Diabetes With Gut Microbial Diversity: A Microbiome-Wide Analysis From Population Studies. *JAMA Netw Open* (2021) 4:e2118811. doi: 10.1001/jamanetworkopen.2021.18811
31. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut Metagenome in European Women With Normal, Impaired and Diabetic Glucose Control. *Nature* (2013) 498:99–103. doi: 10.1038/nature12198
32. Wu H, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, et al. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based Cross-Sectional Study. *Cell Metab* (2020) 32:379–90.e3. doi: 10.1016/j.cmet.2020.06.011
33. Yang Q, Lin SL, Kwok MK, Leung GM, Schooling CM. The Roles of 27 Genera of Human Gut Microbiota in Ischemic Heart Disease, Type 2 Diabetes Mellitus, and Their Risk Factors: A Mendelian Randomization Study. *Am J Epidemiol* (2018) 187:1916–22. doi: 10.1093/aje/kwy096
34. Gou W, Ling CW, He Y, Jiang Z, Fu Y, Xu F, et al. Interpretable Machine Learning Framework Reveals Robust Gut Microbiome Features Associated With Type 2 Diabetes. *Diabetes Care* (2021) 44:358–66. doi: 10.2337/dc20-1536
35. Maskarinec G, Raquinio P, Kristal BS, Setiawan VW, Wilkens LR, Franke AA, et al. The Gut Microbiome and Type 2 Diabetes Status in the Multiethnic Cohort. *PloS One* (2021) 16:e0250855. doi: 10.1371/journal.pone.0250855
36. Menni C, Zhu J, Le Roy CI, Mompeo O, Young K, Rebholz CM, et al. Serum Metabolites Reflecting Gut Microbiome Alpha Diversity Predict Type 2 Diabetes. *Gut Microbes* (2020) 11:1632–42. doi: 10.1080/19490976.2020.1778261
37. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of Gut Microbiota in Type 2 Diabetes Pathophysiology. *EBioMedicine* (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051
38. Shen Z, Zhu C, Quan Y, Yang J, Yuan W, Yang Z, et al. Insights Into Roseburia Intestinalis Which Alleviates Experimental Colitis Pathology by Inducing Anti-Inflammatory Responses. *J Gastroenterol Hepatol* (2018) 33:1751–60. doi: 10.1111/jgh.14144
39. Dagdeviren S, Jung DY, Friedline RH, Noh HL, Kim JH, Patel PR, et al. IL-10 Prevents Aging-Associated Inflammation and Insulin Resistance in Skeletal Muscle. *FASEB J* (2017) 31:701–10. doi: 10.1096/fj.201600832R
40. Zhu C, Song K, Shen Z, Quan Y, Tan B, Luo W, et al. Roseburia Intestinalis Inhibits Interleukin–17 Excretion and Promotes Regulatory T Cells Differentiation in Colitis. *Mol Med Rep* (2018) 17:7567–74. doi: 10.3892/mmr.2018.8833
41. Hoffmann TW, Pham HP, Bridonneau C, Aubry C, Lamas B, Martin-Gallausiaux C, et al. Microorganisms Linked to Inflammatory Bowel Disease-Associated Dysbiosis Differentially Impact Host Physiology in Gnotobiotic Mice. *ISME J* (2016) 10:460–77. doi: 10.1038/ismej.2015.127
42. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-Talk Between Akkermansia Muciniphila and Intestinal Epithelium Controls Diet-Induced Obesity. *Proc Natl Acad Sci USA* (2013) 110:9066–71. doi: 10.1073/pnas.1219451110
43. Yue SJ, Liu J, Wang AT, Meng XT, Yang ZR, Peng C, et al. Berberine Alleviates Insulin Resistance by Reducing Peripheral Branched-Chain Amino Acids. *Am J Physiol Endocrinol Metab* (2019) 316:E73–85. doi: 10.1152/ajpendo.00256.2018
44. Lemas DJ, Young BE, Baker PR, Tomczik AC, Soderborg TK, Hernandez TL, et al. Alterations in Human Milk Leptin and Insulin Are Associated With Early Changes in the Infant Intestinal Microbiome. *Am J Clin Nutr* (2016) 103:1291–300. doi: 10.3945/ajcn.115.126375

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.

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Randomized Clinical Trial: Probiotics Alleviated Oral-Gut Microbiota Dysbiosis and Thyroid Hormone Withdrawal-Related Complications in Thyroid Cancer Patients Before Radioiodine Therapy Following Thyroidectomy

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Background: Thyroid hormone withdrawal (THW) in postoperative thyroid cancer patients who need always accompanied by complications (e.g., dyslipidemia and constipation). At present, there are no effective and safe means to alleviate these complications.

Purpose: We aimed to assess the oral-gut microbiota profiles in THW patients then investigate whether probiotics could alleviating alleviate THW related complications and investigate whether these therapeutic effects were associated with the oral-gut microbiota state.

Methods: Fifty eligible thyroid carcinoma patients undergoing thyroidectomy were randomly assigned to receive probiotics or placebo during THW. Complications were assessed through validated questionnaires and plasma lipid indicators. The complex probiotics preparation was composed of *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, and *Bacillus cereus*.

Results: Probiotics alleviated lack of energy, constipation, weight gain, and dry mouth and decreased the levels of fecal/serum LPS and plasma lipid indicators (total cholesterol, triglycerides, low-density lipoprotein, and apolipoprotein A) ($P < 0.05$). Gut and oral microbial diversity were significantly decreased after THW, while an increased microbial dysbiosis index (MDI) was observed. Probiotics distinctly restored the gut and oral microbial diversity. Increased *Holdemanella*, *Enterococcus*, and *Coprococcus_2*, while decreased *Fusobacterium*, *Eubacterium_ruminantium_group*, *Ruminococcus_1*, and

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Parasutterella in the gut were found after probiotics intervention. Lack of energy, constipation, weight gain, and dyslipidemia were seen to be related to the above microbiota. In addition, probiotics reduced oral *Prevotella_9*, *Haemophilus*, *Fusobacterium*, and *Lautropia*, which were positively correlated with the occurrence of dry mouth.

Conclusion: Probiotics reduce the incidence of complications in patients after THW, which may be related to modifying the oral and gut microbiota.

Clinical Trial Registration: [https://clinicaltrials.gov/], identifier America Clinical Trial Registry NCT03574051.

Keywords: radioiodine therapy, complications, oral microbiota, gut microbiota, probiotics, thyroid hormone withdrawal

INTRODUCTION

Thyroid cancer (TC) is the most prevalent malignant neoplasm in the endocrine system. The incidence of TC has been increasing in the past 25 years (1). The majority of TC patients are differentiated thyroid cancer (DTC) developing from thyroid follicular cells and are classified as papillary or follicular carcinomas. Radioactive iodine (RAI) has been administered following thyroidectomy in patients with DTC for remnant ablation and adjuvant treatment (2). In patients receiving RAI treatment, the uptake of iodine depends on the stimulation of thyrotropin (TSH), which can be achieved by thyroid hormone withdrawal (THW) or injecting recombinant human thyroid-stimulating hormone (rhTSH) (3). Compared with rhTSH injection, THW therapy is low-cost and widely used in Asian countries (4, 5). However, THW always is accompanied by various complications, including fatigue, constipation, weight gain, edema, and hypercholesterolemia (6–9), which have an obvious negative impact on the patients' quality of life (9, 10). Thus, investigating the mechanism for THW related symptom is help to benefit the thyroid cancer patients who need to receive RAI after surgery. Although DTC patients who received recombinant human thyrotropin (rhTSH) to maintain normal thyroid hormone (free triiodothyronine (fT3), free thyroxine (fT4)) levels, these complications still occur (11, 12). Thus, the THW related complications cannot be all be blamed for hypothyroidism, while some other factors should be taken into consideration. Previous studies have shown that constipation, weight gain, fatigue, and dry mouth attribute to the oral and gut microbiota perturbation (13–15). There is accumulating evidence indicating a thyroid-gut axis exists (16, 17). It appears to display an interaction effect between the gut microbiota and thyroid function (16, 18). Therefore, we suspect that THW may cause oral and gut microbiota dysbiosis then induce these complications.

Since our previous study showed the gut microbiota of DTC patients was already disordered (19). This study aims to figure out the gut and oral microbiota characters in postoperative DTC patients after THW. Then, we perform an intervention study to investigate the beneficial effects of probiotics on alleviating the complications of DTC patients with THW.

MATERIALS AND METHODS

Ethics

The authors ensure that the current clinical trial has been carried out by the Code of Ethics of the World Medical Association (Declaration of Helsinki). This study was a randomized, parallel-group, double-blind, placebo-controlled, adaptive randomized controlled trial (RCT). The intervention period was four weeks, while the primary outcome was assessed at week 4. The Ethics Committee approved all protocols applied in this study at the First Affiliated Hospital of Harbin Medical ·University (Eth. 201816). All patients gave written informed consent for participation in the study. In addition, a clinical study was registered with the America Clinical Trial Registry (NCT03574051).

Study Design and Patient Enrollment

Based on the hypothesis that the average incidence of complications of lack of energy in the placebo group and the probiotic group would be 63.4% and 21% (10), respectively, 32 patients were needed (2 - sided $\alpha = 0.05$, 1 - $\beta = 0.9$, and 1:1 ratio). A sample size of 32 was calculated, considering drop-out expected for the follow−up study; the total sample size calculated was 50. We recruited a total of fifty post-thyroidectomy DTC patients awaiting THW therapy from the Department of Nuclear Medicine of the First Affiliated Hospital of Harbin Medical University between January 2017 and April 2018 (**Figure 1**). The inclusion criteria for patients were as follows: 18 to 65 years of age; differentiated thyroid cancer patients who had undergone total thyroidectomy before radioiodine therapy and awaited THW treatment; four weeks of levothyroxine withdrawal after surgery to achieve TSH elevation above 30 mIU/mL; consented to the 4-week probiotic treatment; agreed for serum lipid testing, because this was not routinely performed in our institute. The patient was told to take a low-iodine diet during THW. The random allocation of patients is carried out by a random number code generated by the computer of Harbin Medical University. The patients were randomly divided into 6 blocks with a ratio of 1:1 and received probiotics or a placebo (only the size of the block is known to the statistician). The medicines are distributed and packaged according to random numbers. The parameter

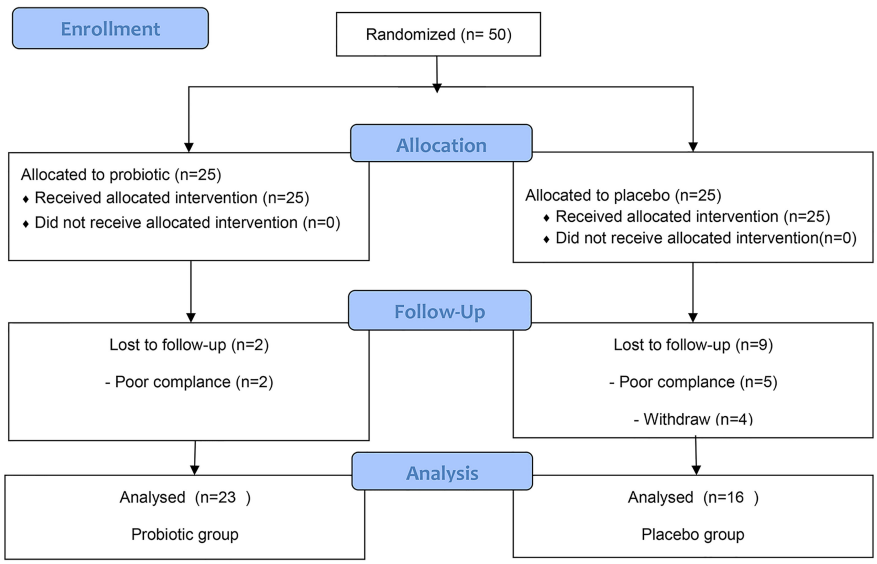

FIGURE 1 | The CONSORT diagram.

description files such as the random number seed blind code, block length, and random number are sealed in the First Affiliated Hospital of Harbin Medical University. Throughout the trial period, the blinding code was not disclosed. The probiotics (Bifidobacterium Tetravaccine Tablets, Hangzhou Yuanda Biopharmaceutical Co., Ltd., SFDA approval number: S20060010; containing > 10^6 CFU/tablet *B. infantis*, > 10^6 CFU/tablet *L. acidophilus*, > 10^6 CFU/tablet *E. faecalis*, > 10^5 CFU/tablet *B. cereus* and > 10^6 CFU/tablet total bacteria). Probiotic or placebo (starch) was supplied from the beginning to the End of treatment for up to 4 weeks (3 capsules two times a day). The shape, color, and other characteristics of the placebo are designed according to the Chinese Pharmacopoeia standards and are the same as the shape and color of the probiotic composition. Probiotics and placebo capsules were randomly numbered, and blinding codes were not disclosed to patients or treating physicians. Finally, 23 patients were included in the probiotics group, and 16 patients were included in the placebo group (**Figure 1**).

Clinical Outcomes

The primary outcome was a change in the incidence of complications after four weeks between probiotics and placebo. The secondary clinical outcomes included oral and gut microbiota, plasma lipid levels, thyroid function, and liver function. The severity of complications was assessed by the Thyroid symptom questionnaire (TSQ) (10), focusing on the following items: lack of energy/fatigue (TSQ-LOE), constipation (TSQ-C), edema (TSQ-E), weight gain (TSQ-WG), and dry mouth (TSQ-DRY) (6, 7). The TSQ was developed based on a previous study (10) and included questions on Scores on a Likert scale were defined as 0-absent, 1-complications absent, 2-rarely present, 3-present, and 4-severely present. A single research assistant administered the questionnaire to all the volunteers.

Sample Collection and Clinical Parameters

For the enrolled patients, we conducted longitudinal sampling, Baseline: 2-4 days after total thyroidectomy (normal thyroid function), End of intervention: 4 weeks after THW treatment (Levothyroxine withdrawn)/THW plus probiotics. Plasma, fecal, and oral washings samples were obtained from each subject (B-THW (before the treatment of THW plus a placebo) group (n = 16), B-THW-P (before the treatment of THW plus the probiotic combination) group (n = 23), A-THW (after treatment with THW plus a placebo) group (n = 16), and A-THW-P [after treatment with THW plus the probiotic) group (n = 23)]. The specific collection and processing methods and a comprehensive description of the clinical parameter analysis are provided in the **Supplementary Material**. Plasma samples were collected for thyroid function detection (free triiodothyronine[fT3], free thyroxine[fT4], and thyroid-stimulating hormone [TSH]) and thyroglobulin [TG], liver function (alanine aminotransferase [ALT], aspartate transaminase [AST], album [ALB], total protein [TP], globulin [GLB], total bilirubin [TBIL], direct bilirubin [DBIL], indirect bilirubin [IBIL], gamma-glutamyl transpeptidase [GGT], alkaline phosphatase [AKP], lactate dehydrogenase [LDH], blood urea nitrogen [BUN], creatinine [Cr], uric acid [UA], glucose [GLU]), plasma lipid (total cholesterol [CHOL], triacylglycerol [TG], low-density lipoprotein [LDL], apolipoprotein A [Apo A], high-density lipoprotein [HDL], very low density lipoprotein [VLDL], apolipoprotein B [Apo B], lipoprotein(a) [Lpa]) and plasma LPS analyses; fecal and oral washings samples were used for 16S rRNA gene sequence and fecal LPS detection.

gDNA Extraction and 16S rRNA Gene Sequencing

DNA isolation on all fecal samples was performed the same, using the AllPrep DNA/RNA Mini kit (TIANGEN Biotech, Beijing, China). DNA isolation on oral washings was performed with the Ultraclean Microbial DNA isolation kit (MO BIO, Carlsbad, California, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified and sequenced using an Illumina HiSeq2500 platform (Illumina, California, USA). Raw sequence data were uploaded to the National Center for Biotechnology Information (NCBI) database and are available through accession number PRJNA784752.

Statistical Analyses

The clinical parameters and linear correlation analysis were performed using the Statistical Package for the Social Sciences (SPSS) version 22.0. Alpha and beta diversity analyses were calculated in our samples using Quantitative Insights Into Microbial Ecology (QIIME, Version 1.7.0) based on rarefied operational taxonomic unit (OTU) count and displayed using R software (Version 2.15.3).

A differential prevalence analysis was performed using the Wilcoxon rank-sum test at the genus levels. The analyses were restricted to taxa with a prevalence > 50% and a minimal proportion > 0.002, and only taxa with $P < 0.05$ were considered statistically significant. The predicted functional composition profiles were collapsed into KEGG pathways (level 3) based on 16S rRNA sequences using PICRUSt. Correlations among the variables (clinical parameters, different microbiota, etc.) were computed using Spearman rank correlation, and the correlation (Student's t-test, $P < 0.05$, |correlation coefficient| > 0.3) are presented using a heatmap or network diagram (Cytoscape, Version 3.2.1) (20).

RESULTS

Baseline Characteristics Examined for All Volunteers

Between January 2018 and April 2019, 50 patients were randomly assigned to either the probiotics or placebo groups. Seven patients who received at least one dose of the drug were automatically withdrawn from the study (the probiotics/placebo group, n = 2/n = 5) because of a failure to undergo follow-up or at their request. In addition, another four patients were excluded from the protocol set because of poor drug compliance in the placebo group (**Figure 1**). All patients were thoroughly informed about their diseases and the treatments they would receive. Their sex, age, baseline characteristics, and tumor classification are summarized in **Table 1**. Twenty-three patients were designated as the probiotics group (n = 23), and 16 patients were assigned as the placebo group (n = 16) (**Figure 1**). The treatment groups were well balanced, and there was no marked difference between the two groups.

TABLE 1 | Clinical and demographic features.

Characteristics	Placebo group (n=16)	Probiotic group (n=23)
Sex (M/F)	4/12	2/21
Age (years, mean ± SD)	41.38 ± 7.07	39.13 ± 9.15
BMI (kg/m^2, mean ± SD)	24.11 ± 3.48	23.70 ± 2.93
Tumor stage, No. (%)		
T1	4	7
T2	6	8
T3	6	8
T4	0	0
Node stage, No. (%)		
N0	0	0
N1	12	14
N2	4	9
N3	0	0

BMI, Body Mass Index; SD, standard deviation.

The Probiotic Reduced the Incidence of Complications and Plasma Lipid Levels

As shown in **Table 2**, our data also indicated that the probiotics reduced the incidence of complications (the percentage of subjects with complications [score 3 and 4 clubbed together]), TSQ-C (62.5% vs. 8.7%, P = 0.004), TSQ-LOE (62.5% vs. 30.4%, P = 0.047), TSQ-WG (68.8% vs. 34.8%, P = 0.037), and TSQ-DRY (68.8% vs. 30.4%, P = 0.018) in A-THW-P group, compared with those of the A-THW group. However, the incidence of TSQ-E was not significantly different between the two groups ($P > 0.05$, **Table 2**). The effect size of reduction in complications scores between two groups is shown separately in **Table 2**. To assess whether the probiotics alleviated dyslipidemia, we monitored the plasma lipid indexes in the patients. We found that the probiotics significantly reduced the total cholesterol (CHOL) index (5.57 ± 0.99 vs. 6.56 ± 1.15, P = 0.006), triglyceride (TG) index (1.79 ± 0.68 vs. 2.41 ± 0.66, P = 0.014), low-density lipoprotein (LDL) index (3.80 ± 0.70 vs. 4.36 ± 0.55, P = 0.017), and apolipoprotein A (Apo A) index (1.56 ± 0.18 vs. 1.68 ± 0.05, P = 0.001) values compared with those of the A-THW group (**Table 3**). Moreover, the probiotics restored the rates of the CHOL index (65% vs. 25%), TG index (74% vs. 38%), LDL index (61% vs. 31%), and Apo A index (74% vs. 31%) in the A-THW-P group to normal levels in comparison with the A-THW group (**Table 4**). Although probiotics were found to slightly increase free triiodothyronine (fT3) levels compared to the A-THW group, there was no statistical difference (1.21 ± 0.25 vs. 1.31 ± 0.29, P = 0.052). However, the probiotics did not affect thyroglobulin and liver function indicators compared with the A-THW group ($P > 0.05$, **Table 3** and **Supplementary Table 1**).

Transitions in the Gut and Oral Microbiome of DTC Patients Following THW

To evaluate whether THW can induce gut and oral microbiota dysbiosis, we performed 16S rRNA sequencing of fecal and oral washings samples. Rarefaction analysis showed that OTU richness in each group approached saturation (**Figures 2A–D**). As estimated by the Sobs and Shannon index, gut and oral microbial diversity were significantly decreased in the A-THW group versus the B-THW group (all $P < 0.05$) (**Figures 2A–D**). Beta diversity results showed a significant distinction of the gut and oral microbial communities between both groups (**Figures 2E, F**). We next calculated the microbial dysbiosis

TABLE 2 | The percentage of subjects with complications (score 3 and 4 clubbed together) and the mean thyroid symptoms score.

Variable	A-THW group (n=16)	A-THW-P group (n=23)	P1 value (The percentage of complications)	P2 value (The symptoms score)
Constipation (TSQ-C)	62.5% (2.69 ± 0.98)	8.7% (1.57 ± 0.88)	0.004	0.001
Lack of energy (TSQ-LOE)	62.5% (2.88 ± 1.05)	30.4% (1.78 ± 1.06)	0.047	0.005
Weight gain (TSQ-WG)	68.8% (2.88 ± 0.93)	34.8% (1.91 ± 1.21)	0.037	0.013
Dry mouth (TSQ-DRY)	68.8% (2.94 ± 0.90)	30.4% (1.87 ± 1.19)	0.018	0.007
Edema (TSQ-E)	26.1% (2.20 ± 1.05)	43.8% (2.00 ± 0.83)	0.250	0.641

All complications (score 3 and 4 clubbed together) are represented as a percentage. All the complications scores are in values (mean ± SD). TSQ, Thyroid symptom questionnaire, P1 Value of the percentage of complications between A-THW vs. A-THW-P group; P2 Value of the complications score between A-THW vs. A-THW-P group; SD, Standard deviation. P-value < 0.05 was considered significant. A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic.

TABLE 3 | Plasma indicators.

Variable	Placebo group (n=16)	Probiotic group (n=23)	P value
Plasma lipid			
CHOL (μmol/L, mean ± SD)			
Baseline	4.68 ± 0.49	4.68 ± 0.47	0.703
End of intervention (week 4)	5.57 ± 0.99	6.56 ± 1.15	0.006*
TG (μmol/L, mean ± SD)			
Baseline	1.20 ± 0.35	1.31 ± 0.42	0.417
End of intervention (week 4)	1.79 ± 0.68	2.41 ± 0.66	0.014*
LDL (μmol/L, mean ± SD)			
Baseline	3.18 ± 0.22	3.20 ± 0.30	0.966
End of intervention (week 4)	3.80 ± 0.70	4.36 ± 0.55	0.017*
ApoA (μmol/L, mean ± SD)			
Baseline	1.43 ± 0.06	1.46 ± 0.07	0.069
End of intervention (week 4)	1.56 ± 0.30	1.68 ± 0.19	0.001*
HDL (μmol/L, mean ± SD)			
Baseline	1.10 ± 0.07	1.16 ± 0.10	0.151
End of intervention (week 4)	1.33 ± 0.21	1.36 ± 0.20	0.516
VLDL (μmol/L, mean ± SD)			
Baseline	0.28 ± 0.33	0.25 ± 0.18	0.582
End of intervention (week 4)	0.33 ± 0.12	0.34 ± 0.13	0.147
ApoB (μmol/L, mean ± SD)			
Baseline	0.94 ± 0.06	0.93 ± 0.06	0.877
End of intervention (week 4)	1.09 ± 0.19	1.21 ± 0.27	0.085
Lpa (μmol/L, mean ± SD)			
Baseline	112.29 ± 36.01	119.03 ± 84.91	0.454
End of intervention (week 4)	125.59 ± 135.63	117.85 ± 129.44	0.903
Thyroid function			
fT3 (pg/mL, mean ± SD)			
Baseline	2.84 ± 0.20	2.71 ± 0.29	0.251
End of intervention (week 4)	1.21 ± 0.25	1.31 ± 0.29	0.052
fT4 (ng/dL, mean ± SD)			
Baseline	1.05 ± 0.07	1.07 ± 0.10	0.682
End of intervention (week 4)	0.43 ± 0.05	0.43 ± 0.03	0.166
TSH (μIU/mL, mean ± SD)			
Baseline	1.79 ± 0.74	1.89 ± 0.65	0.437
End of intervention (week 4)	64.90 ± 24.93	63.26 ± 20.41	0.783
Tg (IU/mL, mean ± SD)			
Baseline	6.36 ± 4.01	6.38 ± 3.43	0.832
End of intervention (week 4)	3.92 ± 3.11	3.72 ± 2.76	0.402

*The measurement data and enumeration data were statistically analyzed with t-test (or one-way ANOVA for multi-group comparison) and $\chi 2$ test, respectively. Measurement data are expressed as the mean ± SD. CHOL, total cholesterol; TG, triacylglycerol; LDL, low-density lipoprotein; ApoA, apolipoprotein A; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; ApoB, apolipoprotein B; Lpa, lipoprotein(a); fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyroid-stimulating hormone; Tg, Thyroglobulin; and SD, standard deviation. P-value < 0.05 was considered significant. *P-value < 0.05*

index (MDI) at the genus level (21). We found that the gut and oral microbiota of the A-THW group showed a higher MDI than that of the B-THW group (Wilcoxon rank-sum test, P < 0.001, **Supplementary Figures 1A, B**). The MDI exhibited an inverse correlation with Shannon index of alpha diversity (Spearman correlation analysis, r = -0.37, -0.49, P < 0.05, **Figures 2G, I**) and a positive correlation with PCI distance of beta diversity (Spearman correlation analysis, r = 0.79, -0.83, P < 0.05, **Figures 2H, J**). These results show a high degree of dysbiosis of DTC patients after THW in the gut and oral

TABLE 4 | Rates of People Recovering to Normal Ranges.

Index	Normal standard	Group	People within normal range, No	Total people, No.	People within normal range, No
CHOL	3.45-5.71, μmol/L	A-THW-P	15	23	65%
		A-THW	4	16	25%
TG	0.48-2.25, μmol/L	A-THW-P	17	23	74%
		A-THW	6	16	38%
LDL	0.36-4.11, μmol/L	A-THW-P	14	23	61%
		A-THW	5	16	31%
ApoA	1.20-1.60, μmol/L	A-THW-P	17	23	74%
		A-THW	5	16	31%

CHOL, total cholesterol; TG, triacylglycerol; LDL, low-density lipoprotein; ApoA, apolipoprotein A. A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic.

microbiota, consistent with the reduced bacterial diversity observed.

Effects of Probiotics on the Gut Microbiota

To evaluate whether probiotics can improve microbiota dysbiosis. The microbial diversity characteristics are shown in **Supplementary Table 2**. The sequencing depths were examined by plotting the rarefaction curve of richness (Sobs index), and the curve in each group was near saturation, suggesting that the sequencing depth was adequate (**Figure 3A**). Alpha diversity was evaluated at the operational taxonomic unit (OTU) level using the Sobs, Chao, and Shannon indexes (**Figure 3B**). The results from the Wilcoxon rank-sum test revealed a significant difference in the Sobs, Chao, and Shannon indexes, which measure richness and evenness, between the B-THW and A-THW groups (**Figure 3B**, $P < 0.05$). With probiotic supplementation, the alpha diversity of the A-THW-P group did not show a difference compared to the B-THW-P group (**Figure 3B**, $P > 0.05$), which shows that the probiotic distinctly restored the gut and oral microbial diversity.

In addition, the gut microbiota structure was analyzed at the phylum level. Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria constituted the 4 most common dominant phyla in the B-THW, B-THW-P, A-THW, and A-THW-P groups (Firmicutes; 62.60%, 74.31%, 72.90%, and 74.05%, respectively; Bacteroidetes: 32.98%, 22.09%, 22.22%, and 17.54%, respectively; Proteobacteria: 2.56%, 1.71%, 2.92%, and 5.64%, respectively; Actinobacteria: 1.23%, 1.07%, 1.05%, and 1.78%, respectively, respectively); (**Figure 3C**). Moreover, the gut microbiota composition was also different among the four groups at the family and genus levels (**Supplementary Figures 2A, B**).

To assess the degree of similarity between the microbiota communities, binary-chord distance matrices were used to calculate the beta diversity values and visualize them in PCoA plots. The diversity captured by the top principal coordinates was 8.05%. The gut microbiota of patients in the A-THW-P group was separated from that of patients in the A-THW group (**Figure 3D**). Similar to the alpha diversity trend, fecal samples of patients in the A-THW-P group were closer to samples of the B-THW/B-THW-P groups than to samples of patients in the A-THW group (**Figure 3E**).

We next combined the relevant taxa that characterized each group of patients and calculated the microbial dysbiosis index (MDI) at the genus level. We found that the gut microbiota of patients from the A-THW-P group showed a lower MDI value than that from the A-THW group ($P < 0.001$, **Figure 4A**). The MDI exhibited a positive correlation with the PC1 distance of beta diversity (Spearman correlation analysis, $r = 0.54$, $P < 0.001$, **Figure 4B**). These results show a lower degree of dysbiosis in the gut microbiota of patients in the A-THW-P group in contrast to the A-THW group, which is consistent with the bacterial diversity observed. The probiotics partially rescued the gut microbiota dysbiosis.

To determine the specific communities associated with patients in the A-THW-P group, we applied the Mann–Whitney U test to compare the gut microbiota at different taxon levels by confining analyses, identifying 16 differentially abundant taxa at the genus level (**Figure 4C**, $P < 0.05$). *Holdemanella*, *Coprococcus_2*, *Ruminococcaceae_UCG-013*, *Enterococcus*, *norank_f:Bacteroidales_S24-7_group*, *unclassified_k:norank*, *Stenotrophomonas*, *Prevotella_7*, *Lactococcus*, and *Senegalimassilia* were enriched in the patients from the A-THW-P group compared with those in the A-THW group (**Figure 4C**). In contrast, the abundances of *Lachnoclostridium, [Eubacterium]_ruminantium_group, Fusobacterium, Prevotella_2, Prevotellaceae_Ga6A1_group*, and *Lachnospiraceae_UCG-004* were decreased in patients from the A-THW-P group compared with those from the A-THW group (**Figure 4C**). Then, these 16 differentially abundance genera were applied to build an interaction network (Spearman's correlation value < -0.3 or > 0.3, $P < 0.05$, **Figure 4D**). The A-THW-P group-enriched species were more highly interconnected than the A-THW group-enriched genera.

Associations Between Gut Microbial Species and Clinical Features

A correlation heatmap was generated to further demonstrate the relationship between the relative abundance of different genera ($n = 16$) and clinical features ($n = 7$) ($P < 0.05$, $|\text{correlation coefficient}| > 0.3$, **Figure 5A**). Correlation networks were generated further to demonstrate the above results (**Figures 5B, C**). Bacteria enriched in the A-THW-P group were negatively correlated with plasma lipid values (CHOL, TG, LDL, and Apo A). The bacteria enriched in the A-THW group were positively correlated with these values. The abundances of the A-THW group-enriched genera, including *Fusobacterium*, *[Eubacterium]_ruminantium_group*, and

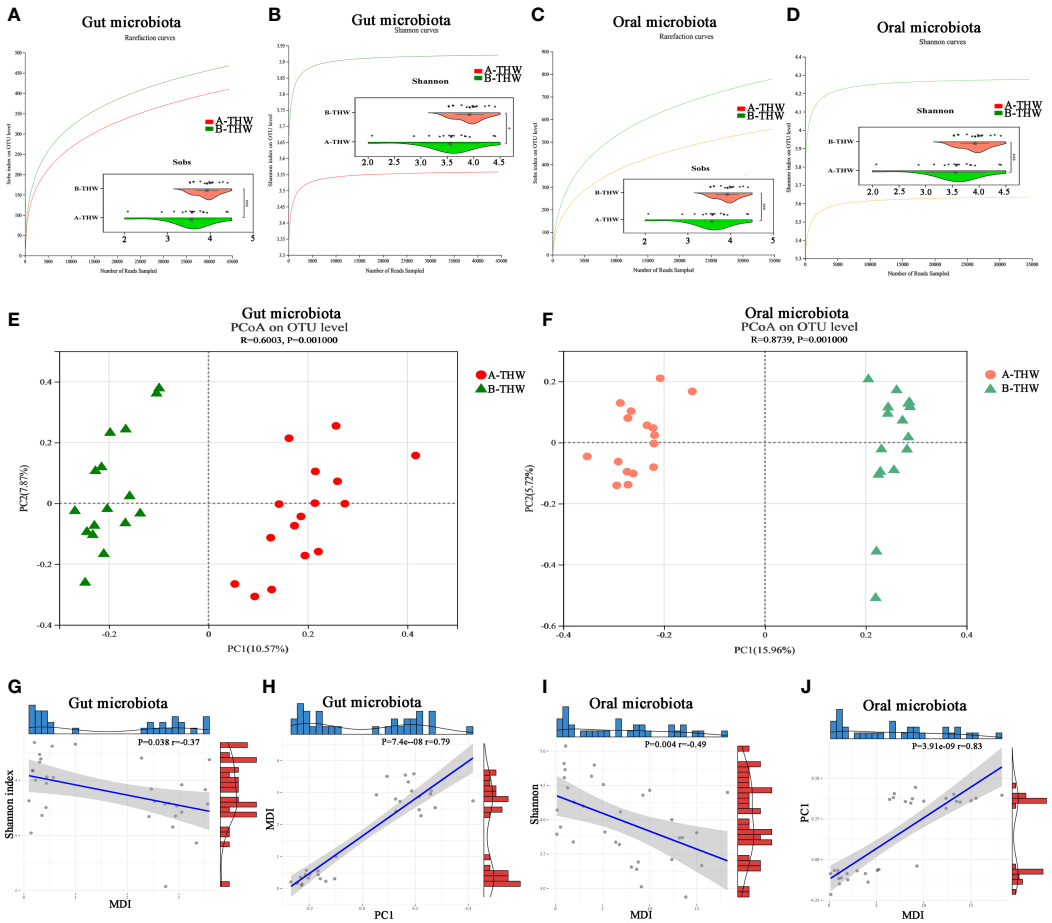

FIGURE 2 | Transitions in the gut and oral microbiome of DTC patients following THW The rarefaction curve reached a plateau, indicating that the sequencing depth was adequate in B-THW (n=16) and A-THW (n=16). As estimated by the Sobs, and Shannon index, gut **(A, B)** and oral **(C, D)** microbial diversity was significantly decreased after THW treatment. The PCoA based on OTU distribution showed that the gut **(E)** and oral **(F)** taxonomic composition was significantly different after THW treatment. The relationship between the MDI and Shannon index of gut **(G)** and oral **(I)** microbiota for each sample. The relationship between the MDI and PC1 distance based on the Binary-chord of gut **(H)** and oral **(J)** microbiota for each sample. PC1, principal coordinate 1; PCoA, principal coordinate analysis; B-THW, before the treatment of THW plus a placebo; B-THW-P, before the treatment of THW plus the probiotic combination; A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic. *P-value < 0.05; ***P-value < 0.001.

Parasutterella, were positively correlated with TSQ-WG and TSQ-LOE values. The abundances of the A-THW-P group-enriched genera, including *Coprococcus_2*, were negatively correlated with TSQ-C values. However, TSQ-C values were positively correlated with some of the A-THW group-enriched genera, including *Fusobacterium*. This finding suggests that the gut microbiota may be involved in the occurrence of the above complications and dyslipidemia.

Functional Alterations in the Gut Microbiota With Probiotics

We carried out PICRUSt analysis to annotate the functions of the microbiota and explore how the microbiota caused complications and dyslipidemia. KEGG Pathways (level 3) of energy metabolism and PPAR signaling pathway were significantly enriched in the A-THW-P group compared with those in the A-THW group ($P < 0.05$, **Figure 5D**). Pathways of lipopolysaccharide (LPS) biosynthesis, LPS biosynthesis proteins, and lipid biosynthesis proteins were enriched in the A-THW group ($P < 0.05$, **Figure 5D**). We observed that fecal/plasma LPS levels of patients in the A-THW group increased simultaneously than those of the A-THW-P group ($P < 0.001$, **Figure 5E**), which is consistent with the enhancement of metabolic pathways related to LPS synthesis (**Figure 5D**). As mentioned above, we identified the changes in the gut microbiota composition, and the abundance of different species was correlated with some plasma lipid levels (e.g., CHOL, TG, LDL, and Apo A) and symptom scores (e.g., TSQ-LOE, TSQ-WG, and TSQ-C). Moreover, we found that the probiotics also improved the function of the gut microbiota. Therefore, we hypothesized that probiotic administration can improve complications, that this effect is related to the recovery of the

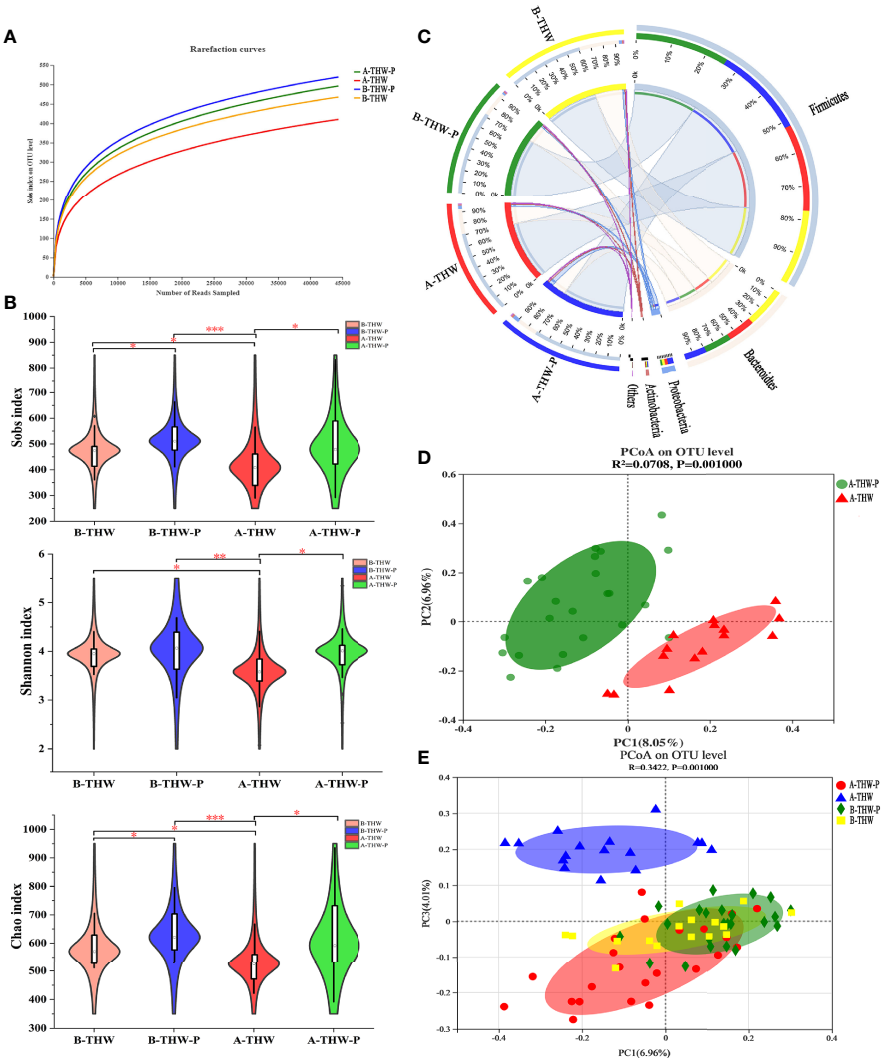

FIGURE 3 | The shift in the gut microbiota architecture in patients with or without probiotic administration. **(A)** The rarefaction curve reached a plateau, indicating that the sequencing depth was adequate. **(B)** The Sobs, Shannon, and Chao indexes of the B-THW, B-THW-P, A-THW, and A-THW-P groups were compared. **(C)** In the Circos plot, the small semicircle (left half-circle) represents the species composition in the sample. The color of the outer ribbon represents the groups, the color of the inner ribbon represents the species, and the length of the ribbon represents the relative abundance of the species in the corresponding sample. The large semicircle (right half-circle) indicates the distribution proportion of species in different samples at this taxonomic level. The color of the outer ribbon represents the species, the color of the inner ribbon represents the groups, and the length of the ribbon represents the relative abundance of the species in the corresponding sample. **(D, E)** Binary-chord principal component analysis; the microbiotas of people from the B-THW, B-THW-P, A-THW, and A-THW-P groups were significantly different. B-THW, before the treatment of THW plus a placebo; B-THW-P, before the treatment of THW plus the probiotic combination; A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic. *P-value < 0.05; **P-value < 0.01; ***P-value < 0.001.

gut microbiota, and that microbiota metabolism might be a regulatory factor.

The Effect of Probiotics on the Oral Microbiota of Patients With THW

The results of distance-based redundancy analysis (dB-RDA) (**Figure 6A**) showed that there is no correlation between TSQ-DRY and gut microbiota, while TSQ-DRY had a significant influence on the oral microbiota (**Figure 6B**). Therefore, we observed improved oral microbiota due to the probiotics and explored its relationship with TSQ-DRY. The alpha diversities were measured by the observed Sobs, Chao, and Shannon indexes. Compared to those in the A-THW group, the Sobs and Chao index values were significantly higher in the A-THW-P group (All, $P < 0.05$, **Figure 6C**). The Shannon index showed a rising trend with an increasing trend in Probiotics supplementation. Still, there was no significant difference ($P > 0.05$, **Figure 6C**). The alpha diversities (Sobs, Chao, and Shannon) of patients from the A-THW-P group returned to the levels of the B-THW/B-THW-P groups compared with the A-THW group (**Figure 6C**). The same

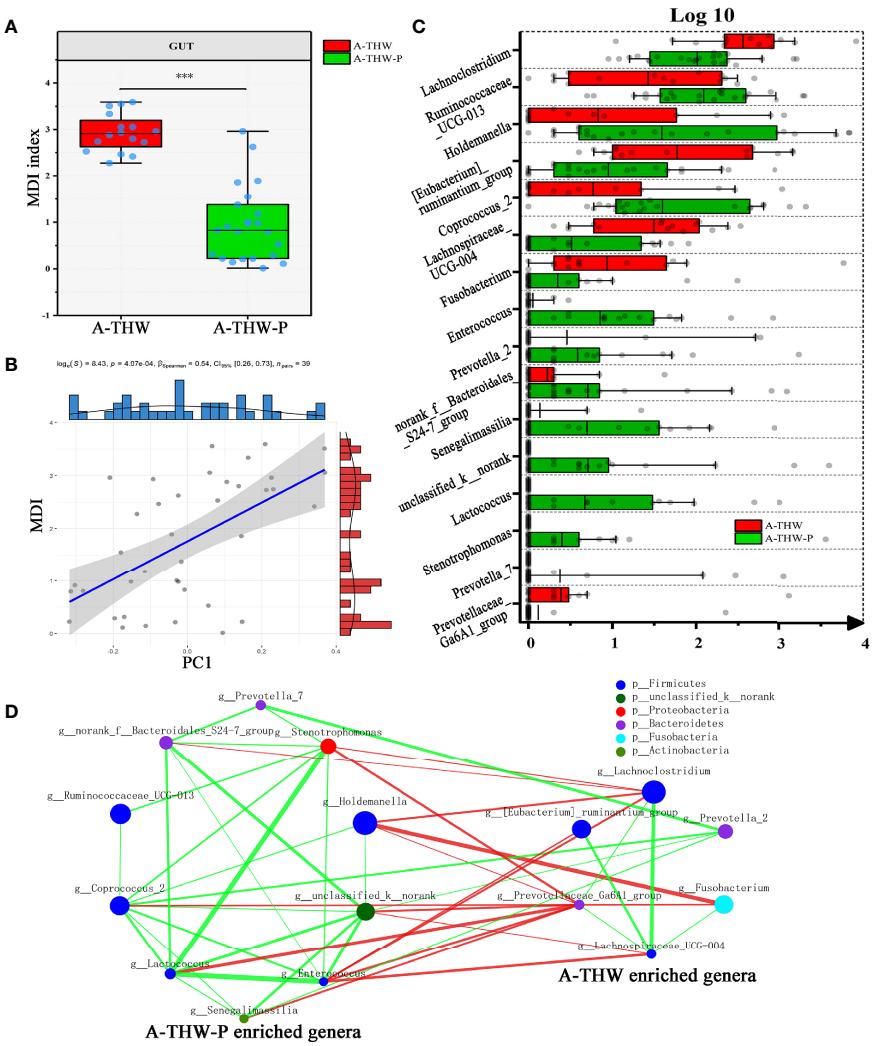

FIGURE 4 | Gut microbiota phylotype alterations at the genus level in patients with or without probiotics. **(A)** Box plot showing the MDI in the A-THW, and A-THW-P groups. Significance was determined by the Kruskal-Wallis method. **(B)** Negative Pearson's correlation between MDI and PC1 distance. **(C)** Comparisons of the relative abundance at the genus level in the A-THW, and A-THW-P groups by Mann–Whitney U-tests ($P < 0.05$). **(D)** A co-occurrence network was deduced from the relative abundance of 16 significantly differentially abundant genera between the A-THW and A-THW-P groups. Species are rearranged on two sides based on their enrichment in the A-THW and A-THW-P group microbiota. Spearman correlation coefficient values below –0.3 (negative correlation) are indicated as red edges, and coefficient values above 0.3 (positive correlation) are indicated as green edges. The node size shows the gene number for each species, and the node color shows their phylum-level classification. A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic. ***P-value < 0.001.

trend was observed in beta diversity measured by binary-chord analysis (**Figure 6D**). Similar to the observation for the gut microbiota, the probiotics significantly reduced the MDI value of the oral microbiota of patients in the A-THW-P group compared to those of the A-THW group (**Supplementary Figure 2D**). The microbial composition of each group is presented at the genus level (**Supplementary Figure 2C**). The dominant genera of the oral microbiota in the A-THW-P group included *Stenotrophomonas* and *Veillonella* (**Figure 6E**). *Haemophilus*, *Fusobacterium, Lautropia, and Prevotella_9* were enriched in the A-THW group (**Figure 6E**). In addition, we regrouped the A-THW-P/A-THW group according to the occurrence of dry mouth, and beta diversity analysis based on the unweighted unifrac distance showed that the samples of the dry mouth group significantly deviated from those of the group without a dry mouth (**Figure 7A**, $P < 0.05$). For investigation of correlations between the microbiota and TSQ-DRY, correlation analysis showed that the levels of *Prevotella_9, Haemophilus, Fusobacterium*, and *Lautropia* in the oral cavity were positively correlated with TSQ-DRY scores. In contrast, *Stenotrophomonas* abundance was negatively correlated with TSQ-DRY scores (**Figure 7B**).

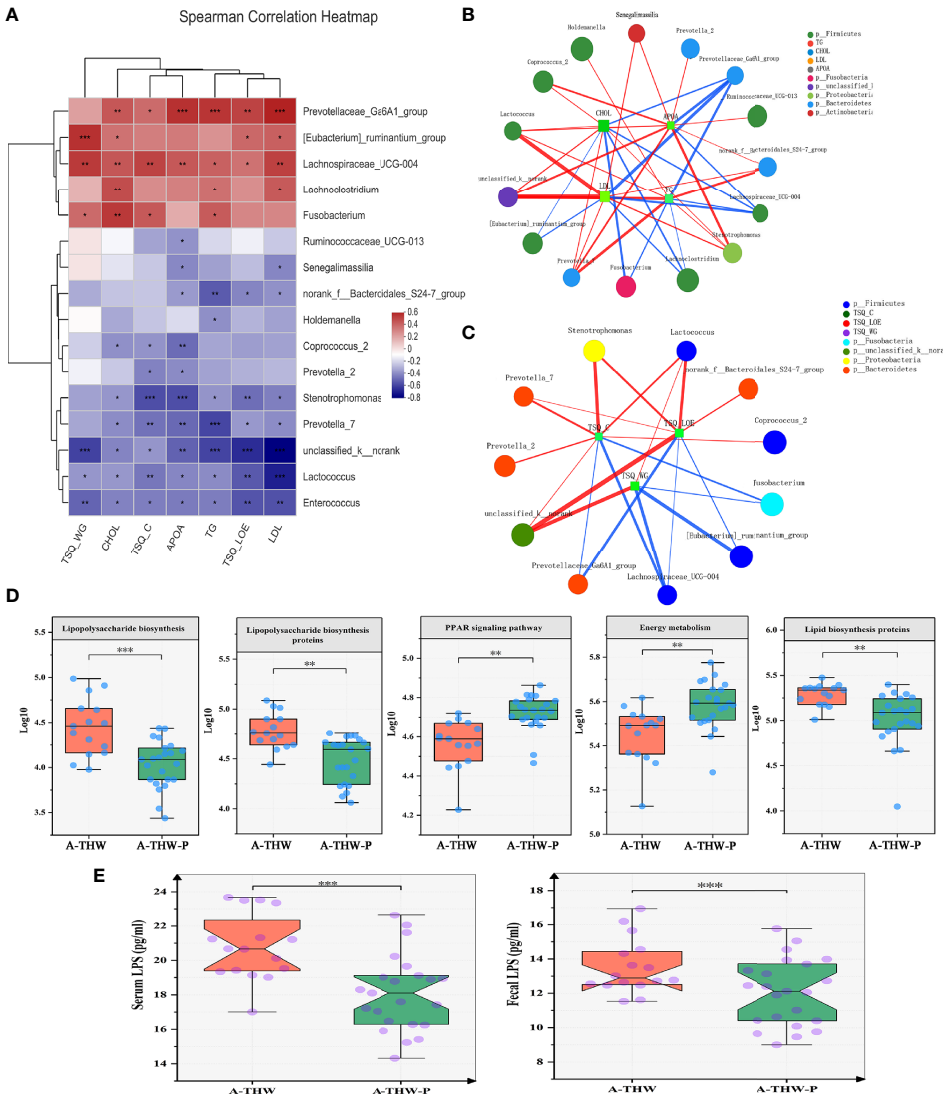

FIGURE 5 | Spearman correlation analysis of environmental factors and characteristics of gut microbiota function. **(A)** The relationships among seven clinical indicators and 16 differentially abundant genera (**Figure 4C**) were estimated using Spearman correlation analysis, and the results **(B, C)** are shown in co-correlation networks. Color intensity represents the magnitude of correlations. Red, positive correlations; blue, negative correlations. Spearman correlation coefficient values below –0.3 (negative correlation) are indicated as red edges, and coefficient values above 0.3 (positive correlation) are indicated as green edges. The node size shows the gene number for each species. The five typically differentially abundant KEGG pathways **(D)** and plasma and fecal LPS levels **(E)** for the A-THW and A-THW-P groups. Wilcoxon rank-sum test. A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic. *P-value < 0.05; **P-value < 0.01; ***P-value < 0.001.

DISCUSSION

The purpose of this study was to assess the impact of probiotics on complications and dyslipidemia in DTC patients with THW. First, we found that probiotics could reduce the incidence of lack of energy, weight gain, constipation, and dry mouth and reduce plasma lipid levels. However, they did not significantly improve the incidence of edema. A significant reduction in fecal/plasma LPS levels and altered oral and gut microbiota were observed, which may account for the protective effect of probiotics supplementation.

Previous studies have shown that variation in thyroid function can affect the gut microbiota (22). In this study, DTC patients with THW had lower gut microbiota diversity (alpha diversity) than before THW treatment. This finding is consistent with a previous study investigating the gut microbiota in Hashimoto's thyroiditis patients with hypothyroidism (23). We found that probiotics could improve the diversity of the gut microbiota.

THW can cause transient dyslipidemia, which will increase the incidence of cardiovascular disease and pancreatitis in DTC patients during THW (24, 25). The range of changes in blood lipids caused by THW is vast: in some patients, the level of change

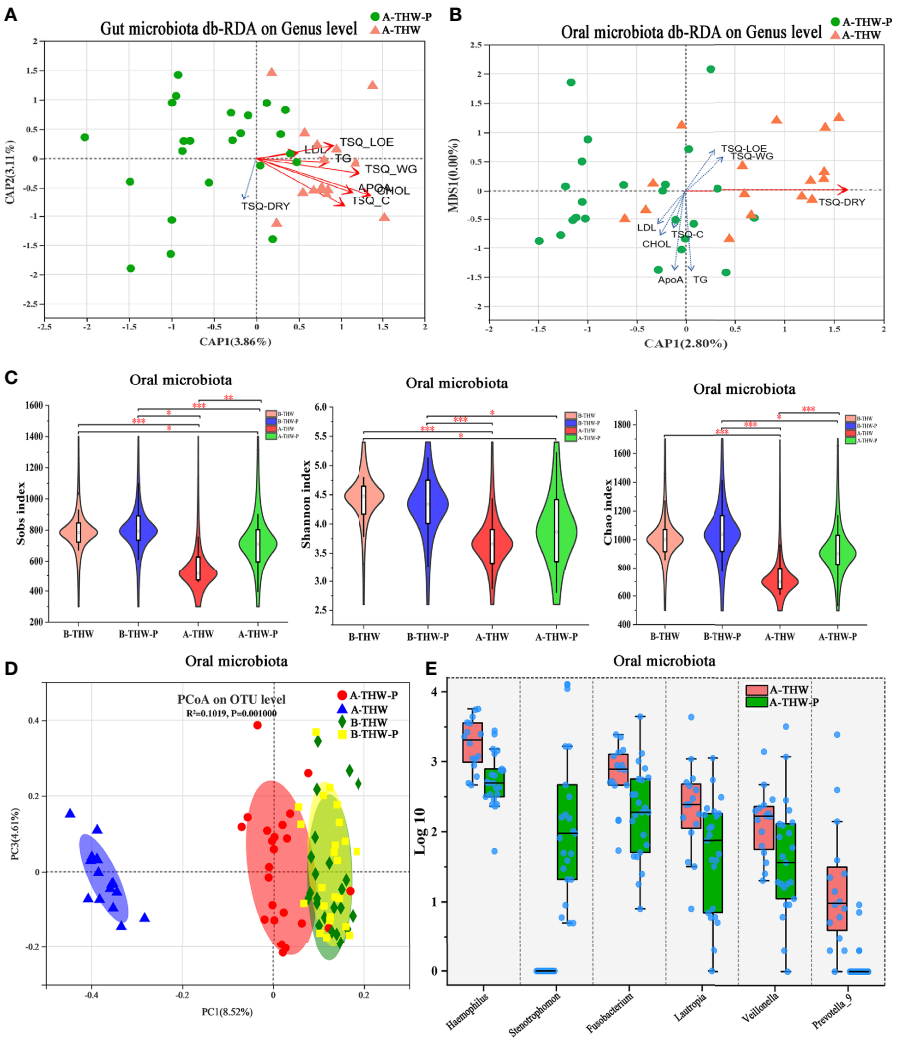

FIGURE 6 | The shift in the oral microbiota composition in patients with or without probiotic administration. dB-RDA shows the relationship of environmental factors to the gut **(A)** and oral **(B)** microbial community structures. **(C)** The Sobs, Chao, and Shannon indexes of the B-THW, B-THW-P, A-THW, and A-THW-P groups were compared. **(D)** Binary-chord principal component analysis; the oral microbiotas of people from the B-THW, B-THW-P, A-THW, and A-THW-P groups were significantly different. **(E)** Comparisons of the oral microbiota relative abundance at the genus level in the A-THW and A-THW-P groups by Mann–Whitney U-tests ($P < 0.05$). B-THW, before the treatment of THW plus a placebo; B-THW-P, before the treatment of THW plus the probiotic combination; A-THW, after treatment with THW plus a placebo; A-THW-P, after treatment with THW plus the probiotic. *P-value < 0.05; **P-value < 0.01; ***P-value < 0.001.

is very slight, while in others, severe hypercholesterolemia is observed. This situation has also been observed in patients with primary thyroid dysfunction (26). The occurrence of dyslipidemia in the THW period cannot be explained by a single mechanism. Several studies have found that TSH and thyroid hormone levels are independently related to total cholesterol levels; in addition, various clinical factors, including gender, age, fasting blood glucose, and BMI, are also considered to have independent effects on total cholesterol levels (27). There is currently no article to evaluate the influence of gut microbiota on blood lipid levels during THW. In our study, plasma lipid levels (e.g., CHOL, TG, HDL, and ApoA) were positively correlated with gut microbiota constituent abundances (e.g., *Coprococcus_2*, and *norank_f:Bacteroidales_S24-7_group*, etc.). According to previous reports, the above microbiota constituents can participate in the production of short-chain fatty acids (SCFAs) (28). One study has shown that SCFAs could regulate plasma lipid metabolism (29). In addition, the lipid biosynthesis protein pathway of the gut microbiota was significantly reduced, and the PPAR metabolism pathway was upregulated after the application of probiotics, according to PICRUSt analysis. According to the report, the PPAR pathway can participate in mediating lipid metabolism (30). We speculated that probiotics might improve the plasma lipid level of patients with THW through the PPAR metabolism and lipid biosynthesis pathway. The accumulation of plasma CHOL, LDL, and other lipids can lead to obesity (31). This effect may be related to the improvement produced by probiotics in weight gain complications.

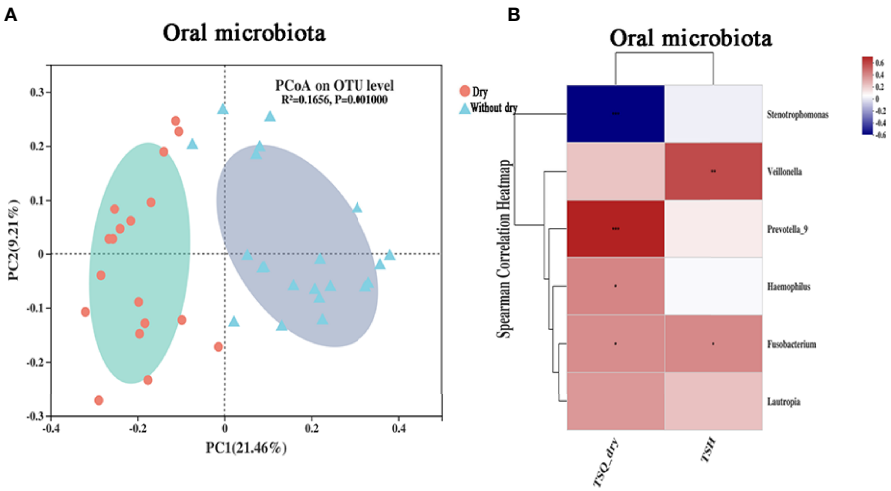

FIGURE 7 | Correlation analysis of the oral microbiota and TSQ-DRY scores. **(A)** Unweighted UniFrac principal component analysis; the oral microbiotas of people from the dry and without dry groups were significantly different. **(B)** The relationships among 2 clinical indicators and 6 differentially abundant genera (**Figure 6E**) were estimated using Spearman correlation analysis. * P-value <0.05; ** P-value <0.01; *** P-value <0.001.

In our study, we found that probiotic administration significantly reduced patients' fecal LPS and plasma LPS concentrations. The abundance of LPS-producing bacteria (e.g., *Fusobacterium*) and LPS synthesis-related pathways were also considerably reduced, which may explain the reduction in fecal LPS concentrations by probiotics. There is a correlation between the fecal/plasma LPS level and the impaired intestinal barrier (32). When intestinal permeability increased, LPS in fecal could pass through the intestine and enter the circulation. Probiotics such as *Lactobacillus* and *Bifidobacterium* protect the gut barrier by increasing tight junction protein expression (e.g., occludin and claudin 3) and reducing inflammatory markers (32–34). Therefore, probiotics may reduce serum LPS level by regulating the intestinal barrier in DTC patients with THW. LPS can bind to Toll-like receptors on thyroid cells and affect the expression of thyroglobulin and sodium iodine transporter (35, 36). This activity may further affect sensitivity to subsequent RAI therapy.

We found that probiotics can reduce the incidence of lack of energy. Patients mainly showed fatigue complications, which may be related to improving the gut microbiota produced by probiotics. A study analyzed chronic fatigue syndrome patients' fecal and plasma samples and healthy volunteers (15). The results showed that compared with healthy people, the bacterial diversity of patients with chronic fatigue syndrome was significantly reduced, and the number of types of anti-inflammatory bacteria was considerably reduced. In addition, bacteria in the intestine can enter the plasma through the damaged intestinal barrier, worsening the disease. The application of probiotics, especially *Bifidobacterium infantis 35624*, can improve the gut microbiota and mucosal barrier function and reduce proinflammatory cytokines and inflammatory biomarkers to delay the progression of chronic fatigue syndrome (37, 38). In our study, probiotic administration increased the alpha diversity of the gut microbiota and reduced the abundance of inflammatory bacteria, such as *Fusobacterium*, while enriching the energy metabolism pathway. Additionally, probiotic administration reduced the fecal and plasma LPS levels, suggesting that probiotics may improve intestinal barrier function. This evidence shows that probiotics reduce the incidence of fatigue in patients with THW, which may be related to the improvement in microbiota diversity, intestinal inflammation, barrier function, and reduction in inflammatory bacterial abundance. In addition, this study showed that probiotic administration could significantly reduce the incidence of constipation in patients with THW. Correlation analysis showed that the occurrence of constipation was negatively correlated with *Prevotella_2*, *Prevotella_7*, and *Lactococcus* abundance, suggesting that probiotics may improve constipation by increasing the abundance of the above bacteria, which is consistent with previous studies (39).

This study showed that probiotics could increase the alpha diversity index of the oral microbiota of patients with THW and reduce the abundances of *Hemophilus, Fusobacterium*, *Lautropia*, and *Prevotella_9*. Compared with healthy volunteers, those with hypothyroidism during pregnancy had significantly higher *Prevotella* abundance (40). In our study, probiotic administration reduced the incidence of dry mouth complications. Beta diversity analysis showed that oral samples from patients with dry mouth complications significantly deviate from those without dry mouth complications. Correlation analysis showed that *Prevotella_9*, *Haemophilus*, and *Fusobacterium* were positively correlated with the occurrence of dry mouth complications. It is reported that LPS produced by oral bacteria such as *Fusobacterium* may cause a decrease in mucin synthesis in salivary acinar cells, which is accompanied by acinar cell apoptosis (41), this may be a potential mechanism for oral bacteria to affect the occurrence of dry mouth. In addition, these oral bacteria were significantly reduced by probiotic administration. We speculate that this effect

may be related to the improvement in the microbiota yielded by probiotic administration. Unfortunately, this study did not measure the salivary gland flow rate of patients with dry mouth complications to reflect their salivary gland function. In the future, additional studies are needed to clarify the relationship between oral bacteria and the occurrence of dry mouth complications in patients with THW.

Iodothyronine-deiodinases play a vital role in the conversion of thyroxine (T4) to its active form triiodothyronine (T3) or reverse T3, its inactive form (42). Deiodinase activity has been found in the gut (43, 44); the presence of gut microbiota might be binding to T3, reducing or eliminating deiodinase activity (43, 44). One study showed that gavage of probiotic yogurt significantly increased serum T3 levels in rats (45). The presence of beneficial bacteria such as probiotics in the gut may accelerate the conversion of T4 to T3 (45–47). In addition, β-glucuronidases and sulfatases enzymes can hydrolyze glucuronide and iodothyronine sulfate metabolites, thereby inactivating thyroid hormones in the liver (35). Gut microbiota expresses β-glucuronidases and sulfatases enzymes (35). Probiotics may affect the activity of these enzymes by modulating the gut microbiota. Although not statistically significant, fT3 levels were higher in the patients treated with probiotics in our study, and this result may be due to probiotics affecting the deiodination of thyroid hormone or the activity of β-glucuronidases and sulfatases enzymes. Many researchers believe that the complications during THW are related to hypothyroidism caused by thyroid hormone deficiency. Since probiotics did not significantly change thyroid hormone levels but did improve complications, we infer that thyroid hormone deficiency during THW may shape a dysbiosis microbiota and cause increased complications. Probiotics can reduce complications by improving dysbiosis microbiota. In addition, probiotics could also be able to prevent serum hormonal fluctuations (48). It is worth noting that iodine levels have been proven to influence the gut microbiota (42). A low-iodine diet and changes in thyroid hormone levels during THW may jointly participate in microbiota transformation.

In addition, although to our knowledge, this study is the first randomized controlled trial showing that DTC patients may have a dysbiosis gut and oral microbiota during THW, and probiotics administration may reduce complications and dyslipidemia in patients after THW by improving the oral and gut microbiota, it must be noted that there were several limitations of this study. To ensure the scientificity and reliability of this study, we implemented strict inclusion and exclusion criteria, which can lead to a dramatic reduction in the number of patients enrolled in the study. This study's sample size is limited. And the subjective evaluation scales and standardized collection of data may lead to potential observer bias. Nevertheless, in most current research on THW-related complications, the evaluation scales are the mainstream evaluation method, and its assessment of complications is relatively reliable (11, 49). There are hardly any other objective evaluation methods in this field. In the future, more effective evaluation methods need to be developed, such as a combination of evaluation scales and laboratory tests. Compared to 16S rDNA amplicon sequencing, shotgun generation sequencing metagenomics has more power to identify a larger number of species. The results of this study are limited, and no major clinical consequences can be proven at this stage. The possible role of probiotics as "adjuvants for THW treatment" may become a starting point for probiotic researchers and endocrinologists to clarify the interaction between the endocrine system and intestinal and oral microecology. So far, this correlation has not been evaluated. Researchers should explore other probiotic strains or longer follow-up times and larger sample sizes in the future.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NCBI BioProject repository, https://www.ncbi.nlm.nih.gov/, accession number PRJNA784752.

ETHICS STATEMENT

The Ethics Committee approved all protocols applied in this study at the First Affiliated Hospital of Harbin Medical University (Eth. 201816). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Guarantee all integrity associated with this study, YW, BL, and FZ. Study concepts/study design or data acquisition or data analysis/interpretation, BL and FZ. Manuscript drafting or manuscript revision for important intellectual content, all authors. Agree to ensure any questions related to the work are appropriately resolved, all authors. Agree to be accountable for the content of the work, all authors. Collect the samples, YLiu, YLu, XW, JF, XJ, WY, and XG. Perform the bioinformatics and statistical analyses and interprets the data, SS, ZL, LL, HC, HW, and SW. Revise the manuscript for important content, YW. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.834674/full#supplementary-material

REFERENCES

1. Cui Y, Mubarik S, Li R, Nawsherwan, Yu C. Trend Dynamics of Thyroid Cancer Incidence Among China and the U.S. Adult Population From 1990 to 2017: A Joinpoint and Age-Period-Cohort Analysis. *BMC Public Health* (2021) 21:624. doi: 10.1186/s12889-021-10635-w
2. Lin JD, Chao TC, Huang MJ, Weng HF, Tzen KY. Use of Radioactive Iodine for Thyroid Remnant Ablation in Well-Differentiated Thyroid Carcinoma to Replace Thyroid Reoperation. *Am J Clin Oncol* (1998) 21:77–81. doi: 10.1097/00000421-199802000-00018
3. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients With Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* (2016) 26:1–133. doi: 10.1089/thy.2015.0020
4. Kowalska A. Comparing the Effectiveness of Stimulation Using rhTSH and Thyroid Hormone Withdrawal in the Treatment of Thyroid Cancer. *Thyroid Res* (2015) 8:A16. doi: 10.1186/1756-6614-8-S1-A16
5. Kannan S, Mahadevan S, Sadacharan D, Thirumurthi K. Is 3-4 Weeks Required for TSH to Rise Post Thyroidectomy? A Prospective Study and Discussion of Its Implications on Patient Care. *Indian J Endocrinol Metab* (2019) 23:452–5. doi: 10.4103/ijem.IJEM_166_19
6. Lee J, Yun MJ, Nam KH, Chung WY, Soh EY, Park CS. Quality of Life and Effectiveness Comparisons of Thyroxine Withdrawal, Triiodothyronine Withdrawal, and Recombinant Thyroid-Stimulating Hormone Administration for Low-Dose Radioiodine Remnant Ablation of Differentiated Thyroid Carcinoma. *Thyroid* (2010) 20:173–9. doi: 10.1089/thy.2009.0187
7. Schroeder PR, Haugen BR, Pacini F, Reiners C, Schlumberger M, Sherman SI, et al. A Comparison of Short-Term Changes in Health-Related Quality of Life in Thyroid Carcinoma Patients Undergoing Diagnostic Evaluation With Recombinant Human Thyrotropin Compared With Thyroid Hormone Withdrawal. *J Clin Endocrinol Metab* (2006) 91:878–84. doi: 10.1210/jc.2005-2064
8. Rubic M, Kuna SK, Tesic V, Samardzic T, Despot M, Huic D. The Most Common Factors Influencing on Quality of Life of Thyroid Cancer Patients After Thyroid Hormone Withdrawal. *Psychiatr Danub* (2014) 26 (Suppl 3):520–7.
9. Sigal GA, Tavoni TM, Silva BMO, Kalil Filho R, Brandao LG, Maranhao RC. Effects of Short-Term Hypothyroidism on the Lipid Transfer to High-Density Lipoprotein and Other Parameters Related to Lipoprotein Metabolism in Patients Submitted to Thyroidectomy for Thyroid Cancer. *Thyroid* (2019) 29:53–8. doi: 10.1089/thy.2018.0190
10. Singh R, Tandon A, Gupta SK, Saroja K. Optimal Levothyroxine Replacement Adequately Improves Symptoms of Hypothyroidism; Residual Symptoms Need Further Evaluation for Other Than Hypothyroidism Causation. *Indian J Endocrinol Metab* (2017) 21:830–5. doi: 10.4103/ijem.IJEM_165_17
11. Tang CYL, Thang SP, Zaheer S, Kwan CK, Ng DC. Recombinant Human Thyrotropin Versus Thyroid Hormone Withdrawal in an Asian Population. *Endocrine* (2020) 69:126–32. doi: 10.1007/s12020-020-02238-z
12. Gamper EM, Wintner LM, Rodrigues M, Buxbaum S, Nilica B, Singer S, et al. Persistent Quality of Life Impairments in Differentiated Thyroid Cancer Patients: Results From a Monitoring Programme. *Eur J Nucl Med Mol Imaging* (2015) 42:1179–88. doi: 10.1007/s00259-015-3022-9
13. Dimidi E, Zdanaviciene A, Christodoulides S, Taheri S, Louis P, Duncan PI, et al. Randomised Clinical Trial: Bifidobacterium Lactis NCC2818 Probiotic vs Placebo, and Impact on Gut Transit Time, Symptoms, and Gut Microbiology in Chronic Constipation. *Aliment Pharmacol Ther* (2019) 49:251–64. doi: 10.1111/apt.15073
14. Perna S, Ilyas Z, Giacosa A, Gasparri C, Peroni G, Faliva MA, et al. Is Probiotic Supplementation Useful for the Management of Body Weight and Other Anthropometric Measures in Adults Affected by Overweight and Obesity With Metabolic Related Diseases? A Systematic Review and Meta-Analysis. *Nutrients* (2021) 13(2):666. doi: 10.3390/nu13020666
15. Giloteaux L, Goodrich JK, Walters WA, Levine SM, Ley RE, Hanson MR. Reduced Diversity and Altered Composition of the Gut Microbiome in Individuals With Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Microbiome* (2016) 4:30. doi: 10.1186/s40168-016-0171-4
16. Knezevic J, Starchl C, Tmava Berisha A, Amrein K. Thyroid-Gut-Axis: How Does the Microbiota Influence Thyroid Function? *Nutrients 12* (2020) 12 (6):1769. doi: 10.3390/nu12061769
17. Ejtahed HS, Angoorani P, Soroush AR, Siadat SD, Shirzad N, Hasani-Ranjbar S, et al. Our Little Friends With Big Roles: Alterations of the Gut Microbiota in Thyroid Disorders. *Endocr Metab Immune Disord Drug Targets* (2020) 20:344–50. doi: 10.2174/1871530319666190930110605
18. Yao Z, Zhao M, Gong Y, Chen W, Wang Q, Fu Y, et al. Relation of Gut Microbes and L-Thyroxine Through Altered Thyroxine Metabolism in Subclinical Hypothyroidism Subjects. *Front Cell Infect Microbiol* (2020) 10:495. doi: 10.3389/fcimb.2020.00495
19. Feng J, Zhao F, Sun J, Lin B, Zhao L, Liu Y, et al. Alterations in the Gut Microbiota and Metabolite Profiles of Thyroid Carcinoma Patients. *Int J Cancer* (2019) 144:2728–45. doi: 10.1002/ijc.32007
20. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res* (2003) 13:2498–504. doi: 10.1101/gr.1239303
21. Jin Y, Geng R, Liu Y, Liu L, Jin X, Zhao F, et al. Prediction of Postoperative Ileus in Patients With Colorectal Cancer by Preoperative Gut Microbiota. *Front Oncol* (2020) 10:526009. doi: 10.3389/fonc.2020.526009
22. Zhou L, Li X, Ahmed A, Wu D, Liu L, Qiu J, et al. Gut Microbe Analysis Between Hyperthyroid and Healthy Individuals. *Curr Microbiol* (2014) 69:675–80. doi: 10.1007/s00284-014-0640-6
23. Liu S, An Y, Cao B, Sun R, Ke J, Zhao D. The Composition of Gut Microbiota in Patients Bearing Hashimoto's Thyroiditis With Euthyroidism and Hypothyroidism. *Int J Endocrinol* (2020) 2020:5036959. doi: 10.1155/2020/5036959
24. Shin DY, Kim KJ, Cho Y, Park KH, Hwang S, Chung WY, et al. Body Mass Index Is Associated With Hypercholesterolemia Following Thyroid Hormone Withdrawal in Thyroidectomized Patients. *Int J Endocrinol* (2014) 2014:649016. doi: 10.1155/2014/649016
25. Sacks FM, Stanesa M, Hegele RA. Severe Hypertriglyceridemia With Pancreatitis: Thirteen Years' Treatment With Lomitapide. *JAMA Intern Med* (2014) 174:443–7. doi: 10.1001/jamainternmed.2013.13309
26. Kuusi T, Taskinen MR, Nikkila EA. Lipoproteins, Lipolytic Enzymes, and Hormonal Status in Hypothyroid Women at Different Levels of Substitution. *J Clin Endocrinol Metab* (1988) 66:51–6. doi: 10.1210/jcem-66-1-51
27. Wang F, Tan Y, Wang C, Zhang X, Zhao Y, Song X, et al. Thyroid-Stimulating Hormone Levels Within the Reference Range Are Associated With Serum Lipid Profiles Independent of Thyroid Hormones. *J Clin Endocrinol Metab* (2012) 97:2724–31. doi: 10.1210/jc.2012-1133
28. Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, et al. A Phylo-Functional Core of Gut Microbiota in Healthy Young Chinese Cohorts Across Lifestyles, Geography and Ethnicities. *ISME J* (2015) 9:1979–90. doi: 10.1038/ismej.2015.11
29. Nogal A, Valdes AM, Menni C. The Role of Short-Chain Fatty Acids in the Interplay Between Gut Microbiota and Diet in Cardio-Metabolic Health. *Gut Microbes* (2021) 13:1–24. doi: 10.1080/19490976.2021.1897212
30. Decano JL, Singh SA, Bueno CG, Lee LH, Halu A, Chelvanambi S, et al. Systems Approach to Discovery of Therapeutic Targets for Vein Graft Disease PPARalpha Pivotally Regulates Metabolism, Activation, and Heterogeneity of Macrophages and Lesion Development. *Circulation* (2021) 143(25):2454–70. doi: 10.1161/CIRCULATIONAHA.119.043724
31. You L, Li F, Sun Y, Luo L, Qin J, Wang T, et al. Extract of Acalypha Australis L. Inhibits Lipid Accumulation and Ameliorates HFD-Induced Obesity in Mice Through Regulating Adipose Differentiation by Decreasing PPARgamma and CEBP/alpha Expression. *Food Nutr Res* (2021) 65. doi: 10.29219/fnr.v65.4246
32. Ballway JW, Song BJ. Translational Approaches With Antioxidant Phytochemicals Against Alcohol-Mediated Oxidative Stress, Gut Dysbiosis, Intestinal Barrier Dysfunction, and Fatty Liver Disease. *Antioxid (Basel)* (2021) 10(3):384. doi: 10.3390/antiox10030384
33. Camilleri M, Vella A. What to Do About the Leaky Gut. *Gut* (2022) 71:424–35. doi: 10.1136/gutjnl-2021-325428

34. Camilleri M. Human Intestinal Barrier: Effects of Stressors, Diet, Prebiotics, and Probiotics. *Clin Transl Gastroenterol* (2021) 12:e00308. doi: 10.14309/ctg.0000000000000308
35. Kunc M, Gabrych A, Witkowski JM. Microbiome Impact on Metabolism and Function of Sex, Thyroid, Growth and Parathyroid Hormones. *Acta Biochim Pol* (2016) 63:189–201. doi: 10.18388/abp.2015_1093
36. Nicola JP, Nazar M, Mascanfroni ID, Pellizas CG, Masini-Repiso AM. NF-kappaB P65 Subunit Mediates Lipopolysaccharide-Induced Na(+)/I(-) Symporter Gene Expression by Involving Functional Interaction With the Paired Domain Transcription Factor Pax8. *Mol Endocrinol* (2010) 24:1846–62. doi: 10.1210/me.2010-0102
37. Lakhan SE, Kirchgessner A. Gut Inflammation in Chronic Fatigue Syndrome. *Nutr Metab (Lond)* (2010) 7:79. doi: 10.1186/1743-7075-7-79
38. Roman P, Carrillo-Trabalon F, Sanchez-Labraca N, Canadas F, Estevez AF, Cardona D. Are Probiotic Treatments Useful on Fibromyalgia Syndrome or Chronic Fatigue Syndrome Patients? *A Systematic Rev Benef Microbes* (2018) 9:603–11. doi: 10.3920/BM2017.0125
39. Tian Y, Zuo L, Guo Q, Li J, Hu Z, Zhao K, et al. Potential Role of Fecal Microbiota in Patients With Constipation. *Therap Adv Gastroenterol* (2020) 13:1756284820968423. doi: 10.1177/1756284820968423
40. Wang B, Xu Y, Zhang M, Zhang J, Hou X, Li J, et al. Oral and Intestinal Microbial Features in Pregnant Women With Hypothyroidism and Their Correlations With Pregnancy Outcomes. *Am J Physiol Endocrinol Metab* (2020) 319:E1044–52. doi: 10.1152/ajpendo.00234.2020
41. Slomiany BL, Slomiany A. Activation of Peroxisome Proliferator-Activated Receptor Gamma Impedes Porphyromonas Gingivalis Lipopolysaccharide Interference With Salivary Mucin Synthesis Through Phosphatidylinositol 3-Kinase/Erk Pathway. *J Physiol Pharmacol* (2003) 54:3–15.
42. Frohlich E, Wahl R. Microbiota and Thyroid Interaction in Health and Disease. *Trends Endocrinol Metab* (2019). doi: 10.1016/j.tem.2019.05.008
43. Nguyen TT, DiStefano JJ, Huang 3LM, Yamada H, Cahnmann HJ. 5'- and 5-Deiodinase Activities in Adult Rat Cecum and Large Bowel Contents Inhibited by Intestinal Microflora. *Am J Physiol* (1993) 265:E521–4. doi: 10.1152/ajpendo.1993.265.3.E521
44. Virili C, Centanni M. "With a Little Help From My Friends" - The Role of Microbiota in Thyroid Hormone Metabolism and Enterohepatic Recycling. *Mol Cell Endocrinol* (2017) 458:39–43. doi: 10.1016/j.mce.2017.01.053
45. Soheilian KM, Khanafari A, Hedayati M. Association of Consuming Probiotic Yoghurt Consumption With Changes in Thyroid Hormones and Weight in Male Wistar Rats. *J Endocrinol Metabol* (2014) 15:430–4.
46. Talebi S, Karimifar M, Heidari Z, Mohammadi H, Askari G. The Effects of Synbiotic Supplementation on Thyroid Function and Inflammation in Hypothyroid Patients: A Randomized, Doubleblind, Placebocontrolled Trial. *Complement Ther Med* (2020) 48:102234. doi: 10.1016/j.ctim.2019.102234
47. Mullur R, Liu YY, Brent GA. Thyroid Hormone Regulation of Metabolism. *Physiol Rev* (2014) 94:355–82. doi: 10.1152/physrev.00030.2013
48. Spaggiari G, Brigante G, De Vincentis S, Cattini U, Roli L, De Santis MC, et al. Probiotics Ingestion Does Not Directly Affect Thyroid Hormonal Parameters in Hypothyroid Patients on Levothyroxine Treatment. *Front Endocrinol (Lausanne)* (2017) 8:316. doi: 10.3389/fendo.2017.00316
49. Tu J, Wang S, Huo Z, Lin Y, Li X, Wang S. Recombinant Human Thyrotropin-Aided Versus Thyroid Hormone Withdrawal-Aided Radioiodine Treatment for Differentiated Thyroid Cancer After Total Thyroidectomy: A Meta-Analysis. *Radiother Oncol* (2014) 110:25–30. doi: 10.1016/j.radonc.2013.12.018

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Composition of the Gut Microbiota in Attention Deficit Hyperactivity Disorder: A Systematic Review and Meta-Analysis

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Background: The latest research accumulates information to explore the correlation between gut microbiota and neurodevelopmental disorders, which may lead to new approaches to treat diseases such as attention deficit/hyperactivity disorder (ADHD). However, the conclusions of previous studies are not completely consistent. The objective of the systematic review and meta-analysis was to identify evidence on the dysbiosis of gut microbiota in ADHD and find potential distinctive traits compared to healthy controls.

Methods: Electronic databases, including PubMed, Embase, Web of Science, Cochrane Library, and PsycINFO, were searched up to August 24, 2021, using predetermined terms. Meta-analysis was performed to estimate the comparison of microbiota profiles (alpha and beta diversity) and the relative abundance of gut microbiota in ADHD patients and healthy controls.

Results: A total of eight studies were included in the meta-analysis, containing 316 ADHD patients and 359 healthy controls. There was a higher Shannon index in ADHD patients than in healthy controls (SMD = 0.97; 95% CI, 0.13 to 1.82; $P = 0.02$; $I^2 = 96\%$), but the significance vanished after sensitivity analysis because of high heterogeneity. No significant differences in other alpha diversity indexes were found. Regarding the relative abundance of gut microbiota, the genus *Blautia* was significantly elevated in ADHD patients compared with controls (SMD = 0.34; 95% CI, 0.06 to 0.63; $P = 0.02$; $I^2 = 0\%$).

Conclusions: Patients with ADHD had gut microbiome alterations compared to healthy controls. Though more studies with strict methodology are warranted due to the high heterogeneity, further studies to translate the findings of gut microbiota dysbiosis to clinical application in ADHD patients are needed and may guide targeted therapies.

Systematic Review Registration: [https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=273993], identifier PROSPERO (CRD42021273993).

Keywords: attention-deficit/hyperactivity disorder, gut microbiota, dysbiosis, *Blautia*, systematic review and meta-analysis

INTRODUCTION

ADHD is one of the most common neurodevelopmental disorders and onset in early childhood, with a prevalence of 5.9% worldwide (1). It is a clinically heterogeneous disease that manifests with different combinations of symptoms, including inattention, hyperactivity, impulsivity, cognitive impairment, and imposes huge burdens on patients and families. The etiologies of ADHD are multifactorial, including genetic (2) and environmental (3) components.

ADHD patients usually have gastrointestinal symptoms (4) such as constipation, abdominal pain, fecal incontinence, accompanied by picky eating (5), and many other diseases (4–6) such as food allergies, asthma, and eczema. All these symptoms have been documented to be influenced by gut microbiota. Possible mechanisms involved microbial metabolites, amino acid metabolites, immune factors, and neurotransmitters (7).

Currently, the major therapeutic interventions for ADHD are medications, behavioral therapy, and cognitive training. While the efficacy of stimulant medications is validated by powered clinical trials, side effects, including decreased appetite, slight sleep delay, and cardiovascular risks, remain a cause for concern. In recent years, researchers have emphasized the importance of environmental factors such as the gut microbiota to investigate novel therapeutic approaches, including probiotics and prebiotics.

To date, several systematic reviews have shown the correlation between ADHD and gut microbiota, but no meta-analysis has been conducted. Thus, we performed this systematic review and meta-analysis to investigate the relationship between ADHD and gut microbiota and find potential distinctive traits in ADHD.

MATERIALS AND METHODS

Protocol and Registration

The study was registered in PROSPERO (CRD42021273993) and strictly followed the PRISMA guidelines (8).

Study Eligibility Criteria

Studies were included based on the following PICOS criteria.

Participants

Participants with confirmed ADHD were selected for the review, irrespective of age, gender, race, the existence of co-morbidities, and the use of medication. Animal studies were excluded in the review.

Interventions, Exposure(s)

No specific exposure was required. We were not interested in interventional studies.

Comparators

Comparator group was healthy controls (HCs) without ADHD diagnosis.

Outcomes

Studies were eligible if they report the differences between ADHD patients and HCs in gut microbiota diversity indices (alpha diversity and beta diversity) and relative or absolute abundance of microbial taxa.

Study Design

Studies were included if they were observational studies or controlled trials. Studies were excluded if they met any of the following criteria: case reports, conference presentations, reviews, expert opinions, or study protocol.

Search Strategy

The most commonly used databases, including PubMed, Embase, Web of Science, Cochrane Library, and PsycINFO, were searched up to August 24, 2021, using the predetermined terms. The search strategy used is available in **Supplementary Material 1**. We did not set restrictions on language, year, or geographical location. Moreover, we manually searched the reference lists of identified articles to find potentially relevant studies and searched the System for Information on Grey Literature in Europe (SEGLE) and WorldCat for grey literature.

Two individual reviewers (NW, XPG) screened the titles and abstracts independently for possible articles. If there was an agreement between the two reviewers regarding a particular study, it was selected for further analysis; however, if there was disagreement, a third reviewer (LY) would determine whether the study qualifies for inclusion. The full texts of these potentially eligible studies were independently evaluated for eligibility by three reviewers (NW, XPG, ZFZ). Any disagreement between them was resolved by discussion or by a third reviewer (LY) when required.

Data Extraction

If studies met the criteria mentioned above, then the data were extracted by one independent reviewer (NW) using a standardized extraction form. The second author (LY) will review all the extracted data with the team to resolve disputes, and the group (NW, XPG, ZFZ, LY) will finalize the data.

For all eligible studies, the following information was extracted: first author; year of publication; country; number, age and sex of ADHD patients as well as healthy controls; definition of ADHD; alpha diversity (microbial diversity within the same group's samples, including observed operational taxonomic units (OTUs), observed species, Shannon diversity, Chao1 diversity, Simpson diversity); beta diversity (community diversity between different groups' samples, including weighted UniFrac distances, unweighted UniFrac distances, Bray–Curtis distance, Jaccard distance); data on microbiota (including the phyla, order, family, genera, and species of microbiota detected and the methodology used for the microbiology assessment); dietary assessment; probiotics usage assessment.

Quality Assessment

The quality of eligible studies was assessed using the Newcastle–Ottawa Quality Assessment Scale (NOS) (9) and evaluated by two reviewers (NW, XPG). The NOS assessed the quality of

studies based on selection, comparability, and exposure, with a total score ranging from 0 to 9. A study of greater than 7 points is defined as a high-quality study.

Data Synthesis

Different studies have investigated the gut microbiota's taxonomic composition at different levels, such as phylum, order, family, genus and species, with a large number and limited overlap of findings. We excluded results if they were reported only in one study.

Data Analysis

Studies included in this meta-analysis reported the comparison of gut microbiota between ADHD patients and controls, including alpha diversity and the relative abundance of bacteria of different phyla, families, and genera. These data were extracted from texts, figures, and supplementary materials. If only figures were given, we used Webplot-digitizer software (https://automeris.io/WebPlotDigitizer/) to extract these parameters from the graphs. Most data are expressed as the means ± standard deviations, and the others are presented as medians and interquartile ranges. We standardized all the data into the form of means ± standard deviations for subsequent analyses using a web-based tool (https://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html).

This meta-analysis was undertaken using Review Manager 5.4 software. Data of gut microbiota were expressed as standardized mean difference (SMD). Heterogeneity was measured using I^2 statistics, with I^2>50% indicating significant heterogeneity. A fixed-effect model was used for initial analyses, and a random effect model was used if I^2>50%. Sensitivity analyses excluding one study at a time were conducted when the heterogeneity was high, but subgroup analyses and meta-regression were not conducted because of limited literature. Two-sided P values were statistically significant if P<0.05. Potential publication biases were detected by funnel plots. Given to the limited capacity of funnel plots when pooling a small number of trials, we further preformed Egger's test to verify the potential publication bias.

RESULTS

Search Results

Up to August 24, 2021, 593 records were found after searching the five databases, and 502 were retained after duplicate manual removal. After screening the title and abstract, 488 studies were removed because of dissatisfaction with the inclusion criteria. After reviewing the full texts of the remaining articles, three were excluded because of a lack of insufficient data, and one was excluded because the data of microbiota is not for gut microbiota. Finally, eight eligible studies were included in this systematic review and meta-analysis (**Figure 1**), and the PRISMA report is presented in **Supplementary Material 2**.

Study Characteristics

Table 1 summarizes the characteristics of the eight studies included in the meta-analysis, among which four were conducted in China (including Taiwan) (12–14, 17), two in the Netherlands (10, 15), one in Germany (11) and one in Spain (16). A total of 316 ADHD patients and 359 healthy controls were included in the analysis, and the sample sizes ranged from 14 to 100. Most studies were age- and gender-matched, and there were no significant differences in demographics, except the study by Aarts, in which the HCs had 39 older adults and caused an older mean age (10). For participants, four studies were conducted in children (12–14, 17), one was in children and adolescents (11), two were in adolescents and adults (10, 15), and the last was in adults (16).

For the clinical diagnosis of ADHD, six studies were assessed according to DSM-IV (10–13, 15, 16), and others followed DSM-5 (14, 17). For the assessment of microbiology, except the one conducted by Wan et al. (14) that used shotgun metagenomics (14), other studies used 16S rRNA gene sequencing (10–13, 15–17). Likewise, there were three pipeline analyses in the included studies, QIIME (10, 12, 17), Mothur (11, 13), and Bowtie2 (14), except for two examinations that did not specify the analyses (15, 16).

We also take care of ADHD medication because it may cause gut microbiota disorders. Three of the included records consisted of medication-naïve participants to compare ADHD patients and HCs (12, 13, 16), one study asked patients to discontinue taking medicine for at least 48 h prior to sampling collection (11), and one explored the effect of medication by removing 19 medicated cases from a regression model (15). For the use of probiotics, two studies asked participants not to receive any probiotics (12, 16). Other studies did not clearly state the usage of probiotics (10, 11, 13–15, 17).

Another aspect to highlight was the preparation of fecal samples. Most studies sequenced each sample of all participants separately. Nevertheless, Zhou et al. (17) made mixed fecal samples of ADHD patients by taking 1.0 g fecal samples from each ADHD child and dissolving them in 10 ml of sterile distilled water (17).

Assessment of Study Quality

All included studies were assessed for quality using the NOS (**Table 2**). All studies were of high quality and were included in the meta-analysis.

Differences in Diversity Outcomes Between ADHD Patients and HCs

Alpha Diversity

Table 3 presents different kinds of alpha diversity indexes used in the included studies to assess the microbial diversity within the same group, including estimated richness (observed OTUs, observed species, Chao1 index), and indexes presented richness and evenness (Shannon index, Simpson index).

For richness, 2 studies (13, 15) provided data on observed OTUs in ADHD patients (n=71) vs HCs (n=78), 2 studies (11, 16) provided observed species in ADHD (n=33) vs HCs (n=94), and 5 studies (10–14) provided Chao1 in ADHD (n=131) vs HCs (n=173). There were no significant differences in SMDs of observed OTUs (SMD = 1.27; 95% CI, −1.21 to 3.75; P = 0.31; I^2 = 97%) (**Figure 2A**), observed species (SMD = 0.02; 95% CI, −0.61 to 0.64; P = 0.96; I^2 = 52%) (**Figure 2B**) or Chao1 (SMD = 0.83; 95% CI, −0.17 to 1.82; P =0.10; I^2 = 93%) (**Figure 2C**) indexes.

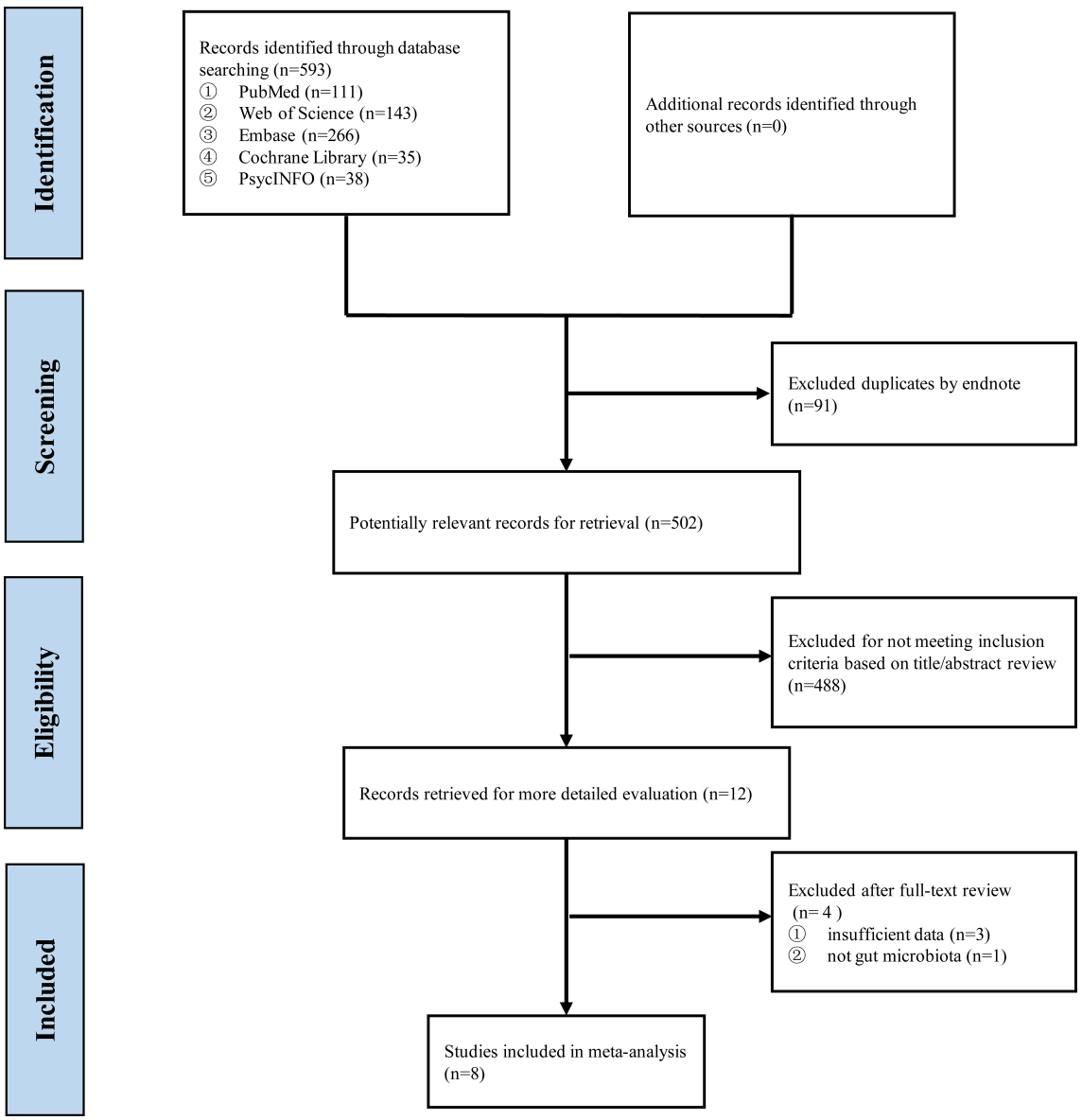


FIGURE 1 | Flow diagram of selected studies.

Regarding richness and evenness, 8 studies (10–17) provided data on the Shannon index in ADHD (n=316) vs HCs (n=359), and 5 studies (12–14, 16, 17) provided the Simpson index in ADHD (n=242) vs HCs (n=217). The estimate demonstrated a higher Shannon index in ADHD patients than in HCs (SMD = 0.97; 95% CI, 0.13 to 1.82; $P = 0.02$; $I^2 = 96\%$) (**Figure 3A**) and no significant difference in the Simpson index (SMD =0.01; 95% CI, −1.58 to 1.60; $P = 0.13$; $I^2 = 96\%$) (**Figure 3B**).

In order to explore the high heterogeneity (I^2) of Chao1 index, Shannon index, and Simpson index, we wanted to perform subgroup analyses and meta-regression but gave up because of limited literature. Then, we found that the heterogeneity was skewed by the results from two outlier studies Wang et al. and Zhou et al. (13, 17), and a sensitivity analysis excluded the two studies and produced a homogeneous study population (**Figure 4**). This high heterogeneity could be due to the preparation of fecal samples (17) and pipeline analyses (13) as described in the study characteristics above. However, there were no significant differences between ADHD patients and HCs in any alpha diversity index.

Beta Diversity

Seven studies reported four types of beta diversity, and the findings were inconsistent (**Table 3**); five records showed no significant difference between ADHD patients and HCs, while two reported the opposite conclusion. We did not conduct a meta-analysis on beta diversity because of few data.

TABLE 1 | Characteristics of the studies included in the meta-analysis.

Study	Country	N[a] (ADHD)	Age (years)	Sex (male, %)	N[b] (Control)	Age (years)	Sex (male, %)	Definition of ADHD	Bacteria		Microbiology Assessment	Dietary Assessment	Probiotics Usage Assessment
									Bacteria Identified	Bacteria Altered			
Aarts et al. (10)	The Netherlands	19	19.5 (2.5)	68.4%	77	27.1 (14.3) (33 older participants)	53.2%	DSM-IV; Schedule for Affective Disorders and Schizophrenia for School-Age Children	**Phylum:** Firmicutes, Actinobacteria, Bacteroidetes **Order:** Clostridiales **Family:** Rikenellaceae, Porphyromonadaceae **Genus:** *Bifidobacterium*, *Eggerthella*	**Phylum:** Firmicutes↓, Actinobacteria↑ **Genus:** *Bifidobacterium*↑	16S rRNA gene sequencing using 454 pyrosequencing; region: V3-V4; Pipeline analysis: QIIME version 1.2	–	–
Prehn-Kristensen et al. (11)	Germany	14	11.9 (2.5)	14 (100%)	17	13.1 (1.7)	17 (100%)	DSM-IV-TR; K-SADS-PL	**Family:** Prevotellaceae, Catabacteriaceae, Porphyromonadaceae, Neisseriaceae, Bacteroidaceae **Genus:** *Bacteroides, Prevotella, Parabacteroides*, *Neisseria*	**Family:** Prevotellaceae↓, Catabacteriaceae↓, Porphyromonadaceae↓, Neisseriaceae↑, Bacteroidaceae↑ **Genus:** *Bacteroides*↑, *Parabacteroides*↓	16S rRNA gene sequencing using Illumina MiSeq; region: V1-V2; Pipeline analysis: Mothur	–	–
Jiang et al. (12)	China	51	8.47 (8.47)	38 (74.51%)	32	8.5 (8.47)	22 (68.75%)	DSM-IV; K-SADS-PL	**Phylum:** Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria **Family**: *Alcaligenaceae*, *Peptostreptococcaceae*, *Moraxellaceae*, *Xanthomonadaceae*, *Peptococcaceae* **Genus**: *Faecalibacterium*, *Lachnoclostridium*, *Dialister*, *Sutterella, Blautia*	**family**: *Alcaligenaceae*↓, *Peptostreptococcaceae*↑, *Moraxellaceae*↑, *Xanthomonadaceae*↑, *Peptococcaceae*↑ **Genus**: *Faecalibacterium*↓, *Lachnoclostridium*↓, *Dialister*↓, *Sutterella*↓, *Blautia*↑	16S rRNA gene sequencing using Illumina MiSeq; region: V3-V4; Pipeline analysis: QIIME version 1.7	Yes	No
Wang et al. (13)	Taiwan	30	8.4 (1.7)	23 (76.7%)	30	9.3 (2.2)	18 (60%)	DSM-IV-TR; K-SADS-E	**Phylum:** Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Actinobacteria **Genus:** *Bacteroidetes, Prevotella, Parabacteroides, Phascolarctobacterium,*	**Phylum:** Fusobacteria↑ **Genus:** *Fusobacteria*↑	16S rRNA gene sequencing using Illumina Miseq sequences; region: V3-V4; Pipeline analysis: Mothur and QIIME	Yes	–

(Continued)

TABLE 1 | Continued

Study	Country	N[a] (ADHD)	Age (years)	Sex (male, %)	N[b] (Control)	Age (years)	Sex (male, %)	Definition of ADHD	Bacteria		Microbiology Assessment	Dietary Assessment	Probiotics Usage Assessment
									Bacteria Identified	Bacteria Altered			
									Escherichia Shigella, *Alistipes, Veillonella, Sutterella, Fusobacteria, Akkermansia*				
Wan et al. (14)	China	17	8 (7,10)	14 (82.3%)	17	8 (7,9.5)	13 (76.5%)	DSM-V; K-SADS	**Genus:** *Faecalibacterium, Veillonellaceae, Odoribacter, Enterococcus* **Species:** *Faecalibacterium prausnitzii, Lachnospiraceae bacterium, Ruminococcus gnavus, Ruminococcaceae, Bacteroides caccae, Odoribacter splanchnicus, Paraprevotella xylaniphila, Veillonella parvula, Odoribacteraceae, Enterococcaceae*	**Genus:** *Faecalibacterium*↓, *Veillonellaceae*↓, *Odoribacter*↑, *Enterococcus*↑ **Species:** *Faecalibacterium prausnitzii*↓, *Lachnospiraceae bacterium*↓, *Ruminococcus gnavus*↓, *Ruminococcaceae*↓, *Bacteroides caccae*↑, *Odoribacter splanchnicus*↑, *Paraprevotella xylaniphila*↑, *Veillonella parvula*↑, *Odoribacteraceae*↑, *Enterococcaceae*↑	Shotgun metagenomics sequencing using Illumina NovaSeq; Platform: Bowtie2	–	–
Szopinska-Tokov et al. (15)	The Netherlands	41	20.2 (4.1)	61%	48	20.4 (3.5)	50%	DSM-IV; K-SADS	**Phylum:** Clostridiales, Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia **Genus:** *Coprococcus_2, Prevotella_9, Intestinibacter*	**Genus:** *Coprococcus_2*↓, *Prevotella_9*↓	16S rRNA gene sequencing using Illumina Hiseq sequences; region: V1-V2	–	–
Richarte et al. (16)	Spain	100	33 (11)	51%	100	30 (8)	47%	Structured Diagnostic Interview for Adult ADHD (DIVA 2.0), the Structured Clinical Interview for DSM-IV Axis I and II Disorders	**Phylum:** Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Verrucomicrobia, Candidatus Melainabacteria **Family**: *Eubacteriaceae, Gracilibacteraceae, Lactobacillaceae, Peptostreptococcaceae, Selenomonadaceae,*	**Family**: *Veillonellaceae*↑ **Genus**: *Dialister*↑	16S rRNA gene sequencing using Illumina Miseq sequences region: V3-V4	–	No

(Continued)

TABLE 1 | Continued

Study	Country	N[a] (ADHD)	Age (years)	Sex (male, %)	N[b] (Control)	Age (years)	Sex (male, %)	Definition of ADHD	Bacteria		Microbiology Assessment	Dietary Assessment	Probiotics Usage Assessment
									Bacteria Identified	Bacteria Altered			
								(SCID-I and SCID-II)	*Veillonellaceae, Verrucomicrobiaceae* **Genus**: *Acetivibrio, Alloprevotella, Anaerotaenia, Dialister, Flintibacter, Fucophilus, Gracilibacter, Herbinix, Leclercia, Megamonas, Megasphaera, Odoribacter, Parasutterella, Porphyromonas, Prevotellamassilia, Romboutsia, Vampirovibrio*				
Zhou et al. (17)	China	44	6.9	–	38	8.6	-	DSM-V	**Genus:** *Bifidobacterium, Gemmiger* **Species:** *Shigella, SMB53, uricibacter, Shigella, Bifidobacterium, Collinsella,Ruminococcus, Clostridium, Roseburia, Gemmiger, Acinetobacter, Enterococcus, Bacteroides, Streptococcus, Faecalibacterium*	**Genus:** *Bifidobacterium*↓ **Species:** *Shigella*↓, *SMB53*↓, *uricibacter*↓, *Shigella*↓, *Bifidobacterium*↓, *Collinsella*↓, *Ruminococcus*↓, *Clostridium*↓, *Roseburia*↑, *Gemmiger*↑, *Acinetobacter*↑, *Enterococcus*↑, *Bacteroides*↑, *Streptococcus*↑, *Faecalibacterium*↑	16S rRNA gene sequencing using Illumina Miseq sequences; region: V3-V4; Pipeline analysis: QIIME2 version 2020.06	–	–

[a]*The number of ADHD patients in each study;* [b]*The number of healthy controls in each study*
↑: indicating the increase of bacterial taxa; ↓: indicating the decrease of bacterial taxa.

TABLE 2 | Newcastle–Ottawa Scale for assessing the quality of the studies included in the meta-analysis.

Author, year	Overall score	Selection				Comparability	Exposure		
		Definition adequate	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate
Aarts et al., 2017 (10)	**8**	**1**	**1**	**1**	**1**	**2**	**0**	**1**	**1**
Prehn-Kristensen et al., 2018 (11)	**9**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**
Jiang et al., 2018 (12)	**9**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**
Wang et al., 2020	**9**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**
Wan et al., 2020 (14)	**9**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**
Szopinska-Tokov et al., 2020 (15)	**9**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**
Richarte et al., 2021 (16)	**9**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**
Zhou et al., 2021 (17)	**8**	**1**	**1**	**1**	**1**	**2**	**0**	**1**	**1**

TABLE 3 | Summary of diversity assessments in the included studies.

Study	α-diversity	Findings	β-diversity	Findings
Szopinska-Tokov et al. (15)	Observed OTUs Shannon index Phylogenetic index	no difference	weighted UniFrac distances	no difference
Prehn-Kristensen et al. (11)	Observed species Shannon diversity Chao1 index	The ADHD group had lower Shannon diversity than HCs.	Bray–Curtis distance	a significant difference
Wan et al. (14)	Shannon index Chao1 index Simpson index	no difference	–	–
Wang et al. (2020)	Chao1 index Observed OTUs Shannon index	The ADHD group had higher Shannon index and Chao index than HCs. However, the Simpson index was lower in ADHD group.	unweighted and weighted unifrac distances	no difference
Aarts et al. (10)	PD whole tree Chao1 index Observed Species Shannon index	no difference	weighted UniFrac distances	no difference
Jiang et al. (12)	Shannon index Simpson	no difference	unweighted and weighted UniFrac distances, Bray–Curtis distance	no difference

(Continued)

TABLE 3 | Continued

Study	α-diversity	Findings	β-diversity	Findings
	index ACE Chao1 index			
Richarte et al. (16)	Simpson index Shannon index	no difference	unweighted and weighted UniFrac distances, Bray–Curtis distance	no difference
Zhou et al. (17)	Shannon index Simpson index Pielou's evenness	The ADHD group had higher indexes than HCs.	weighted UniFrac unweighted UniFrac Jaccard distance Bray–Curtis distance	a significant difference

Differences in Microbial Taxa Between ADHD Patients and HCs

Bacterial Phylum

At the phylum level, five phyla were identified: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia (**Figure 5**). There were no significant differences in phylum.

Because of the high heterogeneity (I^2) of Firmicutes and Actinobacteria, sensitivity analyses excluded the study of Zhou et al. (17) because of the same reason above, and the model was

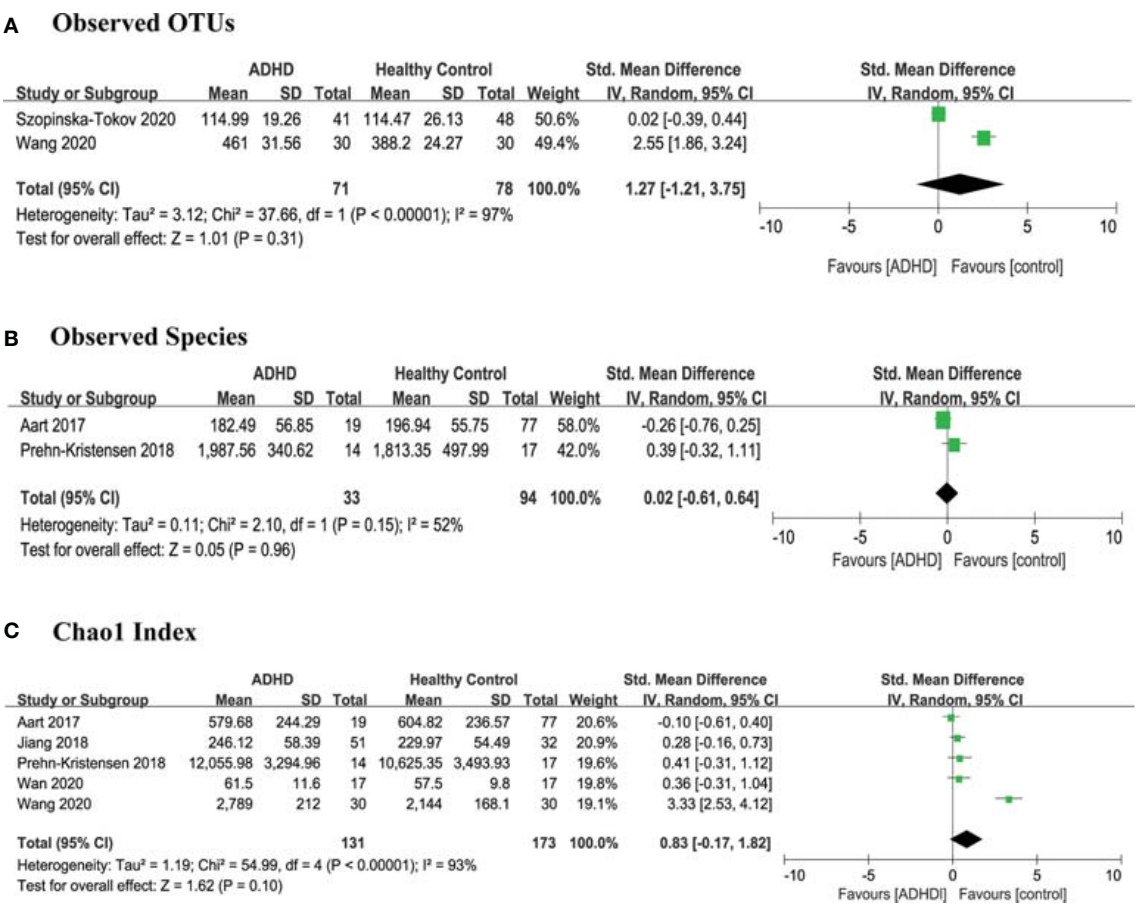


FIGURE 2 | Forest Plots of Alpha Diversity Richness Estimators in the Gut Microbiota of ADHD Compared with HCs. **(A)** Observed OTUs; **(B)** Observed Species; **(C)** Chao1 index. CI, confidence interval; SMD, standardized mean difference.

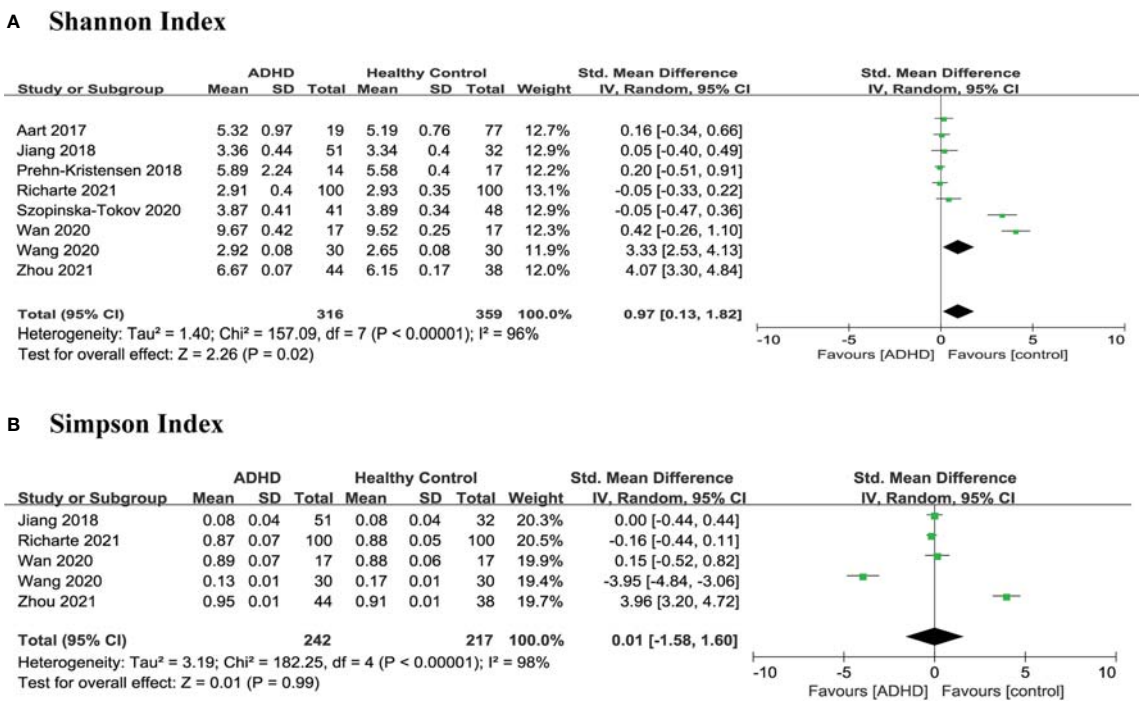


FIGURE 3 | Forest Plots of Alpha Diversity richness and evenness in the Gut Microbiota of ADHD Compared with HCs. **(A)** Shannon index; **(B)** Simpson index. CI, confidence interval; SMD, standardized mean difference.

switched from a random-effects to a fixed-effects model, with a modest impact on the result (**Figure 6**).

Bacterial Family

At the family level, eight families were identified: Alcaligenaceae, Peptostreptococcaceae, Porphyromonadaceae, Veillonellaceae, Rikenellaceae, Lachnospiraceae, Ruminococcaceae and Bacteroidaceae (**Figure 7**). No significant difference was found in family.

Bacterial Genus

Figure 8 shows the fourteen genera that were identified: *Prevotella_9* (12, 15), *Coprococcus_2* (11, 15), *Parabacteroides* (11, 13), *Phascolarctobacterium* (12, 13), *Escherichia Shigella* (12, 13), *Alistipes* (11–13), *Sutteralla* (11, 13), *Veillonella* (13, 14), *Odoribacter* (14, 16), *Faecalibacterium* (11, 12, 14, 17), *Bacteroides* (12, 13), *Bifidobacterium* (12, 17), *Dialister* (11, 12, 16) and *Blautia* (11, 12, 17).

Sensitivity analyses were conducted because of the high heterogeneity of *Alistipes, Faecalibacterium* and *Dialister*, and the model was changed from a random-effects to a fixed-effects model, with a similar result described above (**Figure 9**).

As shown in the forest plot (**Figure 9**), the relative abundance of *Blautia* was significantly higher in ADHD patients than in HCs (SMD = 0.34; 95% CI, 0.06 to 0.63; $P = 0.02$; $I^2 = 0\%$). For other genera, no significant difference was found.

Table 4 summarizes the outcomes of the included studies on microbiota profiles (alpha and beta diversity) and gut microbiota taxa. Different studies did not draw consistent conclusions. For α-diversity, five studies reported nonsignificant differences, but Prehn-Kristensen et al. (11), Wang et al. (13, 18), and Zhou et al. (17) gave different outcomes. Wang et al. (13, 18) and Zhou et al. (17) found a higher Shannon index, but they reached contradictory conclusions on the Simpson index, which may be led by different pipeline analyses of Mothur and QIIME. Prehn-Kristensen et al. (11) disagreed because he found a decrease in the Shannon index. Seven studies addressed β-diversity, with two believed significant differences in all four indexes, while others derived opposite findings. Regarding gut microbiota taxa, different researchers reached different or even contrary conclusions, as shown in **Table 4**.

Publication Bias

Potential publication biases were observed in funnel plots of Chao1 index and Shannon index which were presented in **Supplementary Material 3**. Egger's test further confirmed the significant bias in Shannon index ($P = 0.050$), but not in Chao1 index ($P = 0.218$).

DISCUSSION

To our knowledge, this is the first meta-analysis to identify evidence on the dysbiosis of gut microbiota in ADHD. We searched five important databases to accumulate evidence on whether ADHD patients have a different gut microbial

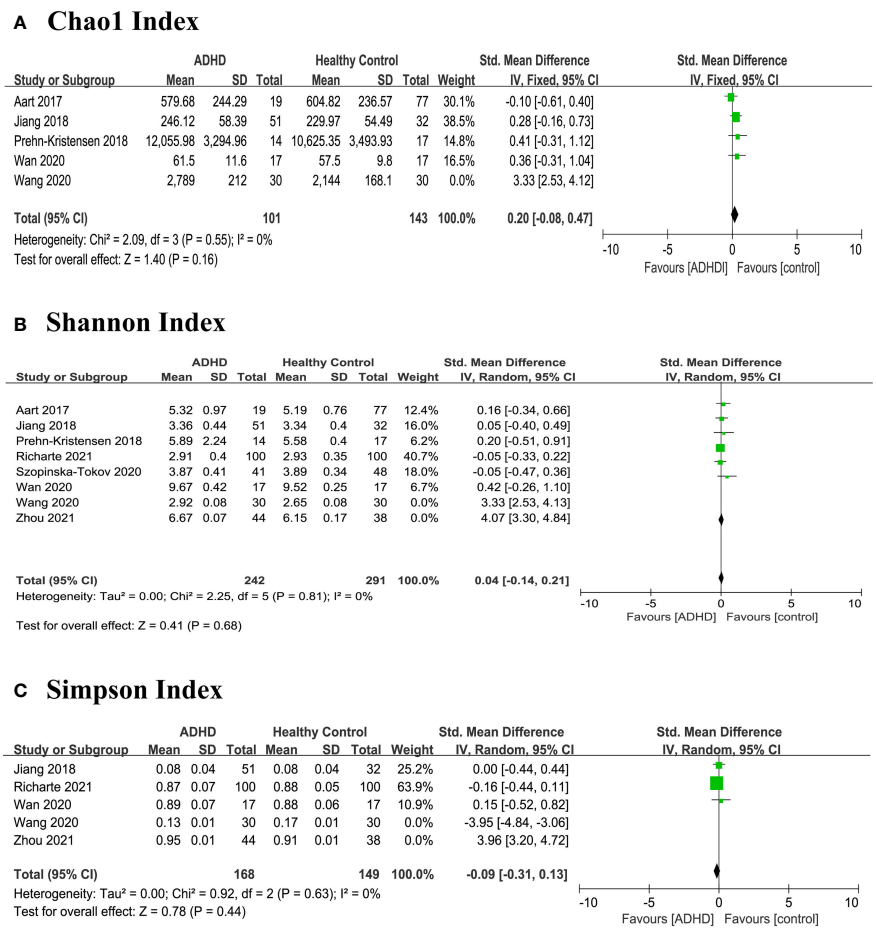


FIGURE 4 | Sensitivity analysis of alpha diversity in the gut microbiota of ADHD compared with HCs after removing heterogeneous studies of Wang 2020 and Zhou 2021 (17). **(A)** Chao1 index; **(B)** Shannon index; **(C)** Simpson index. CI, confidence interval; SMD, standardized mean difference.

composition than healthy controls. A total of eight studies with high quality were included, including 316 ADHD patients and 359 healthy controls. Then, we investigated the diversity and relative abundance of the gut microbiota, more specifically at the 5 phyla, 8 families and 14 genera. Our findings are as follows. First, for the alpha diversity of ADHD patients and HCs, we only found a higher Shannon index in ADHD, but the significance vanished after sensitivity analysis because of high heterogeneity. Second, at the phylum level, no significant difference was found. And at the family level, there was no difference between ADHD and HCs. Finally, at the genus level, *Blautia* was significantly elevated in ADHD patients.

It is worth noting that several systematic reviews (7, 19, 20) summarized differences in gut microbiota between the ADHD group and healthy group but did not draw a final conclusion. They led to a conflicting or even opposite conclusion.

Regarding the alpha diversity of gut microbiota, we found that the Shannon index, which provides information on richness and evenness of gut microbiota, was elevated in ADHD patients, which meant that the within-group diversity was higher in the ADHD group. The result of Shannon index was consistent with reports drawn by Wang et al. (13, 18) and Zhou et al. (17), but we found the heterogeneity was high, and coincidentally, the two studies of Wang et al. (13, 18) and Zhou et al. (17) contributed to it. The possible reasons for this might be the difference in the fecal sampling method of Zhou et al. (17) and pipeline analyses of Wang et al. (13, 18). After sensitivity analysis which excluded the two outlier studies, the difference of Shannon index disappeared. For beta diversity, we did not conduct a meta-analysis due to the inadequate number of studies with available data. Therefore, further studies are needed to explore the association between the diversity of gut microbiota and ADHD.

For specific gut microbiota taxa, we selected bacteria that had two or more studies with sufficient data in the meta-analysis. Our findings that there were no significant differences in bacterial phyla and families were not entirely in tune with previous studies (7, 20). Some studies reported an increased or decreased level of phyla or families, but most studies were in agreement with our study. For the bacterial genus, we found that *Blautia* was significantly higher in ADHD patients, which may serve as a

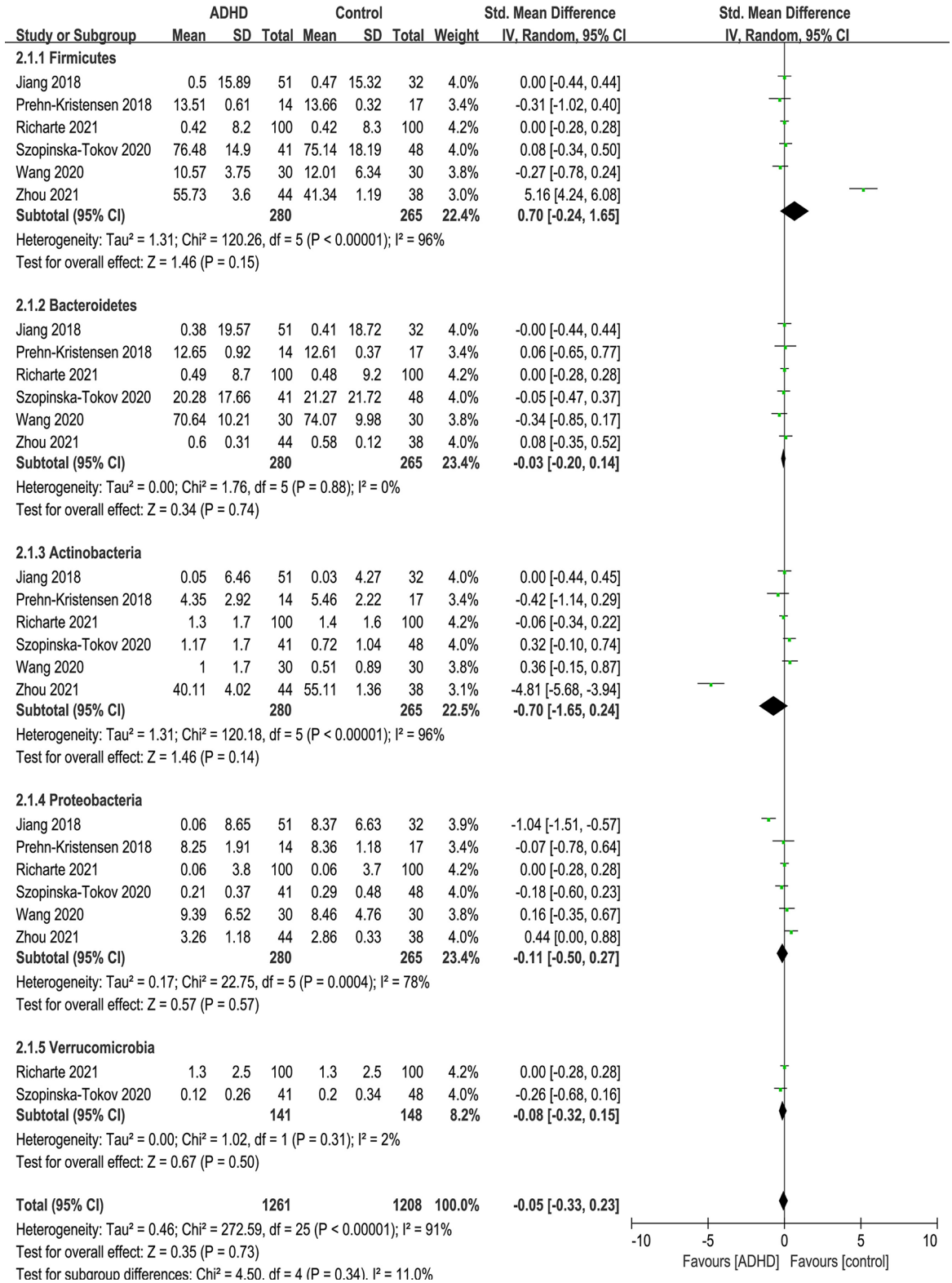


FIGURE 5 | Forest plot of relative abundance of Phylum in the Gut Microbiota of ADHD Compared with HCs. CI, confidence interval; SMD, standardized mean difference.

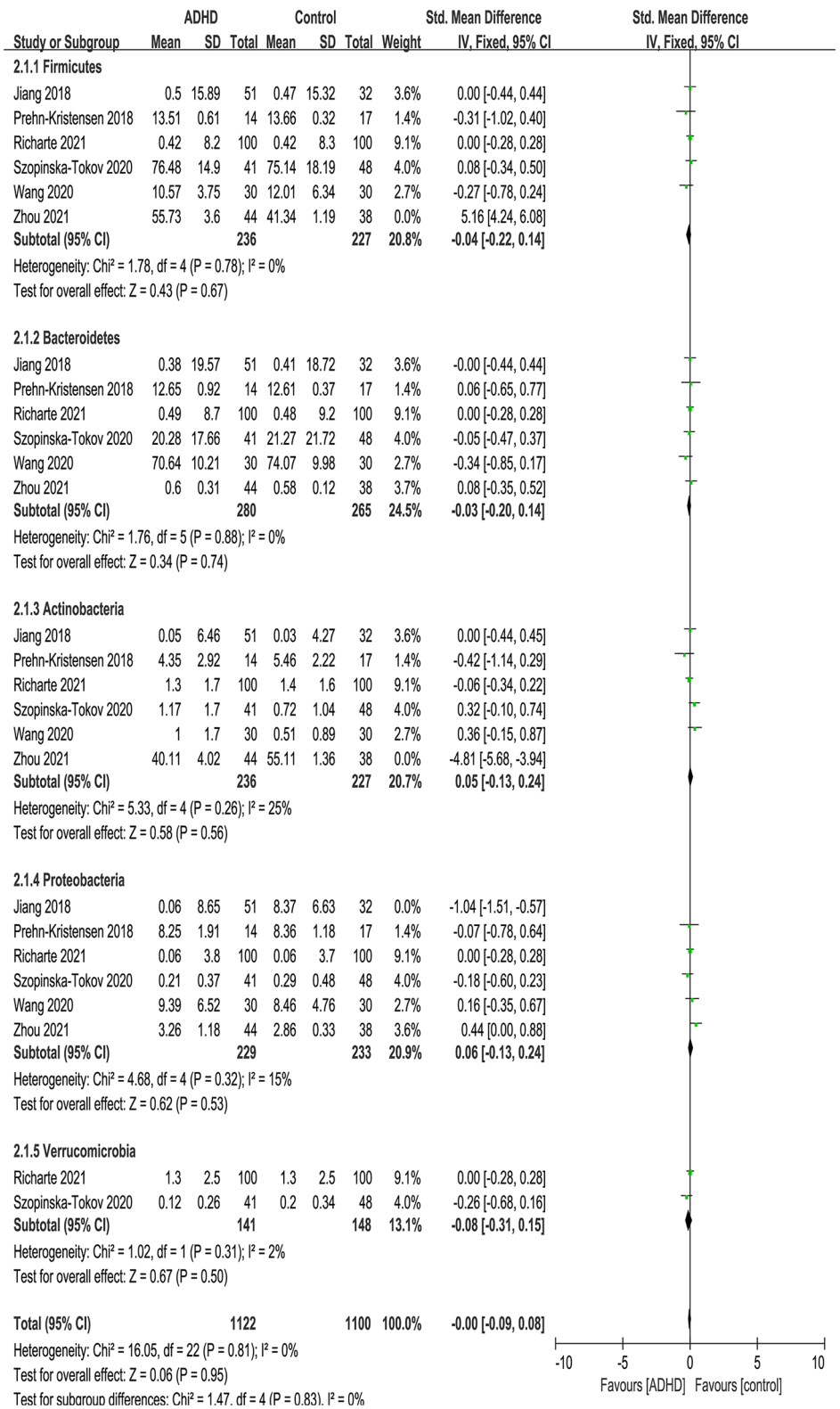


FIGURE 6 | Sensitivity analysis after removing heterogeneous studies of relative abundance of Phylum in the Gut Microbiota of ADHD Compared with HCs. CI, confidence interval; SMD, standardized mean difference.

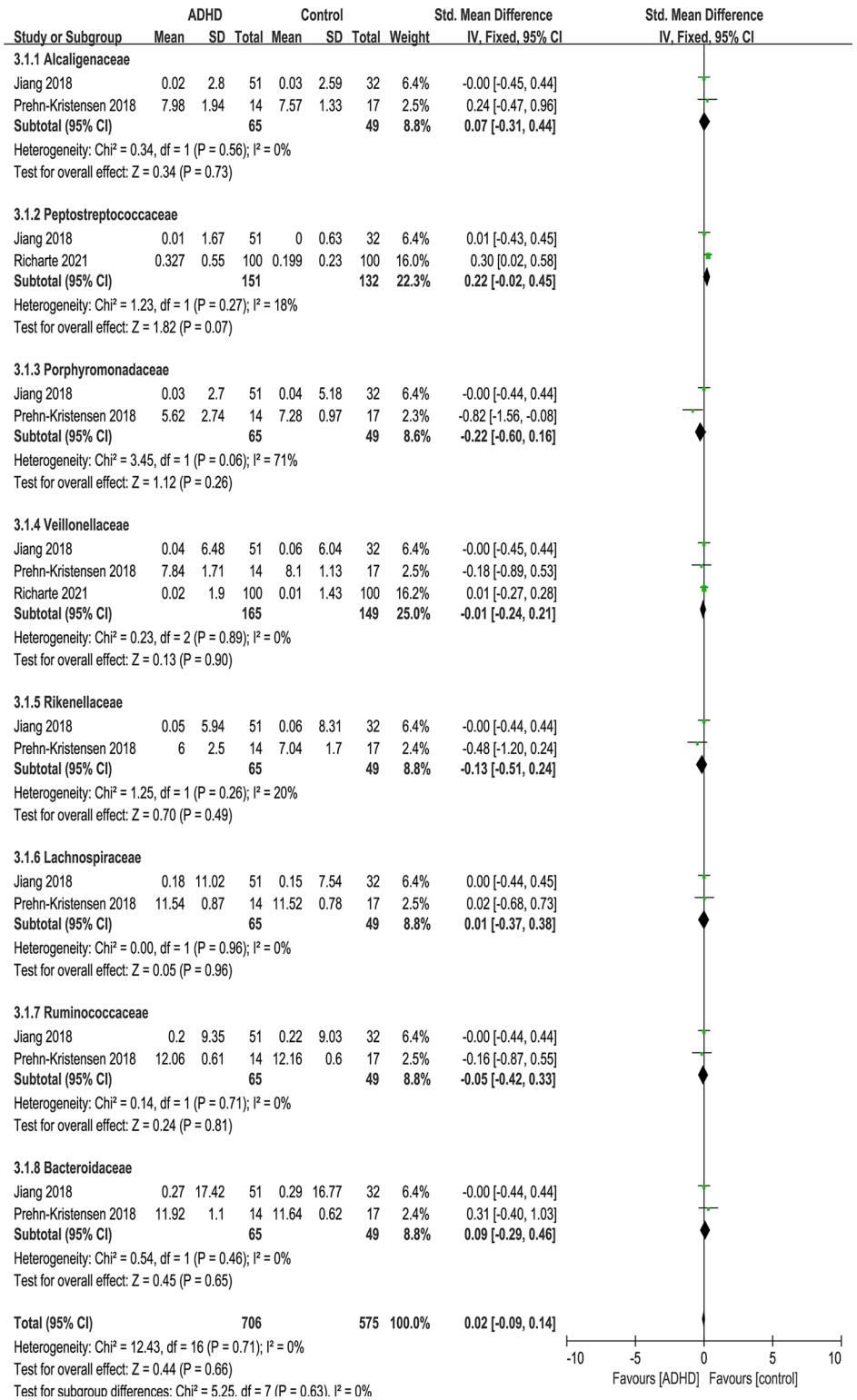


FIGURE 7 | Forest plot of relative abundance of Family in the Gut Microbiota of ADHD Compared with HCs. CI, confidence interval; SMD, standardized mean difference.

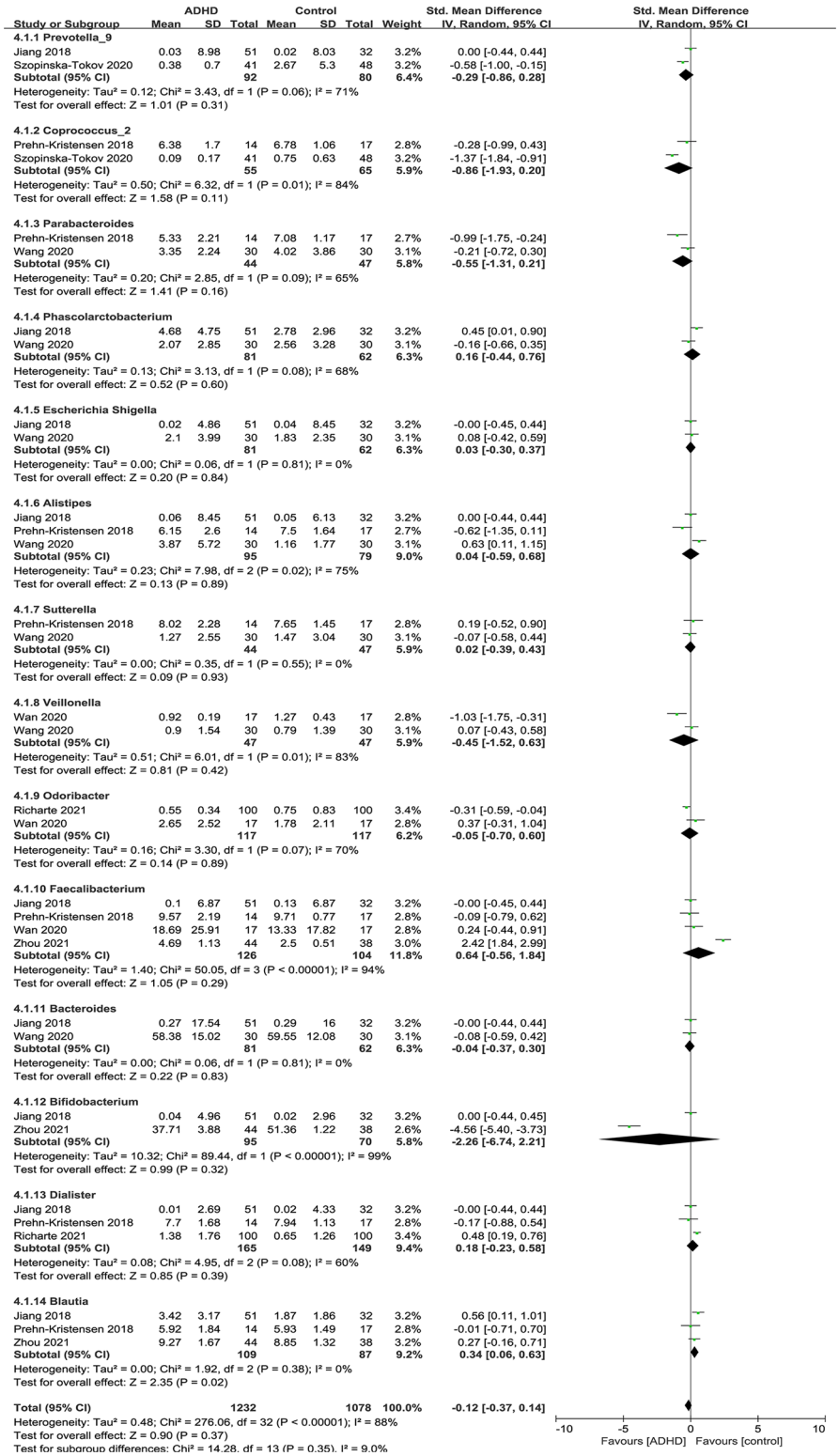


FIGURE 8 | Forest plot of relative abundance of Genus in the Gut Microbiota of ADHD Compared with HCs. CI, confidence interval; SMD, standardized mean difference.

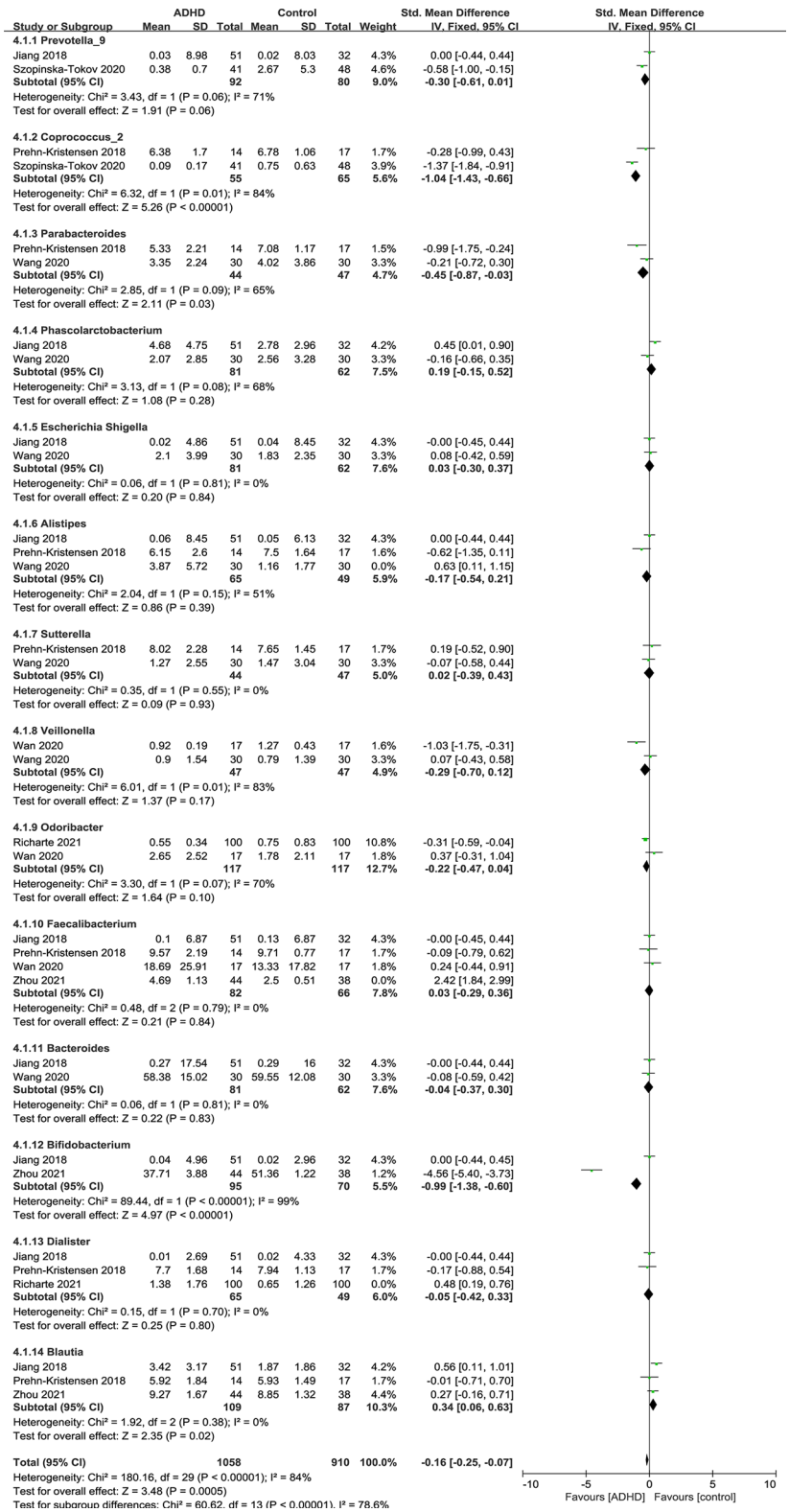
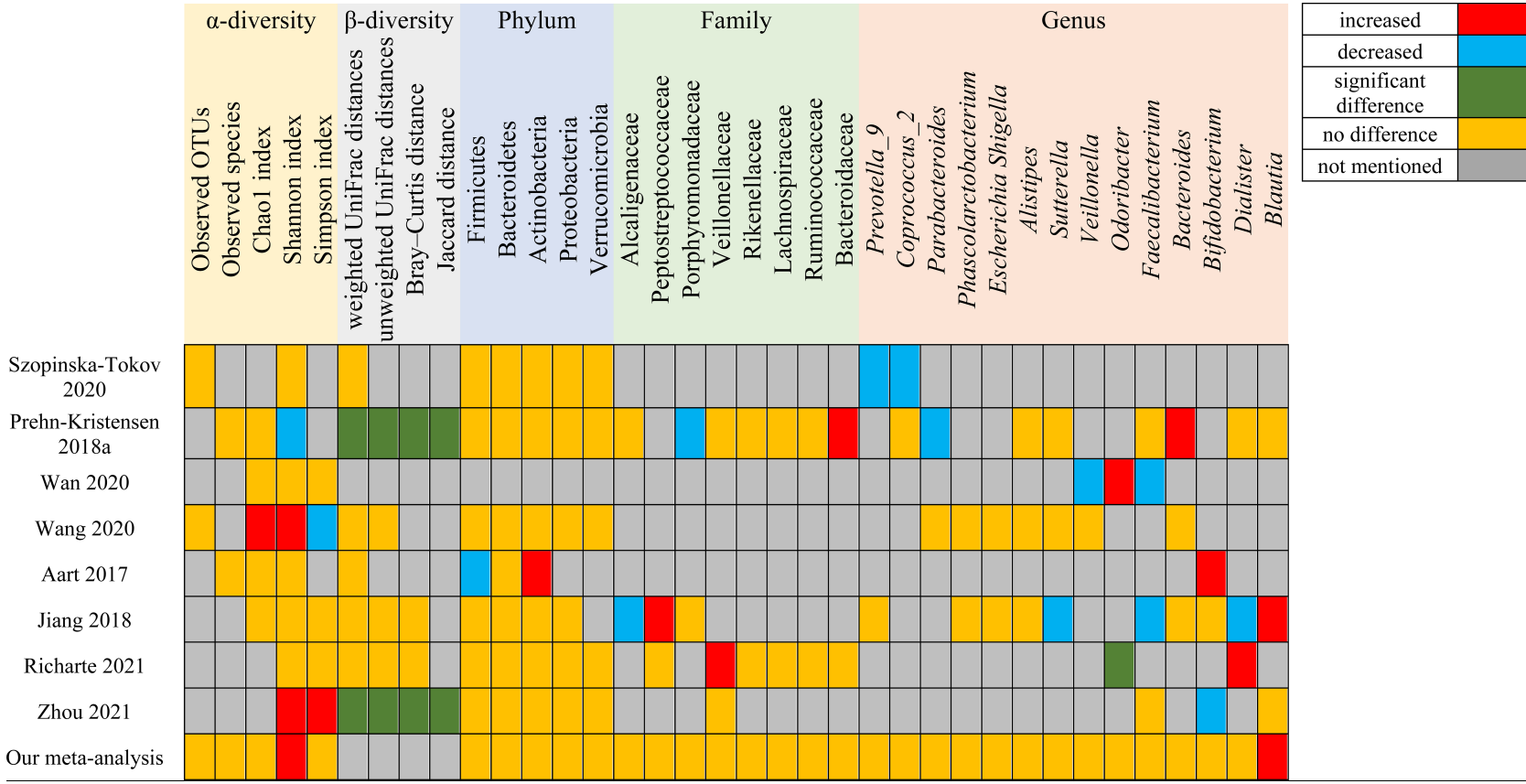


FIGURE 9 | Sensitivity analysis after removing heterogeneous studies of relative abundance of Genus in the Gut Microbiota of ADHD Compared with HCs. CI, confidence interval; SMD, standardized mean difference.

TABLE 4 | Summary of the outcomes of the included studies on microbiota profiles (alpha and beta diversity) and gut microbiota taxa.



Study references: Szopinska-Tokov et al. (15), Prehn-Kristensen (11), Wan et al. (14), Wang et al. (2020), Aarts et al. (10), Jiang et al. (12), Richarte et al. (16), Zhou et al. (17).

biomarker for ADHD. But there still needs more evidence to verify because of the limited number of studies currently.

Blautia belongs to the Lachnospiraceae family, Firmicutes phylum, and contains 20 kinds of species as of now (21). Several recent studies have indicated that *Blautia* is associated with host dysfunctions, such as depression (22, 23), obesity (24, 25), atherosclerosis (26, 27), diabetes (28), and cancer (29), and we now extend these findings to ADHD. This may relate to the functions of physiological of *Blautia*. First, *Blautia* can upregulate T cells (30) in the gut and produce short-chain fatty acids (18) as well as influence the ratio of IFN-γ to IL-4 or TNF-α to IL-4 (31) to achieve anti-inflammatory effects (32). Second, Blautia can produce bacteriocins (33), a kind of secondary metabolite whose function is to prevent the infection of opportunistic pathogens (34). Third, one of the metabolites of *Blautia* is acetic acid, which may modulate other gut microbiota by increasing IgA and changing the capacity of the IgA pool to bind to specific microorganisms (35) and cause a change in gut stability. As inflammation and immunity are substantial etiologies of ADHD, *Blautia* is a possible biomarker of ADHD.

Another point to highlight is that several studies have demonstrated that the use of probiotics or prebiotics may improve ADHD symptoms (19, 36), but we did not conduct an analysis, as most studies included in this meta-analysis did not report on this topic clearly.

In fact, a few limitations should be considered in the meta-analysis. First, the small number of studies and the low to medium sample sizes of each study made the statistical power limited. Other limitations should take into account are geographical location, age, the use of medication, and diet pattern, which may affect outcomes, suggesting that further clinical studies need to be improved to consider these factors. In addition to the reasons described above, a few other factors may also cause high heterogeneity. We did not conduct subgroup analyses of sampling method, sampling time, sequencing, or analysis pipelines because of the limitations of the included literature. However, we performed sensitivity analysis by excluding one or two inappropriate articles when the heterogeneity was high.

CONCLUSION

This is the first meta-analysis to assess gut microbiota and ADHD to date. We found a higher Shannon index and *Blautia* in ADHD patients than in HCs, but there were no significant differences at the phylum and family levels. The result for *Blautia* survived the sensitivity analysis. Further clinical studies need to be taken to consider factors such as geographical location, medication use, diet pattern, sequencing and analysis pipelines to validate these results.

AUTHOR CONTRIBUTIONS

LY took responsibility for the integrity of the data and the accuracy of the data analysis. Study concept, design and supervision: LY and ZZ. Data extraction, analysis and interpretation: all authors. Drafting of the manuscript: NW and XG. Revision of the manuscript: LY and ZZ. All authors interpreted the results, and approved the final version of this article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.838941/full#supplementary-material

REFERENCES

1. Willcutt EG. The Prevalence of DSM-IV Attention-Deficit/Hyperactivity Disorder: A Meta-Analytic Review. *Neurotherapeutics* (2012) 9(3):490–9. doi: 10.1007/s13311-012-0135-8
2. Demontis D, Walters RK, Martin J, Mattheisen M, Als TD, Agerbo E, et al. Discovery of the First Genome-Wide Significant Risk Loci for Attention Deficit/Hyperactivity Disorder. *Nat Genet* (2019) 51(1):63–75. doi: 10.1038/s41588-018-0269-7
3. Sciberras E, Mulraney M, Silva D, Coghill D. Prenatal Risk Factors and the Etiology of ADHD-Review of Existing Evidence. *Curr Psychiatry Rep* (2017) 19(1):1. doi: 10.1007/s11920-017-0753-2
4. Cortese S, Sun S, Zhang J, Sharma E, Chang Z, Kuja-Halkola R, et al. Association Between Attention Deficit Hyperactivity Disorder and Asthma: A Systematic Review and Meta-Analysis and a Swedish Population-Based Study. *Lancet Psychiatry* (2018) 5(9):717–26. doi: 10.1016/S2215-0366(18)30224-4
5. Liu X, Dalsgaard S, Munk-Olsen T, Li J, Wright RJ, Momen NC. Parental Asthma Occurrence, Exacerbations and Risk of Attention-Deficit/Hyperactivity Disorder. *Brain Behav Immun* (2019) 82:302–8. doi: 10.1016/j.bbi.2019.08.198
6. van der Schans J, Aikman B, de Vries TW, Hoekstra PJ, Hak E. Association Between Attention-Deficit/Hyperactivity Disorder and Asthma Among Adults: A Case-Control Study. *Chest* (2017) 151(6):1406–7. doi: 10.1016/j.chest.2017.03.052
7. Boonchooduang N, Louthrenoo O, Chattipakorn N, Chattipakorn SC. Possible Links Between Gut-Microbiota and Attention-Deficit/Hyperactivity Disorders in Children and Adolescents. *Eur J Nutr* (2020) 59(8):3391–403. doi: 10.1007/s00394-020-02383-1
8. Stewart LA, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, et al. Preferred Reporting Items for Systematic Review and Meta-Analyses of Individual Participant Data: The PRISMA-IPD Statement. *JAMA* (2015) 313(16):1657–65. doi: 10.1001/jama.2015.3656
9. Stang A. Critical Evaluation of the Newcastle-Ottawa Scale for the Assessment of the Quality of Nonrandomized Studies in Meta-Analyses. *Eur J Epidemiol* (2010) 25(9):603–5. doi: 10.1007/s10654-010-9491-z
10. Aarts E, Ederveen T, Naaijen J, Zwiers MP, Boekhorst J, Timmerman HM, et al. Gut Microbiome in ADHD and its Relation to Neural Reward Anticipation. *PloS One* (2017) 12(9):e183509. doi: 10.1371/journal.pone.0183509
11. Prehn-Kristensen A, Zimmermann A, Tittmann L, Lieb W, Schreiber S, Baving L, et al. Reduced Microbiome Alpha Diversity in Young Patients

With ADHD. *PloS One* (2018) 13(7):e200728. doi: 10.1371/journal.pone.0200728
12. Jiang HY, Zhou YY, Zhou GL, Li YC, Yuan J, Li XH, et al. Gut Microbiota Profiles in Treatment-Naïve Children With Attention Deficit Hyperactivity Disorder. *Behav Brain Res* (2018) 347:408–13. doi: 10.1016/j.bbr.2018.03.036
13. Wang LJ, Yang CY, Chou WJ, Lee MJ, Chou MC, Kuo HC, et al. Gut Microbiota and Dietary Patterns in Children With Attention-Deficit/Hyperactivity Disorder. *Eur Child Adolesc Psychiatry* (2020) 29(3):287–97. doi: 10.1007/s00787-019-01352-2
14. Wan L, Ge WR, Zhang S, Sun YL, Wang B, Yang G. Case-Control Study of the Effects of Gut Microbiota Composition on Neurotransmitter Metabolic Pathways in Children With Attention Deficit Hyperactivity Disorder. *Front Neurosci* (2020) 14:127. doi: 10.3389/fnins.2020.00127
15. Szopinska-Tokov J, Dam S, Naaijen J, Konstanti P, Rommelse N, Belzer C, et al. Investigating the Gut Microbiota Composition of Individuals With Attention-Deficit/Hyperactivity Disorder and Association With Symptoms. *Microorganisms* (2020) 8(3):1358. doi: 10.3390/microorganisms8030406
16. Richarte V, Sánchez-Mora C, Corrales M, Fadeuilhe C, Vilar-Ribó L, Arribas L, et al. Gut Microbiota Signature in Treatment-Naïve Attention-Deficit/Hyperactivity Disorder. *Transl Psychiatry* (2021) 11(1):382. doi: 10.1038/s41398-021-01504-6
17. Zhou G, Yu R, Ahmed T, Jiang H, Zhang M, Lv L, et al. Biosynthesis and Characterization of Zinc Oxide Nanoparticles and Their Impact on the Composition of Gut Microbiota in Healthy and Attention-Deficit Hyperactivity Disorder Children. *Front Microbiol* (2021) 12:700707. doi: 10.3389/fmicb.2021.700707
18. Wang B, Kong Q, Li X, Zhao J, Zhang H, Chen W, et al. A High-Fat Diet Increases Gut Microbiota Biodiversity and Energy Expenditure Due to Nutrient Difference. *Nutrients* (2020) 12(10):3197. doi: 10.3390/nu12103197
19. Kalenik A, Kardaś K, Rahnama A, Sirojć K, Wolańczyk T. Gut Microbiota and Probiotic Therapy in ADHD: A Review of Current Knowledge. *Prog Neuropsychopharmacol Biol Psychiatry* (2021) 110:110277. doi: 10.1016/j.pnpbp.2021.110277
20. Lacorte E, Gervasi G, Bacigalupo I, Vanacore N, Raucci U, Parisi P. A Systematic Review of the Microbiome in Children With Neurodevelopmental Disorders. *Front Neurol* (2019) 10:727. doi: 10.3389/fneur.2019.00727
21. Liu X, Mao B, Gu J, Wu J, Cui S, Wang G, et al. Blautia-A New Functional Genus With Potential Probiotic Properties? *Gut Microbes* (2021) 13(1):1–21. doi: 10.1080/19490976.2021.1875796
22. Cheung SG, Goldenthal AR, Uhlemann AC, Mann JJ, Miller JM, Sublette ME. Systematic Review of Gut Microbiota and Major Depression. *Front Psychiatry* (2019) 10:34. doi: 10.3389/fpsyt.2019.00034
23. Yang J, Zheng P, Li Y, Wu J, Tan X, Zhou J, et al. Landscapes of Bacterial and Metabolic Signatures and Their Interaction in Major Depressive Disorders. *Sci Adv* (2020) 6(49):eaba8555. doi: 10.1126/sciadv.aba8555
24. 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(3):e02392–17. doi: 10.1128/mBio.02392-17
25. Kong C, Gao R, Yan X, Huang L, Qin H. Probiotics Improve Gut Microbiota Dysbiosis in Obese Mice Fed a High-Fat or High-Sucrose Diet. *Nutrition* (2019) 60:175–84. doi: 10.1016/j.nut.2018.10.002
26. Wu M, Yang S, Wang S, Cao Y, Zhao R, Li X, et al. Effect of Berberine on Atherosclerosis and Gut Microbiota Modulation and Their Correlation in High-Fat Diet-Fed ApoE-/- Mice. *Front Pharmacol* (2020) 11:223. doi: 10.3389/fphar.2020.00223
27. Yue C, Li M, Li J, Han X, Zhu H, Yu G, et al. Medium-, Long- and Medium-Chain-Type Structured Lipids Ameliorate High-Fat Diet-Induced Atherosclerosis by Regulating Inflammation, Adipogenesis, and Gut Microbiota in ApoE(-/-) Mice. *Food Funct* (2020) 11(6):5142–55. doi: 10.1039/d0fo01006e
28. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of Gut Microbiota in Type 2 Diabetes Pathophysiology. *Ebiomedicine* (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051
29. Peters BA, Wilson M, Moran U, Pavlick A, Izsak A, Wechter T, et al. Relating the Gut Metagenome and Metatranscriptome to Immunotherapy Responses in Melanoma Patients. *Genome Med* (2019) 11(1):61. doi: 10.1186/s13073-019-0672-4
30. Xie Y, Sun J, Wei L, Jiang H, Hu C, Yang J, et al. Altered Gut Microbiota Correlate With Different Immune Responses to HAART in HIV-Infected Individuals. *BMC Microbiol* (2021) 21(1):11. doi: 10.1186/s12866-020-02074-1
31. Benítez-Páez A, Gómez DPE, López-Almela I, Moya-Pérez Á, Codoñer-Franch P, Sanz Y. Depletion of Blautia Species in the Microbiota of Obese Children Relates to Intestinal Inflammation and Metabolic Phenotype Worsening. *Msystems* (2020) 5(2):e00857–19. doi: 10.1128/mSystems.00857-19
32. Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, et al. Intestinal Microbiota-Derived Short-Chain Fatty Acids Regulation of Immune Cell IL-22 Production and Gut Immunity. *Nat Commun* (2020) 11(1):4457. doi: 10.1038/s41467-020-18262-6
33. Perez M, Ntemiri A, Tan H, Harris H, Roager HM, Ribière C, et al. A Synthetic Consortium of 100 Gut Commensals Modulates the Composition and Function in a Colon Model of the Microbiome of Elderly Subjects. *Gut Microbes* (2021) 13(1):1–19. doi: 10.1080/19490976.2021.1919464
34. Kim SG, Becattini S, Moody TU, Shliaha PV, Littmann ER, Seok R, et al. Microbiota-Derived Lantibiotic Restores Resistance Against Vancomycin-Resistant Enterococcus. *Nature* (2019) 572(7771):665–9. doi: 10.1038/s41586-019-1501-z
35. Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, et al. Acetate Differentially Regulates IgA Reactivity to Commensal Bacteria. *Nature* (2021) 595(7868):560–4. doi: 10.1038/s41586-021-03727-5
36. Rosi E, Grazioli S, Villa FM, Mauri M, Gazzola E, Pozzi M, et al. Use of Non-Pharmacological Supplementations in Children and Adolescents With Attention Deficit/Hyperactivity Disorder: A Critical Review. *Nutrients* (2020) 12(6):1573. doi: 10.3390/nu12061573

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Serum Bilirubin Level Is Increased in Metabolically Healthy Obesity

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Objectives: Bilirubin is a biochemical substance with metabolic benefits. The objective of this research was to elucidate the association between serum bilirubin levels and metabolic alterations in different obesity phenotypes.

Methods: In total, 1,042 drug-naive participants were included in the study. Of them, 541 were obese patients and 501 were age-matched and sex-matched healthy control subjects. The obese patients were divided into metabolically healthy obesity (MHO) group and metabolically unhealthy obesity (MUHO) group according to the levels of fasting plasma glucose (FBG), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and blood pressure (BP). Clinical and biochemical parameters including total bilirubin (TBil), indirect bilirubin (IBil) and direct bilirubin (DBil) were measured. ANOVA or Kruskal-Wallis H test was used to test differences among the three groups. Pearson and Spearman correlations were used to analyze the relationships between two parameters. The relationships between bilirubin and other variables were analyzed using Multivariate regression analysis.

Results: MHO group had favorable blood pressure, glucose and lipids profiles, along with increased TBil and DBil, and decreased high-sensitivity C-reactive protein (hsCRP) and homeostasis model assessment of insulin resistance (HOMA-IR) levels when compared to MUHO group ($P < 0.05$ for all). TBil and DBil were negatively correlated with total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), fasting insulin (FINS), hsCRP and HOMA-IR, even after adjusted for age, gender and BMI (all P <0.01). Multivariate regression analysis demonstrated that HOMA-IR was independently correlated with TBil and DBli levels (β = -0.400, $P < 0.01$).

Conclusion: MHO group harbors increased bilirubin level compared with MUHO group. HOMA-IR was independently correlated with TBil and DBli levels.

Keywords: bilirubin, obesity, metabolically benign, morbid, MHO

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INTRODUCTION

Obesity has become a global health problem due to its epidemic proportions and health hazard (1–3). Obesity is confirmed as one of the most important risk factors for dyslipidemia, type 2 diabetes and metabolic syndrome (1–3). Interestingly, a part of obese subjects show normal metabolism, which is defined as "metabolically healthy obesity (MHO)" (4, 5). However, exact mechanisms of MHO remain unclear.

Bilirubin, a product of heme metabolism, exerts anti-inflammatory and antioxidative effects (6, 7). Circulating bilirubin level is reported to be negatively correlated with the risk of cardio-metabolic diseases, including type 2 diabetes, NAFLD etc (7–11). As an intermediate state between health and metabolic disorders, MHO would manifest different bilirubin levels (12). However, the association between bilirubin and MHO is rarely reported.

The research aimed to elucidate the associations between serum bilirubin levels and metabolic parameters in different obesity phenotypes.

MATERIAL AND METHODS

Study Subjects

A total of 541 obese patients were consecutively enrolled in our study. The included patients were no less than 18 years, with BMI (body mass index) ≥ 28.0 kg/m^2. All of the subjects received physical examinations in Beijing Chao-Yang Hospital, Capital Medical University between January 2018 and January 2019 (2). Meanwhile, 501 healthy individuals with normal weight (18.5 kg/m^2 ≤ BMI < 24.0 kg/m^2) were recruited as controls, and they were matched with obese cases in age and gender (2). The subjects had no evidences for alcohol abuse, cardiovascular disease, thyroid dysfunction, anemia, hematological disease, chronic hepatitis/cirrhosis, biliary obstruction disease, acute or chronic infections, renal insufficiency, systemic inflammation, or cancer. Individuals in pregnancy or taking medications known to influence bilirubin, liver function, insulin, glucose, lipid or blood pressure were excluded, such as glycyrrhizic acid, ursodeoxycholic acid, potassium magnesium aspartate, "Yinzhihuang" granules, Metformin, Reserpine, Guanethidine, etc. In addition, persons meeting any one of the following conditions were also removed: serum bilirubin levels ≥ 2 times of upper limit of normal (ULN) (ULNs: TBIL: 21.0 μmol/L and DBIL : 6.8 μmol/L) or serum ALT and/or AST and/or GGT levels ≥ 3ULN (ULNs: alanine aminotransferase (ALT) : 50 U/L, aspartate aminotransferase (AST) : 40 U/L, and gamma-glutamyl transferase (GGT) : 60 U/L).

When obese individuals (BMI ≥28 kg/m^2) did not conform to the following criteria, they were categorized as MHO (13): (1) elevated FBG (≥5.6 mmol/L), (2) elevated TG (≥1.7 mmol/L), (3) reduced HDL-C (<1.0 mmol/L for men and <1.3 mmol/L for women), and (4) elevated SBP (≥130 mmHg) or/and DBP (≥85 mmHg). Obese patients who had one or more of these four metabolic risk components were categorized as metabolically unhealthy obesity (MUHO) (13).

This research was performed according to the Declaration of Helsinki ethical principles. Ethics approval was given by the Institutional Review Board (IRB) of Beijing Chao-Yang Hospital, Capital Medical University. Written informed consents were obtained from all subjects.

Clinical and Biochemical Indicators' Measurement

Both health status and medical history were collected through a standard questionnaire during a face-to-face interview, including alcohol consumption, medication status, physical activity, and history of diseases. Height was measured, with an accuracy of 0.1 cm, and weight was accurate to 0.1 kg, by professional medical staff, while participants were wearing light clothing without shoes. Body mass index (BMI) was calculated as weight/body height (kg/m^2). We employed a sphygmomanometer to measure sitting blood pressure for non-dominant arm after at least ten-minute rest.

After overnight fasting, blood samples were collected from median cubital vein. All biochemical indicators were measured using an automatic biochemical analyzer (Hitachi 747, Roche Diagnostics, Germany), except for fasting plasma insulin (FINS), glycated hemoglobin A1c (HbA1c) and high-sensitivity C-reactive protein (hsCRP). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) were measured *via* velocity method. Total bilirubin (TBil) and direct bilirubin (DBil) were measured through vanadate oxidation method. Indirect bilirubin (IBil) was calculated based on TBil minus DBil. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were assessed adopting colorimetric enzymatic methods. Plasma TC and TG were tested employing enzymatic cholesterol oxidase reaction and glycerol lipase oxidase reaction, respectively. HDL-C and LDL-C were assessed *via* direct measurement. Fasting plasma glucose (FBG) were estimated utilizing glucose oxidase assay. FINS concentrations were tested using chemiluminescence assay (Dimension Vista, Siemens Healthcare Diagnostics, Germany). HbA1c was measured applying high-performance liquid chromatography (HPLC) on an automatic biochemical analyzer (HLC-723G7, Tosoh Corporation, Tokyo, Japan). hsCRP levels were determined with immunonephelometric analysis. To estimate insulin resistance, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated: HOMA-IR = [FINS (μIU/mL) * FBG (mmol/L)/22.5] (14).

Statistical Analysis

All statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA). Continuous variables in normal distribution were recorded as mean values ± standard deviation (SD). Because values for ALT, GGT, TG, FINS, hsCRP and HOMA-IR did not conform to normal distribution, they were represented by the median, 25% quartile and 75% quartile. Chi-squared test was adopted to analyze categorical variables. ANOVA or Kruskal-Wallis H test was used to compare differences among the three groups. *Post hoc* analyses were performed. The relationships between two parameters were analyzed using Pearson and Spearman correlations. The relationship between bilirubin and other variables was analyzed using Multivariate regression analysis. $P < 0.05$ (two-tailed) revealed statistical significance of results.

RESULTS

Baseline Characteristics of control, MHO and MUHO Groups

Baseline characteristics of the included subjects were summarized in **Table 1**. Analysis results showed that age and gender were similar

among control, MHO and MUHO groups. Among these three groups, no significant differences were observed in serum IBil levels. Significant differences were identified in BMI, SBP, DBP, ALT, AST, GGT, TBil, DBil, TC, TG, HDL-C, LDL-C, FBG, FINS, HbA1c, hsCRP and HOMA-IR levels among control, MHO and MUHO groups (all $P < 0.01$).

Further *post hoc* analysis showed that both of MHO and MUHO groups had significantly increased BMI, FINS, hsCRP and HOMA-IR compared with control group (**Table 1**). Moreover, patients in MUHO group had significantly increased SBP, DBP, ALT, AST, GGT, TC, TG, LDL-C, FBG and HbA1c levels, and decreased Tbil, DBil and HDL-C levels when compared to healthy controls (**Table 1**). MHO and MUHO groups showed similar tendencies in BMI, ALT, AST, GGT, IBil, TC, LDL-C and FINS levels (**Table 1**). Interestingly, MHO group had relatively lower SBP, DBP, TG, FBG, HbA1c, hsCRP and HOMA-IR levels, and higher TBil, DBil and HDL-C levels than MUHO group (**Table 1**).

Comparison on Bilirubin Levels Among Control, MHO and MUHO Groups

We compared TBil, DBil and IBil levels among control, MHO and MUHO groups. As displayed in **Supplementary Figure 1**, MUHO group showed significantly lower level of TBil than control ($P<0.01$) and MHO ($P<0.05$) groups. MHO and control groups showed similar TBil values ($P>0.05$). As for IBil level, there were no significant differences among control, MHO and MUHO groups ($P>0.05$ for all) (**Supplementary Figure 2**). In addition, the level of DBil was decreased in MUHO group, compared with control and MHO groups ($P<0.01$ for both). Meanwhile, control and MHO groups showed insignificant difference in DBil level ($P>0.05$) (**Supplementary Figure 3**).

The Associations Between Bilirubin and Clinical Parameters in All Subjects

TBil level showed negative association with BMI, GGT, TC, TG, LDL-C, FINS, hsCRP and HOMA-IR, and positive association with HDL-C level (**Table 2**). Moreover, these significant correlations did not show remarkable alteration after adjusted for age, gender and BMI ($P <0.01$ for all).

Serum DBil level was negatively correlated with age, BMI, ALT, GGT, TC, TG, LDL-C, FBG, FINS, hsCRP and HOMA-IR, and positively associated with HDL-C levels (**Table 2**). After adjusted for age, gender and BMI, the associations of DBil level with TC, LDL-C, FINS, hsCRP and HOMA-IR were still significant (TC: $r = - 0.493$; LDL-C: $r = - 0.530$; FINS: $r = - 0.441$; hsCRP: $r = - 0.335$; HOMA-IR: $r = - 0.380$; all $P <0.01$).

Serum IBil level was negatively correlated with hsCRP and HOMA-IR, and positively correlated with HDL-C and LDL-C levels (**Table 2**). However, these associations were insignificant after adjusted for age, gender and BMI.

Multivariate Stepwise Regression Analysis on Relationships Between Serum Bilirubin Level and Other Clinical Parameters

Multivariate regression analysis was used to evaluate the correlation between serum bilirubin level and clinical and biochemical parameters, including age, gender, BMI, TG, FBG, hsCRP and HOMA-IR. Results showed HOMA-IR was independently correlated with TBil and DBil levels ($\beta = -0.400$, $P < 0.01$).

DISCUSSION

Compared with MUHO group, MHO group had favorable blood pressure, glucose and lipid profiles, increased TBil and DBil levels, and decreased hsCRP and HOMA-IR levels, even in a comparable BMI level. Serum TBil and DBil levels were negatively correlated with TC, LDL-C, FINS, hsCRP and HOMA-IR. HOMA-IR was independently correlated with TBil and DBil levels.

Apart from favorable metabolic parameters, MHO group had decreased hsCRP and HOMA-IR levels when compared to MUHO group, even in a comparable BMI level. Insulin resistance is a major mechanism for metabolic syndrome (15, 16). Chronic overfeeding induces adipocyte hypertrophy, and then activates inflammatory pathways and accelerates inflammatory cells' infiltration in adipose tissue, which further promote chronic low-grade inflammation and systemic insulin resistance in diet-induced obese mice (15–17). A recent human study showed that serum bilirubin level was negatively associated with inflammatory cytokines such as TNF-α, IL-6 and CRP, and positively associated with anti-inflammatory adiponectin (18). Consistently, our study showed that relatively reduced inflammatory state and heightened insulin sensitivity predicted favorable metabolic parameters in MHO patients.

Bilirubin has been recognized as a biochemical substance with metabolic benefits in recent years (19). Bilirubin includes two subtypes: DBil and IBil. DBil could be converted from IBil by UDP-glucuronyl transferase 1A1 (UGT1A1) in liver. The present research displayed that MHO patients had higher Tbil and DBil levels compared with MUHO group. Moreover, serum DBIL level was negatively correlated with HOMA-IR level. Multivariate regression analysis displayed that HOMA-IR was independently correlated with TBil and DBil levels. Consistently, previous studies showed that higher plasma TBil and DBil levels were associated with better metabolic parameters and lower risk of NAFLD (10, 20, 21). Morbid obesity decreases UGT1A1 activity, which may lead to low DBil level (22). In our study, both of MHO and MUHO groups showed decreased TBil and DBil levels. Moreover, IBil level in MHO group was similar to that in MUHO group. So significant down-regulation of TBil level in MUHO group could be attributed to decreased DBil level caused by UGT1A1 defect. Bilirubin administration for 14 days significantly reduced body weights, improved glucose tolerance and elevated insulin sensitivity in DIO mice (23). Bilirubin treatment reduced macrophage infiltration, and inhibited the expressions of TNF-α, IL-1β and MCP-1 in adipose tissue of diet-induced obese mice (24). Bilirubin also regulated T helper type 17 (Th17) immune responses and inhibited the generation of ROS induced by toll-like receptor 4 (25, 26). In the present study, correlation analysis found that serum DBil level was negatively associated with hsCRP level. These findings from our study and previous ones suggested that increased TBil and DBil levels predicted normal metabolism among obese subjects. In normal physiological pH condition, bilirubin is a fat soluble

TABLE 1 | Baseline characteristics of control, MHO and MUHO groups.

Parameters	Control group (n = 501)	MHO group (n = 51)	MUHO group (n = 490)	*P*
Age,y	36.7 ± 9.0	36.7 ± 8.8	36.7 ± 7.5	.998
Gender, M/F, n	418/83	9/42	410/80	.965
BMI, kg/m^2	22.06 ± 1.61	30.03 ± 1.66**	30.40 ± 2.20**	.000
SBP, mmHg	116.9 ± 8.2	121.3 ± 5.3	132.0 ± 12.89**$$	.000
DBP, mmHg	71.7 ± 7.6	73.7 ± 6.3	81.6 ± 10.1**$$	.000
ALT, U/L	20.0 (16.0 – 28.0)	24.0 (20.5 – 35.0)	37.5 (29.0 – 57.2)**	.000
AST, U/L	20.1 ± 6.4	21.5 ± 4.6	25.6 ± 9.23**	.000
GGT, U/L	19.0 (14.0 – 27.0)	24.0 (19.5 – 43.0)	40.0 (26.7 – 57.2)*	.000
TBil, μmol/L	16.78 ± 4.01	15.29 ± 3.92	14.70 ± 3.67**$	.002
DBil, μmol/L	5.90 ± 1.87	5.30 ± 1.89	4.72 ± 1.80**$$	.000
IBil, μmol/L	10.88 ± 3.17	9.98 ± 3.54	9.97 ± 3.07	.076
TC, mmol/L	4.70 ± 0.83	4.82 ± 0.91	5.21 ± 1.08**	.000
TG, mmol/L	0.96 (0.75 – 1.19)	1.03 (0.75 – 1.26)	2.04 (1.46 – 2.91)**$$	.000
HDL-C, mmol/L	1.33 ± 0.25	1.21 ± 0.16	1.03 ± 0.24**$$	.000
LDL-C, mmol/L	2.74 ± 0.68	2.98 ± 0.70	2.99 ± 0.73**	.002
FBG, mmol/L	5.19 ± 0.22	5.18 ± 0.30	5.74 ± 1.12**$$	.000
FINS, mIU/L	9.00 (6.41 – 13.01)	15.22 (12.20 – 16.90)*	19.95 (15.73 – 31.52)**	.000
HbA1c, %	5.27 ± 0.17	5.35 ± 0.49	6.08 ± 0.64*$	.000
hsCRP, mg/L	0.19 (0.08 – 0.81)	0.82 (0.10 – 2.57)*	3.94 (1.49 – 6.81)**$$	.000
HOMA-IR	2.01 (1.48 – 2.66)	3.50 (2.71 – 4.00)*	5.06 (3.71 – 7.85)**$$	.000
Metabolic components				
Elevated BP[a]	0	0	286 (58.4%)	
Hyperglycemia[b]	0	0	226 (46.1%)	
Dyslipidemia[c]	0	0	397 (81.0%)	

Data are means ± standard deviation unless indicated otherwise. ALT, GGT, TG, FINS, hsCRP, and HOMA-IR are shown as medians (upper and lower quartiles). MHO, metabolically healthy obesity; MUHO, metabolically unhealthy obesity; M, males; F, females; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HbA1c, hemoglobin A1c; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.

**comparison between MUHO group and control group, *P < 0.05, **P < 0.01.*

$comparison between MUHO group and MHO group, $P < 0.05, $$P < 0.01.

[a]Elevated BP were defined as, elevated SBP (≥130 mmHg) or/and DBP (≥85 mmHg);

[b]Hyperglycemia, elevated FBG (≥5.6 mmol/L);

[c]Dyslipidemia, elevated TG (≥1.7 mmol/L) or/and reduced HDL-C (<1.0 mmol/L for men and <1.3 mmol/L for women).

substance difficult to dissolve in water. In blood, bilirubin binds to albumin for transportation. It was reported that DBil was weakly bound to albumin, while IBil was strongly bound to albumin (27). So DBil might be easily separated from albumin, and played protective roles in metabolic processes.

In addition, serum levels of TBil and DBil were negatively correlated with atherogenic blood lipids (TC and LDL-C). In diet-induced obese mice, bilirubin treatment significantly reduced TC level, accompanied by reduced hepatic expression of SREBP-1, a factor required for *de novo* lipogenesis (23). Consistently, patients

TABLE 2 | Correlation between bilirubin and clinical parameters in all participants.

Parameters	TBil		DBil		IBil	
	r	P	*r*	*P*	*r*	*P*
Age	.016	.616	-.069	.030	.057	.075
BMI	-.082	.010	-.134	.000	-.052	.104
SBP	-.006	.842	-.034	.286	.008	.812
DBP	-.022	.482	-.029	.370	.046	.148
ALT	-.036	.261	-.098	.002	-.003	.920
AST	.019	.550	.015	.631	.020	.535
GGT	-.073	.022	-.087	.006	-.010	.751
TC	-.078	.014	-.352	.000	.060	.059
TG	-.089	.005	-.291	.000	-.017	.592
HDL-C	.138	.000	.117	.000	.140	.000
LDL-C	-.030	.347	-.229	.000	.069	.030
FBG	-.043	.174	-.089	.005	-.018	.566
FINS	-.206	.000	-.236	.000	-.176	.000
HbA1c	-.158	.122	-.194	.057	-.132	.196
hsCRP	-.364	.000	-.408	.000	-.313	.000
HOMA-IR	-.205	.000	-.244	.000	-.171	.000

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HbA1c, hemoglobin A1c; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin.

with Gilbert's syndrome had reduced TC and LDL-C levels when compared to matched controls (19, 28, 29). Bilirubin could promote lipid catabolism and inhibit lipid accumulations (30, 31). Bilirubin might bind to PPARα and promote β-hydroxybutyrate, and then activate hepatic β-oxidation pathway, thus boosting lipid metabolism (32, 33).

Several limitations in our research should be acknowledged here. First, this cross-sectional study cannot render causal inferences. Besides, Gilbert's syndrome, caused by reduced activity of UGT enzyme, is a common hereditary disease featured by hyperbilirubinemia. Although participants with serum bilirubin level ≥ 2ULN were excluded, Gilbert's syndrome cases with serum bilirubin levels ≤ 2ULN might be recruited due to the absence of genetic examination. Finally, we only observed the association between bilirubin and metabolic parameters, and precise mechanism was not explored. In addition, only 51 patients were included in MHO group that might reduce statistical power of our analysis. Moreover, the prevalence of MHO was 9.43% in our study population, which was lower than previously reported occurrence rate (10%-30%) (5). Selection bias might contribute to this difference. More prospective and larger-scale studies are needed to determine the function and mechanisms of bilirubin in the progression of metabolic disorders in obese patients.

CONCLUSIONS

Comparing with MUHO group, MHO group has favorable blood pressure, glucose and lipid profiles, apart from increased TBil and DBil levels and decreased hsCRP and HOMA-IR levels. Multivariate regression analysis shows that HOMA-IR is independently correlated with TBil and DBil levels.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board (IRB) of Beijing Chao-Yang Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JF, QW, and LZ conceived and designed the experiments, analyzed the data, and wrote the paper. JL and GW performed the experiments. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.792795/full#supplementary-material

REFERENCES

1. Expert Panel Members, Jensen MD, Ryan DH, Donato KA, Apovian CM, Ard JD. Executive Summary: Guidelines, (2013) for the Management of Overweight and Obesity in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Obesity Society Published by the Obesity Society and American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Based on a Systematic Review From the The Obesity Expert Panel 2013. *Obes (Silver Spring)* (2014) 22(Suppl 2):S5–39. doi: 10.1002/oby.20821
2. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation* (2014) 129:S102–138. doi: 10.1161/01.cir.0000437739.71477.ee
3. Joshy G, Korda RJ, Attia J, Liu B, Bauman AE, Banks E. Body Mass Index and Incident Hospitalisation for Cardiovascular Disease in 158 546 Participants From the 45 and Up Study. *Int J Obes (Lond)* (2014) 38:848–56. doi: 10.1038/ijo.2013.192
4. Stefan N, Haring HU, Hu FB, Schulze MB. Metabolically Healthy Obesity: Epidemiology, Mechanisms, and Clinical Implications. *Lancet Diabetes Endocrinol* (2013) 1:152–62. doi: 10.1016/S2213-8587(13)70062-7
5. Bluher M. Metabolically Healthy Obesity. *Endocr Rev* (2020) 41:bnaa004. doi: 10.1210/endrev/bnaa004
6. Stocker R. Antioxidant Activities of Bile Pigments. *Antioxid Redox Signal* (2004) 6:841–9. doi: 10.1089/ars.2004.6.841
7. Hinds TDJr., Stec DE. Bilirubin, A Cardiometabolic Signaling Molecule. *Hypertension* (2018) 72:788–95. doi: 10.1161/HYPERTENSIONAHA.118.11130
8. Jung CH, Lee MJ, Kang YM, Hwang JY, Jang JE, Leem J, et al. Higher Serum Bilirubin Level as a Protective Factor for the Development of Diabetes in Healthy Korean Men: A 4 Year Retrospective Longitudinal Study. *Metabolism* (2014) 63:87–93. doi: 10.1016/j.metabol.2013.09.011
9. Nano J, Muka T, Cepeda M, Voortman T, Dhana K, Brahimaj A, et al. Association of Circulating Total Bilirubin With the Metabolic Syndrome and Type 2 Diabetes: A Systematic Review and Meta-Analysis of Observational Evidence. *Diabetes Metab* (2016) 42:389–97. doi: 10.1016/j.diabet.2016.06.002
10. Tian J, Zhong R, Liu C, Tang Y, Gong J, Chang J, et al. Association Between Bilirubin and Risk of Non-Alcoholic Fatty Liver Disease Based on a Prospective Cohort Study. *Sci Rep* (2016) 6:31006. doi: 10.1038/srep31006

11. Kunutsor SK, Frysz M, Verweij N, Kieneker LM, Bakker SJL, Dullaart RPF. Circulating Total Bilirubin and Risk of non-Alcoholic Fatty Liver Disease in the PREVEND Study: Observational Findings and a Mendelian Randomization Study. *Eur J Epidemiol* (2020) 35:123–37. doi: 10.1007/s10654-019-00589-0
12. Camhi SM, Must A, Gona PN, Hankinson A, Odegaard A, Reis J, et al. Duration and Stability of Metabolically Healthy Obesity Over 30 Years. *Int J Obes (Lond)* (2019) 43:1803–10. doi: 10.1038/s41366-018-0197-8
13. Ortega FB, Lavie CJ, Blair SN. Obesity and Cardiovascular Disease. *Circ Res* (2016) 118:1752–70. doi: 10.1161/CIRCRESAHA.115.306883
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis Model Assessment: Insulin Resistance and Beta-Cell Function From Fasting Plasma Glucose and Insulin Concentrations in Man. *Diabetologia* (1985) 28:412–9. doi: 10.1007/BF00280883
15. Heilbronn LK, Campbell LV. Adipose Tissue Macrophages, Low Grade Inflammation and Insulin Resistance in Human Obesity. *Curr Pharm Des* (2008) 14:1225–30. doi: 10.2174/138161208784246153
16. Olefsky JM, Glass CK. Macrophages, Inflammation, and Insulin Resistance. *Annu Rev Physiol* (2010) 72:219–46. doi: 10.1146/annurev-physiol-021909-135846
17. Chawla A, Nguyen KD, Goh YP. Macrophage-Mediated Inflammation in Metabolic Disease. *Nat Rev Immunol* (2011) 11:738–49. doi: 10.1038/nri3071
18. Petelin A, Jurdana M, Jenko Praznikar Z, Ziberna L. Serum Bilirubin Correlates With Serum Adipokines in Normal Weight and Overweight Asymptomatic Adults. *Acta Clin Croat* (2020) 59:19–29. doi: 10.20471/acc.2020.59.01.03
19. Seyed Khoei N, Grindel A, Wallner M, Molzer C, Doberer D, Marculescu R, et al. Mild Hyperbilirubinaemia as an Endogenous Mitigator of Overweight and Obesity: Implications for Improved Metabolic Health. *Atherosclerosis* (2018) 269:306–11. doi: 10.1016/j.atherosclerosis.2017.12.021
20. Chang Y, Ryu S, Zhang Y, Son HJ, Kim JY, Cho J, et al. A Cohort Study of Serum Bilirubin Levels and Incident non-Alcoholic Fatty Liver Disease in Middle Aged Korean Workers. *PLoS One* (2012) 7:e37241. doi: 10.1371/journal.pone.0037241
21. Kwak MS, Kim D, Chung GE, Kang SJ, Park MJ, Kim YJ, et al. Serum Bilirubin Levels are Inversely Associated With Nonalcoholic Fatty Liver Disease. *Clin Mol Hepatol* (2012) 18:383–90. doi: 10.3350/cmh.2012.18.4.383
22. Rougée LR, Miyagi SJ, Collier AC. Obstetric Obesity is Associated With Neonatal Hyperbilirubinemia With High Prevalence in Native Hawaiians and Pacific Island Women. *Hawai'i J Med Public health: J Asia Pacific Med Public Health* (2016) 75:373–8.
23. Liu J, Dong H, Zhang Y, Cao M, Song L, Pan Q, et al. Bilirubin Increases Insulin Sensitivity by Regulating Cholesterol Metabolism, Adipokines and PPARgamma Levels. *Sci Rep* (2015) 5:9886. doi: 10.1038/srep09886
24. Dong H, Huang H, Yun X, Kim DS, Yue Y, Wu H, et al. Bilirubin Increases Insulin Sensitivity in Leptin-Receptor Deficient and Diet-Induced Obese Mice Through Suppression of ER Stress and Chronic Inflammation. *Endocrinology* (2014) 155:818–28. doi: 10.1210/en.2013-1667
25. Idelman G, Smith DLH, Zucker SD. Bilirubin Inhibits the Up-Regulation of Inducible Nitric Oxide Synthase by Scavenging Reactive Oxygen Species Generated by the Toll-Like Receptor 4-Dependent Activation of NADPH Oxidase. *Redox Biol* (2015) 5:398–408. doi: 10.1016/j.redox.2015.06.008
26. Longhi MS, Vuerich M, Kalbasi A, Kenison JE, Yeste A, Csizmadia E, et al. Bilirubin Suppresses Th17 Immunity in Colitis by Upregulating CD39. *JCI Insight* (2017) 2:e92791. doi: 10.1172/jci.insight.92791
27. Nakagami T, Toyomura K, Kinoshita T, Morisawa S. A Beneficial Role of Bile Pigments as an Endogenous Tissue Protector: Anti-Complement Effects of Biliverdin and Conjugated Bilirubin. *Biochim Biophys Acta* (1993) 1158:189–93. doi: 10.1016/0304-4165(93)90013-X
28. Tapan S, Karadurmus N, Dogru T, Ercin CN, Tasci I, Bilgi C, et al. Decreased Small Dense LDL Levels in Gilbert's Syndrome. *Clin Biochem* (2011) 44:300–3. doi: 10.1016/j.clinbiochem.2010.12.003
29. Wallner M, Marculescu R, Doberer D, Wolzt M, Wagner O, Vitek L, et al. Protection From Age-Related Increase in Lipid Biomarkers and Inflammation Contributes to Cardiovascular Protection in Gilbert's Syndrome. *Clin Sci (Lond)* (2013) 125:257–64. doi: 10.1042/CS20120661
30. Hana CA, Tran LV, Mölzer C, Müllner E, Hörmann-Wallner M, Franzke B, et al. Serum Metabolomics Analysis Reveals Increased Lipid Catabolism in Mildly Hyperbilirubinemic Gilbert's Syndrome Individuals. *Metabolism: Clin Exp* (2021) 125:154913. doi: 10.1016/j.metabol.2021.154913
31. Hana CA, Klebermass EM, Balber T, Mitterhauser M, Quint R, Hirtl Y, et al. Inhibition of Lipid Accumulation in Skeletal Muscle and Liver Cells: A Protective Mechanism of Bilirubin Against Diabetes Mellitus Type 2. *Front Pharmacol* (2021) 11:636533. doi: 10.3389/fphar.2020.636533
32. Stec DE, John K, Trabbic CJ, Luniwal A, Hankins MW, Baum J, et al. Bilirubin Binding to Pparα Inhibits Lipid Accumulation. *PLoS One* (2016) 11:e0153427. doi: 10.1371/journal.pone.0153427
33. Hinds TD, Creeden JF, Gordon DM, Stec DF, Donald MC, Stec DE. Bilirubin Nanoparticles Reduce Diet-Induced Hepatic Steatosis, Improve Fat Utilization, and Increase Plasma β-Hydroxybutyrate. *Front Pharmacol* (2020) 11:594574. doi: 10.3389/fphar.2020.594574

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REVIEW
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Effects of Oral Glucose-Lowering Agents on Gut Microbiota and Microbial Metabolites

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The current research and existing facts indicate that type 2 diabetes mellitus (T2DM) is characterized by gut microbiota dysbiosis and disturbed microbial metabolites. Oral glucose-lowering drugs are reported with pleiotropic beneficial effects, including not only a decrease in glucose level but also weight loss, antihypertension, anti-inflammation, and cardiovascular protection, but the underlying mechanisms are still not clear. Evidence can be found showing that oral glucose-lowering drugs might modify the gut microbiome and thereby alter gastrointestinal metabolites to improve host health. Although the connections among gut microbial communities, microbial metabolites, and T2DM are complex, figuring out how antidiabetic agents shape the gut microbiome is vital for optimizing the treatment, meaningful for the instruction for probiotic therapy and gut microbiota transplantation in T2DM. In this review, we focused on the literatures in gut microbiota and its metabolite profile alterations beneficial from oral antidiabetic drugs, trying to provide implications for future study in the developing field of these drugs, such as combination therapies, pre- and probiotics intervention in T2DM, and subjects with pregestational diabetes and gestational diabetes mellitus.

Keywords: gut microbiota, microbial metabolites, T2DM, antidiabetic drugs, SCFA

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INTRODUCTION

The International Diabetes Federation Diabetes Atlas 10th edition shows a continued global increase in diabetes prevalence, estimating that 537 million adults are living with diabetes worldwide, most of which is type 2 diabetes mellitus (T2DM) (1). T2DM is a metabolic disorder with multiple pathogenic factors, including genetic elements, sedentary behaviors, and overeating (2). Once without effective treatment, it might lead to a composite of microvascular or macrovascular complications, for instance chronic kidney disease, diabetic eye disease, and cardiovascular disease (CVD) (3). Differing from insulin-dependent type 1 diabetes mellitus, T2DM is closely interrelated with insulin resistance (IR) and strongly intertwined with obesity, non-alcoholic fatty liver disease, and metabolic syndrome (4). Nowadays, more than 10 types of medicines are approved by the USA Food and Drug Administration for the glycemic treatment (5). Thousands of clinical trials and basic research are proceeding worldwide for diabetes

pharmacotherapy, including looking for potential intervention targets (6). In addition to the reduction in HbA_1c, results from a vast number of clinical and experimental studies have shown the potential effects of glucose-lowering drugs, such as weight reduction, cardiovascular safety, and lipid-lowering and antihypertensive effects; however, the mechanisms behind these benefits need to be further revealed (5, 6).

Gut microbiota has become a hot topic in metabolic disorders in the past decade, including T2DM (7–9). Accumulating evidence confirms that gut microbiota has emerged as a large complex ecological community and a vital regulator of host physical condition, *via* microbial metabolites and host interactions (10, 11). Among 100 trillion of microorganisms, which is 10 times the number of human body cells, including bacteria, fungi, viruses, and protozoa, the bacterial component is characterized as the most well-investigated group (11, 12) and will be the chief spotlight of this review. There are nearly 500–1,000 species of bacteria within the gastrointestinal tract and more than 90% of the total community are Firmicutes and Bacteroidetes at the phylum level, followed by Proteobacteria, Actinobacteria, Verrucomicrobiota, Fusobacteria, Cyanobacteria, and Tenericutes (11, 12). The gut microbiota homeostasis is preserved with control of pathogenic microbe growth and protection of beneficial microbes (11, 13). The gut microbiome is considered as a modifiable "new organ" that plays a crucial role in shaping the metabolic and immunological functions of T2DM (14). Although with wide interindividual variation, once the gut microbiota composition was destroyed, an imbalanced gut microbiome community leads to an abnormal production of metabolites, lipid and carbohydrate metabolism disturbance, IR, oxidative stress, and low-grade chronic inflammatory state in T2DM (7, 8, 15–18).

Therefore, understanding how antidiabetes agents influence the gut microbiome might be of importance for optimizing T2DM treatment. Microbiota and host metabolism might deliver promising and novel constructive aspects of commonly used oral antidiabetic drugs (19). In addition, fecal microbiota transplantation (FMT) has become a promising strategy for patients with T2DM (20, 21). In this review, we focus on the literatures in gut microbiota and metabolite profile alterations beneficial from oral antidiabetic drugs in diabetes and metabolic disorder state, in both basic research and clinical studies. We aim to figure out the similarities and differences in the literatures of gut microbiota and the metabolite-related effect of oral antidiabetic drugs, in order to deliver some leads for future studies in these developing fields of these drugs and T2DM treatment.

GUT MICROBIOTA AND METABOLITES ALTERED IN T2DM

Although the definite microbial signatures linked to T2DM have not been discovered yet, a large number of studies have found that gut microbiota dysbiosis in T2DM is highly associated with specific intestinal microbial taxa or certain enrichment of gene functional pathways (22–28). In a metagenome-wide association study from 345 Chinese individuals, T2DM-related gut flora dysbiosis was characterized by a decreased abundance in a cluster of butyrate-producing bacteria, such as *Roseburia intestinalis*, *Faecalibacterium prausnitzii*, *Clostridiales* sp. SS3/4, and *Eubacterium rectale*, and an increased abundance of opportunistic pathogens, such as *Bacteroides caccae*, *Escherichia coli*, and some *Clostridium* species (*Clostridium symbiosum*, *Clostridium bolteae*, *Clostridium hathewayi*, and *Clostridium ramosum*) (22). Another large-scale metagenome analysis study which recruited a population of 145 70-year-old European women with metagenomic profiles showed increases in the abundance of four *Lactobacillus* species (including *Lactobacillus gasseri*), *Streptococcus mutans*, and *Clostridium hathewayi* and decreases in the abundance of five *Clostridium* species (including *Clostridium beijerinckii*, *Clostridium botulinum*), *Roseburia_272*, and *Bacteroides intestinalis* in the T2DM group (23). Due to the difference in genetic inheritance, diet, and lifestyle factors, the connections among gut microbial communities, microbial metabolites, and T2DM are intricate. Despite the obvious discrepancy in metagenomic clusters between these two populations, the similar microbial functions enriched in T2DM included an increased level in lipid or glucose metabolism-related membrane transport and oxidative stress resistance and a decreased level in metabolism of vitamins and cofactors, butyrate production, and cell motility (22, 23). To recognize the core gut microbial features of T2DM, a machine learning framework totally recruited more than 9,000 people revealed that a microbiome risk score including 14 microbial features was positively associated with risk of T2DM and the future glucose increment after adjustment for traditional risk factors (such as age, sex, parental history of diabetes, body mass index, systolic blood pressure, and triglycerides) (28). In the meantime, a downward trend of butyrate-producing genus (*Roseburia* spp.) and a rising trend of chronic inflammation-associated genus (*f:lactobacillaceae*) were confirmed in this interpretable machine learning framework (28). Among a substantial body of experimental and clinical research, the genera of *Bifidobacterium*, *Akkermansia*, *Bacteroides*, *Roseburia*, and *Faecalibacterium* were inversely correlated with T2DM, while the genera of *Ruminococcus*, *Blautia*, *Lactobacillus*, and *Fusobacterium* were positively correlated with T2DM (8, 22, 23).

Although the underlying mechanism between complex gut microbiota and T2DM is still unclear, evidence has shown that a variety of metabolites derived from gut flora, including short-chain fatty acids (SCFAs), glycolipid lipopolysaccharides (LPS), bile acids (BAs), trimethylamine-N-oxide (TMAO), indole derivatives, amino acids, vitamins, and one-carbon metabolites, interacted with the host as signaling molecules and were further involved in the pathophysiological process of metabolic diseases (29–40) (**Figure 1**). SCFAs (including butyrate, acetate, and propionate) are the major microbial metabolites produced by dietary fiber fermentation within the intestinal lumen (41). SCFAs were found reduced in T2DM in both clinical and experimental research (42–45). By activation of specific G protein-coupled receptor 41 and 43 (GPR41 and GPR43), SCFAs could stimulate the secretion of peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) from intestinal enteroendocrine L cells (39, 46). PPY is an important neuroendocrine hormone, regulating food intake and energy

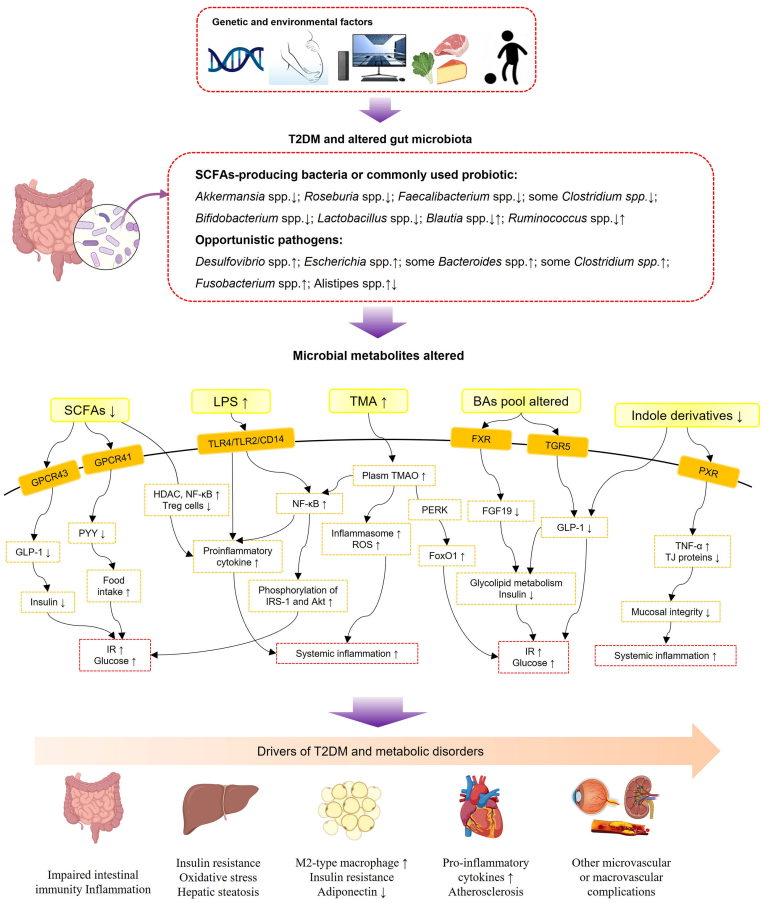

FIGURE 1 | Schematic view of gut microbiota, microbial metabolites, and T2DM-associated metabolic disorders. SCFAs, short-chain fatty acids; LPS, lipopolysaccharides; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; BAs, bile acids; GPCR43, G-protein-coupled receptor 43; GPCR41, G-protein-coupled receptor 41; TLR4, toll-like receptor 4; TLR2, toll-like receptor 2; CD14, cluster of differentiation 14; FXR, farnesoid X receptor; TGR5, Takeda G protein-coupled receptor 5; PXR, pregnane X receptor; GLP-1, glucagon-likepeptide-1; PYY, peptide tyrosine-tyrosine; HDAC, histone deacetylases; NF-κB, nuclear factor-kappaB; IRS-1, insulin receptor substrate-1; ROS, reactive oxygen species; PERK, protein kinase-like ER kinase; FoxO1, forkhead box-O1; FGF19, fibroblast growth factor 19; TNF-α, tumor necrosis factor alpha; TJ proteins, tight-junction proteins; IR, insulin resistance.

balance; reduced secretion of GLP-1 in T2DM leads to a reduction of insulin and thus impaired glucose and energy metabolism (47). Besides, SCFAs have been identified as vital mediators in maintaining intestinal immunity and systemic inflammation through upregulating anti-inflammatory regulatory T cells, inhibition of histone deacetylase, and further inhibition of inflammatory signaling pathways and proinflammatory cytokines, such as nuclear factor-kappaB (NF-κb) and tumor necrosis factor alpha (TNF-α) (37, 48).

LPSs, the main compounds of gram-negative bacterial membranes, are known as potent stimulators of inflammation (49). Evidence shows that T2DM subjects possess a high enrichment of gram-negative bacteria, particularly those belonging to Proteobacteria at the phylum level (50). Notably, the Bacteroidetes phylum also belongs to a large part of gram-negative bacteria, but a decreased abundance of Bacteroidetes was found in obesity and diabetes conditions (24, 51–54). This contradiction might be explained by the fact that the LPS produced by the Bacteroidetes phylum has a lower endotoxic activity than other gram-negative bacteria such as the Proteobacteria phylum (55). Subsequently, a high concentration of LPS produced within the gut (metabolic endotoxemia) might lead to chronic low-grade inflammation in diabetic subjects through upregulating inflammatory signaling pathways and proinflammatory cytokine secretion (56, 57). LPSs produced by gut bacteria might damage the intestinal barrier leading to a "leaky gut" syndrome, for instance, a weakened tight junction and reduced gut secretory immunoglobulin A (58). Besides, LPSs have been confirmed to result in IR due to increased IRS-1 and Akt phosphorylation (59) (**Figure 1**).

Originally synthesized from cholesterol in the liver, BAs were revealed to have a reciprocal interaction with gut microbiota *via* the gut-to-liver axis (40). Primary BAs are converted into secondary BAs by gut microbiota (40). BAs are important

signaling mediators regulating energy metabolism and systematic inflammation *via* the nuclear farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) (40). In subjects with diabetes and metabolic diseases, BAs' pool composition altered (60). The altered proportion of FXR antagonistic BAs leads to an altered expression of fibroblast growth factor 19 (FGF19), which were both vital molecules for BAs and glycolipid metabolism (60). Activation of TGR5 by secondary BAs stimulates GLP-1 secretion from L cells to increase insulin secretion and glucose tolerance (61). Evidence shows that modifications of the BA pool presented a beneficial effect in bariatric surgery and antidiabetic treatment (62–64).

TMAO is predominantly generated from dietary choline, which is transformed to trimethylamine in the gut and then oxidized in the liver (31). Elevated plasma concentrations of TMAO were reported positively related with metabolic dysfunction, such as insulin resistance, CVDs, and T2DM (31, 34, 65), and various bacteria (such as *Clostridium hathewayi*, *Escherichia fergusonii*, *Providencia alcalifaciens*, and *Providencia rustigianii*) have been recognized as contributing to the production of TMAO (66). TMAO was found to play a proinflammatory role by activating the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 inflammasome, accelerating reactive oxygen species generation and various proinflammatory cytokines (67). In addition, evidence in experimental research shows that TMAO promoted metabolic dysfunction by directly binding and activating protein kinase-like ER kinase, a key sensor of intracellular stress, and then enhanced transcription activity of forkhead box-O1 in the liver (31).

Indole derivatives are produced from tryptophan by the gut microflora (33). In the recent years, indole derivatives have exhibited anti-inflammatory and antidiabetic effects (68). Evidence shows that indole derivatives were able to stimulate the secretion of GLP-1 from L cells (32). Various indole derivatives have been synthesized to investigate their bioactivities and biological functions (68). Microbe-specific indoles, such as indole 3-propionic acid, were found to regulate mucosal integrity through activating the xenobiotic sensor, pregnane X receptor, to downregulate enterocyte TNF-α expression and upregulate junctional protein expression (36). In addition to the abovementioned metabolites, vitamins and cofactors produced by probiotics, such as *Bifidobacterium* and *Lactobacillus*, yield greater health benefits on patients with T2DM and metabolic diseases (69). Amino acids synthesized by the gastrointestinal microbiota were also vital factors to energy metabolism and glucose homeostasis (70). For instance, *Prevotella copri* and *Bacteroides vulgatus* were discovered as the main species mediating the association between biosynthesis of branched-chain amino acids (BCAAs) and IR, and *Prevotella copri* could induce IR, aggravate glucose intolerance, and increase circulating BCAAs levels (70).

Overall, a vast body of human studies and plentiful animal studies have suggested that T2DM was characterized by gut microbiota dysbiosis and alterations of gut microbiota-derived metabolites, which are important contributors to the pathological injury of T2DM.

THE EFFECTS OF ORAL ANTIDIABETIC DRUGS ON GUT MICROBIOTA AND MICROBIAL METABOLITES

Metformin

Metformin can alleviate patients' hyperglycemia mainly by significant suppression of glucose production in the liver (71). Activation of the master cellular energy sensor AMP-activated protein kinase (AMPK) is well documented in the mechanism of metformin but may not interpret for its complex beneficial effects (72–75). In fact, metformin was found to modify the intestinal flora community in T2DM in a vast body of clinical research and experimental animal studies (76–80) (summarized in **Table 1**).

Metagenomics combined with targeted metabolomic data in a randomized, placebo-controlled, double-blind study showed that metformin strongly altered the gut microbiome and its function in individuals with treatment-naive T2DM (79). Subsequently, the authors transplanted fecal samples from three donors (treatment-naive condition compared with 4-month metformin-treated condition) into germ-free mice and observed that glucose tolerance was improved in mice that received 4-month metformin-treated fecal samples, indicating a direct beneficial effect on glucose homeostasis (79). This effect might be mediated by increased SCFA-producing bacteria and the abundance of *Akkermansia muciniphila*, enriched pathways of the metabolism of vitamins and cofactors, and metalloproteins or metal transporters (79). In line with this research, a large study aimed at disentangling metformin treatment signatures in T2DM recruited 784 subjects from Denmark, Switzerland, and China and illustrated that metformin treatment significantly increased the abundance of *Escherichia* spp. and reduced that of *Intestinibacter* spp. The functional enrichment analyses demonstrated that SCFA-producing pathways and enrichment of virulence factors and gas metabolism genes were significantly enhanced, while intestinal lipid absorption and LPS-triggered intestinal inflammation were reduced (77). A randomized clinical trial which recruited 450 T2DM subjects uncovered that metformin altered the gut microbiota composition, increased the beneficial bacteria, such as *Blautia* and *Faecalibacterium*, and inhibited potential pathogen-like microbiota, for example, *Oscillibacter*, *Alistipes*, and *Bacteroides* (78). As summarized in **Table 1**, most clinical studies revealed that microbes mediated the therapeutic effects of metformin chiefly through improvement in SCFA production, BA pool composition alteration, or reduction in LPS production.

In addition to clinical studies on T2DM patients, a clinical trial which recruited 20 healthy Korean participants found that metformin treatment altered the abundances of *Clostridium*, *Escherichia*, *Intestinibacter*, and *Romboutsia*, and the relative abundances of metabolites changed including carbohydrate, fatty acid, and amino acid metabolism (95). In experimental animal models, treatment with metformin was revealed to increase SCFA production, to reduce circulation LPS, to inhibit intestinal proinflammatory signaling activities, which was in line with clinical studies (80, 96, 97) (**Figure 2**). The activation of SCFA receptors, GPR41 and GPR43, stimulated the secretion of

TABLE 1 | Clinical research exploring the effects of oral anti-diabetic drugs on gut microbiota in T2DM.

Anti-diabetic drugs	Subjects	Key results
Metformin (77)	784 subjects from Denmark, Switzerland and China	*Escherichia* spp.↑ *Lactobacillus* spp. ↓ Functional enrichment: SCFAs producing↑, virulence factors and gas metabolism genes↑ intestinal lipid absorption↓ LPS triggered local inflammation↓
Metformin (78)	450 subjects	Simpson's diversity index↑ *Blautia* spp. and *Faecalibacterium* spp.↑ *Alistipes* spp., *Oscillibacter* spp., and *Bacteroides* spp.↓
Metformin (79)	40 treatment-naive T2DM	Firmicutes, *Escherichia coli, Bifidobacterium adolescentis, Akkermansia muciniphila*↑ SCFA-producing genus↑ Fecal SCFAs and plasma bile acid concentrations↑
Metformin (45)	121 subjects	*Escherichia coli* and *Ruminococcus torques*↑; *Intestinibacter bartlettii*↓ Fecal SCFAs increased at 6 mouths
Metformin (81)	23 T2DM patients	Enterobacteriaceae↑
Metformin (76)	22 newly diagnosed T2DM	*Bacteroides fragilis*↓ bile acid glycoursodeoxycholic acid↑
Metformin (82)	60 adults with a BMI ≥ 25 kg/m^2	*Bacteroides caccae, Lachnospiraceae bacterium*↑*Bacteroides uniformis*↓ butyrate↑zonulin↓microbial butyrate-producing pathways↑
Metformin (83)	14 males with T2DM	Firmicutes↓ GLP-1, lithocholic and deoxycholic acids↑ primary bile acid↓
Metformin (84)	112 subjects	*Akkermansia muciniphila*, *Prevotella*, *Butyrivibrio, Bifidobacterium bifidum, Megasphaera*↑ *Clostridiaceae 02d06*↓
Metformin (85)	130 T2DM subjects	*Spirochaete, Turicibacter, and Fusobacterium*↑ Taurine and hypotaurine metabolism↑
Metformin (86)	30 T2DM subjects	*Bifidobacterium*
Dapagliflozin (87)	24 subjects	No significant effect on microbial composition
Empagliflozin (88)	67 T2DM with risk factors for CVD	SCHA-producing bacteria↑ Several harmful bacteria including Escherichia-Shigella, Bilophila, and Hungatella↓
Sitagliptin (89)	51 subjects	No significant effect on microbial composition
Sitagliptin (90)	57 T2DM subjects	Fecal chenodeoxycholic acid, cholic acid and ursodeoxycholic acid ↑
Vildagliptin (91)	30 T2DM subjects	*Pseudomonas*, *Klebsiella*, *Blautia, Faecalibacterium* and *Roseburia* levels altered
Saxagliptin (91)	30 T2DM subjects	*Megamonas* spp.↑; *Turicibacter* spp. ↓
Acarbose (62)	51 treatment-naive subjects	*Lactobacillus and Bifidobacterium*↑*Bacteroides*↓ Altered plasm BAs pool composition
Acarbose (92)	18 subjects	*Bifidobacterium*, *Eubacterium*, and *Lactobacillus*↑*Bacteroides*↓
Acarbose (93)	95 subjects	*Bifidobacterium longum* and *Enterococcus faecalis*↑ Plasm LPS↓
Acarbose (91)	30 T2DM subjects	Butyricimonas level increased first and then decreased during treatment
Acarbose (94)	52 prediabetes patients	*Lactobacillus* spp. and *Dialister* spp.↑ *Butyricicoccus* spp., *Phascolarctobacterium* spp. and *Ruminococcus* spp.↓
Glipizide (62)	43 treatment-naive subjects	No effect on intestinal microbiota composition
Gliclazide (87)	17 subjects	No significant effect on microbial composition

SCFAs, short-chain fatty acids; CVD, cardiovascular disease; LPS, lipopolysaccharides; GLP-1, glucagon-likepeptide-1.

PYY and GLP-1, inhibiting appetite and improving insulin secretion. At the same time, increased-circulation SCFAs are responsible for improving energy metabolism, suppressing fat accumulation and insulin signaling in adipose tissue, and regulating the intestinal immunity and systemic inflammation (38, 39, 98). Accompanied by decreased LPS produced in the gut, metformin intervention increased goblet cell mass, mucin production, and tight-junction (ZO1 and occludin) proteins in obese gut, thereby relieving intestinal inflammation, decreasing leaky gut, and repairing the intestinal barrier structure (80, 96). In addition, the metabolic benefits of metformin might also be mediated by gut microbiota and bile acid homeostasis (76). Evidence shows that *Bacteroides fragilis* was decreased in samples from newly diagnosed T2DM patients after metformin treatment for 3 days, meanwhile the BA pool was altered (76). Bile acid glycoursodeoxycholic acid was increased, accompanied by inhibition of intestinal FXR signaling and decreased serum FGF19 levels (76). Reduced circulating FGF19 was found in subjects with metabolic disorders and hepatic steatosis, and FGF19 analogues have been identified as promising therapeutic methods in metabolic improvement (60). However, research associated with FGF19 was inconsistent, and the underlying mechanism still needs further research. Among the numerous gut flora altered during the metformin treatment in both clinical and experimental studies, *Akkermansia muciniphila*, a mucin-degrading bacterium, is related to healthy intestinal mucosa (79, 84, 96, 99). Furthermore, oral administration of *Akkermansia muciniphila* to high-fat diet-induced mice without metformin treatment significantly improved glucose homeostasis and reduced visceral adipose tissue inflammation by inducing Tregs, indicating the promising treatment value of *Akkermansia* spp. for T2DM (99).

In brief, in addition to activation of the master cellular energy sensor AMPK (74), metformin might act partly through gut microbiota and its metabolites to improve metabolic health. Notably, the metformin concentration in the gastrointestinal lumen is 30–300 times higher than in the circulation (100). High concentrations of metformin in the gastrointestinal lumen

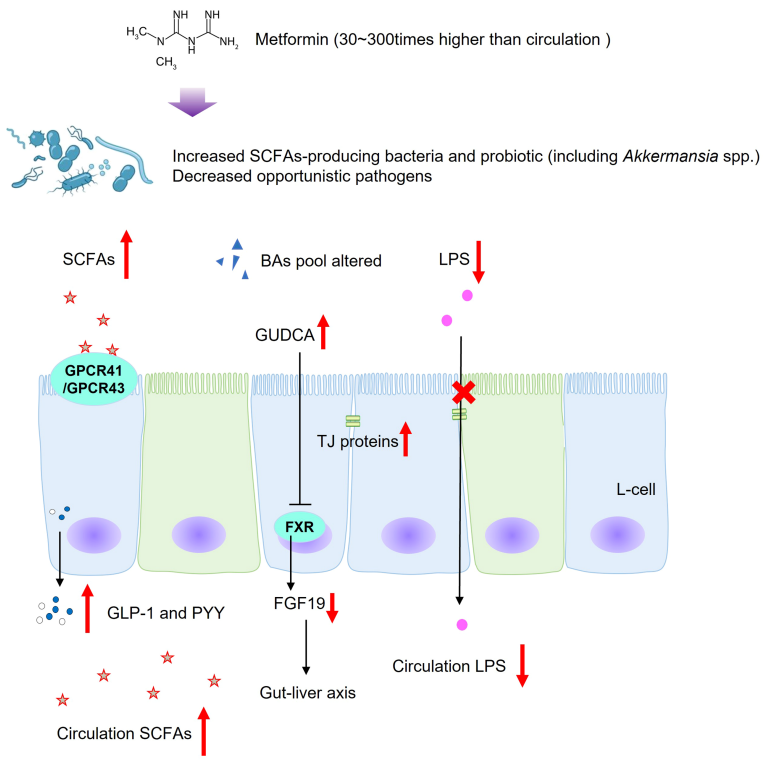

FIGURE 2 | Possible regulatory mechanisms of metformin on gut microbiota and microbial metabolites in T2DM. SCFAs, short-chain fatty acids; BAs, bile acids; LPS, lipopolysaccharides; GUDCA, glycoursodeoxycholic acid; GPCR43, G-protein-coupled receptor 43; GPCR41, G-protein-coupled receptor 41; FXR, farnesoid X receptor; TGR5, Takeda G protein-coupled receptor 5; GLP-1, glucagon-likepeptide-1; PYY, peptide; tyrosine-tyrosine; FGF19, fibroblast growth factor 19; TJ proteins, tight-junction proteins.

can increase glucose uptake and inhibit mitochondrial oxidative phosphorylation in enterocytes then accelerate glucose utilization through glycolysis and overproduction of lactate, the reason why metformin might contribute to gastrointestinal intolerance in a minority of people (71, 101, 102). Previous studies also hint that overproduction of lactate might also be microbially mediated (71, 103). Therefore, the potential mechanisms and contradiction of gastrointestinal intolerance and gut microbiota-related benefits need further investigation.

SGLT2 Inhibitors

Sodium-glucose cotransporter 2 (SGLT2) inhibitors improve glycemic control by increasing renal glucose excretion, accompanied by pleiotropic non-glycemic properties, such as reductions in body weight and cardiovascular and renal protection effects (104–107). However, the underlying mechanism of the pleiotropic benefits was still not clear. Evidence shows that the protective effect might be explained for increased ketone body production in CVD, a clear fuel to improve the cardiac function of the energy-starved myocardium (108). As an orally ingested antidiabetic agent, experimental animal studies have found that SGLT2 inhibitor intervention slightly altered the microbiota composition in experimental animal studies (109–111) (summarized in **Table 2**).

Dapagliflozin treatment showed minor beneficial alterations of gut microbiota in T2DM mice, a trend for decreased *Oscillospira* spp. and Firmicutes/Bacteroidetes ratios and increased *Akkermansia muciniphila* in the treatment group (109). In the butyrate-supplemented diet-fed db/db mice, the dapagliflozin-treated mice were also characterized by a decreased trend in Firmicutes/Bacteroidetes ratios, as well as a decreased trend in *Adlercreutzia* spp. and *Alistipes* spp. and an increased trend in *Streptococcus* spp (111).. In addition to slight alterations in gut microbiota, SGLT2 inhibitor intervention significantly improved intestinal SCFA production in animal models (110, 113). However, the results were inconsistent, and dapagliflozin treatment was found to have no beneficial effects on gut bacteria in diabetic rats (112). Only two clinical studies explored the alteration of fecal microbiome with SGLT2 inhibitor treatment (87, 88). Seventy-six treatment-naive T2DM with risk factors for CVD were included in a randomized, open-label, two-arm clinical trial (88). After a 3-month intervention, empagliflozin improved glucose metabolism and reduced CVD-related risks, while it significantly altered the gut microbiota, including an increase in SCFA-producing bacteria and a reduction in several harmful bacteria such as *Escherichia–Shigella*, *Bilophila*, and *Hungatella* (88). However, another clinical study found no significant effect on microbial alpha diversity or composition

TABLE 2 | Experimental animal studies analyzing the effects of SGLT2 inhibitors on gut microbiota.

Anti-diabetic drugs	Animal model	Dose	Duration	Key results	Mechanism of action
Dapagliflozin (109)	C57BLKS/J-lepr^{db}/lepr^{db}	60 mg/kg diet	8 weeks	Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia altered *Oscillospira*, Firmicutes/Bacteroidetes ratios↓	Vascular function improvements effects not conclusively mediated by gut microbiota
Dapagliflozin (111)	Butyrate-supplemented db/db mice	1 mg/kg/day	6 weeks	*Streptococcus* spp.↑ *Adlercreutzia* spp. and *Alistipes* spp., Firmicutes/Bacteroidetes ratios↓	No big difference in the microbiota composition with Dapagliflozin intervention
Dapagliflozin (112)	STZ-induced HFD-fed Sprague Dawley rats	1 mg/kg/day	4 weeks	no effects on beneficial bacteria Proteobacteria (especially *Desulfovibrionaceae*)↑	No effects on beneficial bacteria
Dapagliflozin (110)	MafA-deficient mice	1 mg/kg/day	6 weeks	*Blautia*↑ *Clostridium perfringens, enterococci, Enterobacteriaceae*, and *intestinal enterococci*↓ Intestinal SCFAs↑	Regulated the intestinal microecological balance of the body and promoted blood glucose and energy homeostasis.
Canagliflozin (113)	CE-2 diet-induced mice	10 mg/kg/day	2 weeks	Actinobacteria, *Oscillospira*↓ Cecal SCFAs↑	Increased bacterial carbohydrate fermentation; Reduced the accumulation of uremic toxins including p-cresyl sulfate

STZ, streptozocin; HFD, high-fat diet; SCFA, short-chain fatty acids.

(87). It might be due to the fact that all of the subjects included had already been treated with metformin, which might have overshadowed the possible impact of dapagliflozin on the gut microbiome (87). Experimental studies found that dapagliflozin increased the abundance of *Desulfovibrionaceae*, which was increased in the fecal microbiota of animal models with metabolic disorders (114, 115), while metformin reduced *Desulfovibrionaceae*, suggesting that the combination drug therapy of dapagliflozin and metformin might have complementary actions on the gut microbiota in diabetes (112). Given all this, the pleiotropic beneficial effects of the SGLT2 inhibitor might be slightly mediated by gut microbiota or not be mediated by gut microbiota, and the potential mechanism of the pleiotropic beneficial effects of SGLT2 inhibitors need to be further uncovered (116).

Thiazolidinedione Insulin Sensitizers

Thiazolidinedione (TZD) drugs are effective oral agents for T2DM in improving insulin sensitivity (117). TZDs are ligands of peroxisome proliferator-activated receptor gamma (PPAR-γ), leading to the activation of various pathways related to glycemic homeostasis and lipid metabolism (117, 118). The expression of PPAR-γ is abundant in the intestinal tract; thus, it is possible that PPAR-γ agonists straightly impact on gut microbiome homeostasis to improve energy metabolism (119, 120). However, only a few experimental animal studies explored whether TZD treatment can modify gut microbiota homeostasis (119, 121, 122). In a high-fructose-fed mouse model, pioglitazone partly altered gut microbiota and relieved the intestinal inflammation and epithelial barrier impairment, such as preventing the increment of the pathogenic bacteria *Deferribacteraceae* (*Mucispirillum*) (121). In diabetic mice, treatment with rosiglitazone promoted insulin sensitivity without modifying the composition of gut flora but improved the gene expression related to lipid and carbohydrate metabolism as well as immune regulation in the ileum and colon (119). Another experimental study discovered that microbial metabolites, for example, hippurate and indole-3-ethanol, were decreased by pioglitazone intervention in iNOS knockout mice (122). These experiment research suggested that TZDs might have mild protective effects on gut microbiota, mainly focused on lipid and carbohydrate metabolism and inflammation. However, no clinical study focused on gut microbiota and microbial metabolites alterations with TZDs treatment in T2DM subjects; further research is still needed.

Dipeptidyl Peptidase-4 Inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitors inhibit the degradation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide to stimulate insulin secretion, reserve β-cell function, and maintain glucose homeostasis (123). A series of experimental studies have shown that DPP-4 inhibitors might be able to improve energy metabolism through shaping the gut microbial composition and increasing fecal SCFAs (124–127) (summarized in **Table 3**). In high-fat diet-induced obesity mice, DPP-4 inhibitors exerted an important impact on gut microbial composition and fecal metabolites, particularly the increased abundance of Bacteroidetes (124). Researchers then transplanted the fecal microbiota of DPP-4 inhibitor-treated patients to germ-free mice and observed an improved glucose intolerance (124). Compared with that in GLP-1 receptor agonist liraglutide-treated mice, the gut microbiota differed substantially in mice treated with DPP-4 inhibitors, indicating that the hypoglycemic mechanism of DPP-4 inhibitors on gut microbiota is at least not primarily by GLP-1 and the other potential benefit of DPP-4 inhibitors needs further research (124, 128). In addition to increment of SCFA-producing flora, DPP-4 inhibitors were found to reduce Toll-like receptor ligands and improve the production of antimicrobial peptides, exerting immunomodulatory and anti-inflammatory effects and maintaining intestinal homeostasis in obese mice, as well as

TABLE 3 | Experimental animal studies analyzing the effects of DPP-4 inhibitors on gut microbiota.

Anti diabetic drugs	Animal model	Dose	Duration	Key results	Mechanism of action
DPP-4 inhibitor (124)	HFD-fed C57BL/6	300 mg/kg/day of saxagliptin or 4 g/kg of sitagliptin	4 weeks	The changes of 68.6% genera induced by HFD were rescued by the DPP-4 inhibitor. Bacteroidetes↑ Firmicutes↓ Bacteroidales S24–7 group, Bacteroidaceae, Ruminococcaceae, Desulfovibrionaceae and Streptococcaceae↓ Fecal SCFAs (especially succinate) ↑	Increasing the production of succinate contributed to the hypoglycemic effect of DPP-4 inhibitor
DPP-4 inhibitors (125)	HFD-fed C57BL/6	15 mg/kg/day	12 weeks	Firmicutes/Bacteroidetes ratios↓ Ruminococcus, Dorea, Verrucomicrobia↑ Plasma sphingomyelin, phosphatidylcholine and lysophosphatidylcholine entities↓	Elevated levels of butyrate-producing flora Reduced levels of certain plasma sphingomyelin, phosphatidylcholine and lysophosphatidylcholine entities
Vildagliptin (127)	WD-fed C57BL/6	50 mg/kg/day	8 weeks	*Oscillibacter* spp., Ruminococcaceae↓ *Lactobacillus* spp.↑ Cecal propionate↑ Cecal TLR ligands↓	Promoted antimicrobial peptide production and increased crypt depth in the ileum Indirectly reduced the expression of proinflammatory cytokines in the liver
Sitagliptin (94)	Zucker diabetic fatty rats	10.76 mg/kg/day	4 weeks	*Lactobacillus* spp.↑Firmicutes↑ Firmicutes to Bacteroidetes ratios↑	Selectively increased the beneficial flora
Saxagliptin (128)	STZ-induced ApoE-/- C57BL/6 mice	80 mg/kg/day	8 weeks	No significant effect on microbial composition	No significant effect on microbial composition
Linagliptin (126)	HFRU-fed C57BL/6 mice	15 mg/kg/day	5 weeks	*Bacteroidetes* spp.↑ *Proteobacteria* spp.↓ *Zo-1* mRNA, *Mucin* mRNA↑	Attenuated hepatic steatosis by gut-liver axis modulation
Vildagliptin (129)	STZ-induced diabetic Sprague-Dawley rats	20 mg/kg/day	12 weeks	Firmicutes/Bacteroidetes ratios↓ *Baceroides* and *Erysipelotrichaeae*↑	Increased SCFAs production
Sitagliptin (130)	HF/HC-STZ Sprague-Dawley rat	10 mg/kg/day	12 weeks	Firmicutes↓ Bacteroidetes, Tenericutes↑	Increased SCFAs-producing bacteria and probiotic

STZ, streptozocin; HFD, high-fat diet; WD, Western diet; HFRU, high-fructose diet; HF/HC, high fat or high carbohydrate; SCFA, short-chain fatty acids; TLR, Toll-like receptors.

cross talk with the liver and the whole-host health (126, 127). Some studies exhibited a decreased trend in the Firmicutes/Bacteroidetes ratio with treatment of DPP-4 inhibitors (124, 125, 127), while one experimental animal study found an enlarged abundance of Firmicutes and increased ratios of Firmicutes/Bacteroidetes (94). Although the relation between metabolic disorders and the Firmicutes/Bacteroidetes ratio is currently contradictory, more literatures considered it as a characteristic of obesity and T2DM (55).

There existed a few clinical studies that explored the gut flora modifying the effect of DPP-4 inhibitors (89–91). However, in a clinical study which included 51 T2DM patients, the advantageous effect of sitagliptin on glucose control, weight loss, and BA metabolism was not related to alterations in the gut microbiota (89, 90). No significant effect on microbial composition was found, which is possibly due to the fact that these subjects previously used metformin or sulphonylureas as hypoglycemic therapies, and it might have covered the possible effects of DPP-4 inhibitors (89, 124). Another clinical study which included 90 T2DM subjects found that both vildagliptin and saxagliptin altered the composition of gut microbiota, respectively (91). Thus, the microbiota-shaping effects of DPP-4 inhibitors in clinical studies and its additional hypoglycemic mechanism need further investigation.

α-Glucosidase Inhibitors

α-Glucosidase inhibitors are antidiabetic drugs, including acarbose, miglitol, and voglibose, which delay the absorption of carbohydrates in the intestinal tract to inhibit the rise in postprandial plasma glucose concentration (131). α-Glucosidase inhibitors are inhibitors of both human and bacterial α-glucosidases, and because of its high intestinal drug concentration, α-glucosidase usually has noticeable impacts on the intestinal flora (132, 133). Large amounts of research revealed that α-glucosidase inhibitors could shape the composition of the gut microbiome in both animal studies and clinical studies (62, 92–94, 134). Evidence shows that acarbose modulated the gut microbiota and corresponding shaped fecal and plasma BA composition, which may improve host energy metabolism (62, 135). A clinical study which recruited 51 treatment-naive T2DM patients showed that a three-mouth treatment with acarbose increased *Lactobacillus* and *Bifidobacterium* abundances and reduced *Bacteroides* abundances, along with altered plasm BA pool composition (62). Another clinical study which included 95 T2DM patients found that acarbose treatment improved the abundance of *Enterococcus faecalis* and *Bifidobacterium longum*, along with the reduction of plasma inflammatory factors, such as prothrombin activator inhibitor-1 and LPS levels (93). As summarized in **Table 4**, intervention with α-glucosidase inhibitors in experimental animal studies also confirmed significant impacts on gut microbiota and relevant metabolites. In addition to their glucose-lowering and energy metabolism-improving effects, α-glucosidase inhibitors were found to reverse joint inflammation on collagen-induced arthritis mice and the underlying mechanism might be due to the alteration of host–commensal interactions, which have been confirmed to be

TABLE 4 | Experimental animal studies analyzing the effects of α-glucosidase inhibitors on gut microbiota.

Anti-diabetic drugs	Animal model	Dose	Duration	Key results	Mechanism of action
Acarbose (94)	Zucker diabetic fatty rats	32.27 mg/kg/day	4 weeks	Actinobacteria↑ *Bifidobacterium, Ruminococcus 2, Lactobacillus intestinalis*↑ Metagenomic functional prediction: elevated carbohydrate transport and metabolism.	Selectively increased the beneficial flora
Acarbose (134)	Old mice	1,000 ppm	8 months	*Muribaculaceae*↑ SCFA↑	Modulated the fermentation products of the gut flora
Acarbose (136)	HS or PP-fed mice	400 ppm	28 days	Diet-dependent gut community structure alteration and SCFA increasing	Increased SCFA production
Acarbose (137)	STZ-induced HFHSD-fed SD rats	30 mg/kg/day	7 weeks	*Escherichia-Shigella*↓ *Muribaculaceae, Lachnospiraceae, Bifidobacterium, Ruminococcaceae_UCG-014, Ruminococcus_1, Romboutsia, Eggerthellaceae, Alistipes, Faecalibaculum, Ruminococcaceae_UCG-013* and *Peptococcaceae*↑	Beneficial composition of gut microbiota restored
Acarbose or miglitol (138)	Collagen-induced arthritis mice	500 mg/kg/day	55 days	Firmicutes↑*Oscillospira* spp., *Desulfovibrio* spp. and *Ruminococcus* spp.↑ *Lactobacillus* spp., *Anaeroplasman* spp., *Adlercreutzia* spp., and *RF39* spp.↓	Regulated immunity *via* Th17/Treg cells in the intestinal lamina propria
Voglibose (135)	HFD-fed C57BL/6 mice	1 mg/kg/day	12 weeks	the ratio of Firmicutes to Bacteroidetes↓ Plasm taurocholic, cholic acid and deoxycholic acid↑	Downregulated gene expression of CYP8B1 and HNF4α Upregulated gene expression of PGC1α
Miglitol (139)	HFHSD-fed rats	0.04% miglitol plus in diet	12 weeks	*Erysipelotrichaceae* and *Coriobacteriaceae*↓ Plasm LPS↓	Reduced LPS levels in portal plasma
Miglitol (140)	ChREBP-knockout mice	0.08% miglitol plus in diet	8 weeks	Lactobacillales and *Bifidobacterium*↑ clostridium cluster XIVa↓ Fecal lactate↑	Increased cecal lactate contents and altered intestinal flora

STZ, streptozocin; HFD, high-fat diet; HS, high-starch; PP, plant polysaccharides; HFHSD, high-fat, high-sucrose diet; SCFAs, short-chain fatty acids; HNF4α, hepatocyte nuclear factor 4alpha; PGC1α, peroxisome proliferator-activated receptor-γ co-activator-1α; LPS, lipopolysaccharide.

correlated with rheumatoid arthritis, such as several butyrate-producing species, *Lactobacillus* spp. and *Oscillospira* spp (48, 138, 141).. These results suggested a promising prospective of α-glucosidase inhibitors due to its potential antiarthritis effect mediated by the gut microbiome (134, 138).

In fact, over 95% of the acarbose dose was not absorbed in the gut, coupled with its feature to inhibit microbial α-glucosidases, and subjects' treatment response to acarbose is dependent on several factors, such as dietary intake, genetic factor, and microbiota composition before treatment (also named enterotypes) (62, 91–93, 136, 142, 143). The acarbose-shaped gut microbial composition might be related to the dietary intake in a small Japanese population with T2DM (92). Moreover, hierarchical clustering showed that the habitual dietary intake of sucrose, fat, and carbohydrate was associated with three distinct microbial clusters, and even the abundance alteration of *Faecalibacterium* was positively related to dietary rice intake but negatively related to bread intake (92). A previous study also found that patients with a gut flora driven by *Bacteroides* displayed more beneficial modifications in gut microbiota, plasma BA composition, and more metabolic metabolism enhancement after acarbose treatment than those with *Prevotella* (62). In addition, researchers revealed that acarbose resistance has spread in certain host gut microbiomes, which contributed an emerging layer to the multifaceted network of carbohydrate-mediated cross talk among various human microbiomes (132, 144). Besides, in antibiotic pretreatment mice, whose gut microbial enzyme activities have been weakened, the metabolism of voglibose was reduced and more significantly glucose-lowering effects were presented (143). In brief, from currently clinical and experimental studies, α-glucosidase inhibitors have obvious effects on gut microbiota and its effects significantly depend on host diet and the original composition of the gut microbiome.

Other Oral Glucose-Lowering Agents

Other less researched oral antidiabetes medications, such as sulfonylurea and glinide insulin secretagogues, have been noticed to cross talk with probiotic bacteria or microbial metabolic profiles (145–147). Nevertheless, two clinical studies which were designed to assess the effects of sulfonylureas on gut microbiota in T2DM subjects found no beneficial impacts on gut microbiota composition even in treatment-naive subjects, but with enhanced glycemic control (62, 87). At the same time, acarbose showed beneficial effects on the composition of the gut microbiome, suggesting that the detected metabolic modifications of sulfonylureas might not be intermediated by their impacts on the gut microbiota (62, 87). Recently, a few newly invented oral anti-glucose agents were discovered and used in clinical application, such as chiglitazar and imeglimin (148, 149). Activating as a peroxisome proliferator-activated receptor pan-agonist for glucose control, chiglitazar was found to improve insulin sensitivity and lipid homeostasis and reduce circulating levels of inflammatory parameters (150, 151).

Imeglimin was confirmed to have the effects of modulating mitochondrial bioenergetics, enhancing mitochondrial function, improving insulin sensitivity, and preserving β-cell function (152–154). However, the associations of these anti-glucose agents and gut microbiota composition were still lacking.

In addition, newly identified exciting targets, including glucokinase activators and G-protein-coupled receptor 40 agonists, have also been researched, although not clinically usable (155, 156). Therefore, with the development of novel glucose-lowering agents, further research is still needed to uncover the complex interaction among gut microbiota, glucose-lowering agents, and the microbial-host metabolic cross talk.

CONCLUSIONS AND FUTURE PROSPECTS

Antidiabetic agents modify the gut flora and thereby alter gastrointestinal and plasma metabolite profiles, further improving metabolic health. Knowledge and studies so far indicate that oral antidiabetes drugs, including metformin, DPP-4 inhibitors, and α-glucosidase inhibitors, have obvious effects on gut microbiota and microbial metabolites, while SGLT2 inhibitors and TZDs have slighter effects (62, 77, 87, 89). Even if the definite microbial signatures linked to certain antidiabetic agents have not been discovered yet, understanding how antidiabetes drugs influence the gut microbiome might be vital for identifying their potential mechanisms and optimizing their treatment. Although different hypoglycemic drugs shape gut microbiota differently, they have been confirmed to have some similar effects in regulating microbiota and metabolites. Among various microbiota and metabolites derived from gut flora, metformin, SGLT2 inhibitors, DPP-4 inhibitors, and α-glucosidase inhibitors have been demonstrated to have similar effects on increased SCFA-producing bacteria and SCFA production, which may partly explain their beneficial effects in the regulation of insulin sensitivity enhancement, energy metabolism, and systemic inflammation (77, 78, 110, 124, 134). Notably, among various SCFA-producing bacteria, *Akkermansia muciniphila* has been proven increased particularly during the metformin treatment in both clinical and experimental studies, which also related to healthy intestinal mucosa and anti-inflammatory action (79, 84, 96, 99). In addition, alteration of the BA pool was commonly displayed in both metformin and α-glucosidase inhibitors, corresponding with decreased *Bacteroides fragilis* in metformin-treated individuals and increased *Lactobacillus* and *Bifidobacterium* abundances and reduced *Bacteroides* abundances in α-glucosidase inhibitor-treated individuals, respectively (62, 76). In addition, reduction of opportunistic pathogen and attenuated intestinal inflammation could be seen in intervention research on metformin, DPP-4 inhibitors, and α-glucosidase inhibitors (77, 126, 138). Therefore, manipulation of gut microflora composition could be a potential and promising target to improve metabolic outcomes in subjects with T2DM. The microbiota–host cross talk might convey novel and potential ideas of generally used oral glucose-lowering drugs.

Firstly, combination therapy might have additional benefits, due to the fact that different antidiabetes drugs shape gut microbiota with distinct effects (62, 94, 112), For example, dapagliflozin increased the abundance of *Desulfovibrionaceae* in a T2DM rat model, which is an unfriendly sulfate-reducing bacteria in the gut, while metformin reduced it on the contrary, revealing a rationality and complementary action of combined pharmacotherapy between dapagliflozin and metformin (112). However, the definite combination effects of metformin and SGLT2 inhibitors need further investigation. T2DM is a chronic disease with progressive features and possible complex complications; a satisfactory treatment effect is hard to achieve with monotherapy. Besides metformin and SGLT2 inhibitor combined treatment, combination therapies, such as metformin with pioglitazone or metformin with DPP-4 inhibitors, might exhibit a synergetic role in gut microbiome benefits (157, 158). Further investigations in both experimental and clinical are needed to figure out the combined pharmacotherapy effects on gut microbiota.

Secondly, pre- and probiotics could be a promising treatment for T2DM in the modulation of gut microbiota (159). For example, *Actinoplanes* spp. and *Lactobacillus* spp. have been definitively demonstrated to effectively inhibit the alpha-glucosidase activity to reduce glucose levels (160, 161). The combination of hypoglycemic agents and certain probiotics or prebiotics may further enhance the glucose-lowering effects (82, 162). Prebiotics, such as inulin and galacto-oligosaccharide, could be fermented by the gut flora, leading to modulation of intestinal microbiota and the production of various microbial metabolites including SCFAs (163–165). Besides, evidence shows that combination of metformin and gastrointestinal microbiome modulator (consisting of inulin, beta-glucan, and polyphenols) treatment significantly relieved metformin tolerance than the placebo combination (166). Notably, for patients with pregestational diabetes and gestational diabetes mellitus (GDM), the dominating pharmacotherapy is insulin, while only metformin and glyburide are used in some countries (167, 168). Other oral hypoglycemic agents are limited in these patients. Hyperglycemia during pregnancy is associated with significantly increased maternal and fetal metabolic disturbance and morbidity (167). Therefore, dietary modification and physical activity are particularly important for glycemia control (167). A systematic review and meta-analysis revealed that probiotic supplementation in GDM could significantly reduce homeostasis model assessment of the insulin resistance index with no adverse effects reported (169). Evidence shows that inulin-type fructan supplementary improved glucose and lipid metabolism in HFD-induced GDM mouse models associated with gut flora modification (170). Our research team found that maternal inulin treatment improved glucose metabolism in adult male offspring *via* regulation of the hepatic long non-coding RNA profile (164). However, results are inconsistent showing that probiotics, including *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subspecies *lactis*, did not prevent GDM in overweight and obese pregnant women (171). Thus, more clinical studies are needed to verify these results and explore the ideal bacterial composition of pre- and probiotics that might positively alter glucose metabolism in GDM or pregestational diabetes.

Thirdly, FMT from normal glucose tolerance or antidiabetes treatment subjects to mice revealed a significant improvement in gut microbiota composition, glucose homeostasis, and metabolic

health (17, 124, 172). Despite that this promising treatment was still in its infancy (173–175), FMT combined with antidiabetes drugs might bring novel interventions and perspectives in T2DM management. The effects and mechanisms underneath these potential treatment schedules are still unclear, and it is vital to further develop meaningful and applicable interventions combined with intestinal microbiota in the future study.

AUTHOR CONTRIBUTIONS

DW: writing—original draft preparation. JL, LZ, QZ, ML, and XX: writing—review and editing. XX: supervision. XX and QZ: funding acquisition. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, Regional and Country-Level Diabetes Prevalence Estimates for 2021 and Projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119
2. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 Diabetes: Principles of Pathogenesis and Therapy. *Lancet* (2005) 365(9467):1333–46. doi: 10.1016/s0140-6736(05)61032-x
3. Gregg EW, Sattar N, Ali MK. The Changing Face of Diabetes Complications. *Lancet Diabetes Endocrinol* (2016) 4(6):537–47. doi: 10.1016/s2213-8587(16)30010-9
4. Ferguson D, Finck BN. Emerging Therapeutic Approaches for the Treatment of NAFLD and Type 2 Diabetes Mellitus. *Nat Rev Endocrinol* (2021) 17(8):484–95. doi: 10.1038/s41574-021-00507-z
5. Draznin B, Aroda VR, Bakris G, Benson G, Brown FM, Freeman R, et al.9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2021. *Diabetes Care* (2021) 44(Suppl 1):S111–s124. doi: 10.2337/dc21-S009
6. Perreault L, Skyler JS, Rosenstock J. Novel Therapies With Precision Mechanisms for Type 2 Diabetes Mellitus. *Nat Rev Endocrinol* (2021) 17(6):364–77. doi: 10.1038/s41574-021-00489-y
7. Bordalo Tonucci L, Dos Santos KM, De Luces Fortes Ferreira CL, Ribeiro SM, De Oliveira LL, Martino HS. Gut Microbiota and Probiotics: Focus on Diabetes Mellitus. *Crit Rev Food Sci Nutr* (2017) 57(11):2296–309. doi: 10.1080/10408398.2014.934438
8. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of Gut Microbiota in Type 2 Diabetes Pathophysiology. *EBioMedicine* (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051
9. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human Microbiome Project. *Nature* (2007) 449(7164):804–10. doi: 10.1038/nature06244
10. Canfora EE, Meex RCR, Venema K, Blaak EE. Gut Microbial Metabolites in Obesity, NAFLD and T2DM. *Nat Rev Endocrinol* (2019) 15(5):261–73. doi: 10.1038/s41574-019-0156-z
11. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, Stability and Resilience of the Human Gut Microbiota. *Nature* (2012) 489(7415):220–30. doi: 10.1038/nature11550
12. Sommer F, Bäckhed F. The Gut Microbiota–Masters of Host Development and Physiology. *Nat Rev Microbiol* (2013) 11(4):227–38. doi: 10.1038/nrmicro2974
13. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the Normal Gut Microbiota. *World J Gastroenterol* (2015) 21(29):8787–803. doi: 10.3748/wjg.v21.i29.8787
14. Tilg H, Moschen AR. Microbiota and Diabetes: An Evolving Relationship. *Gut* (2014) 63(9):1513–21. doi: 10.1136/gutjnl-2014-306928
15. Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, et al. Gut Bacteria Selectively Promoted by Dietary Fibers Alleviate Type 2 Diabetes. *Science* (2018) 359(6380):1151–6. doi: 10.1126/science.aao5774
16. Jia J, Dou P, Gao M, Kong X, Li C, Liu Z, et al. Assessment of Causal Direction Between Gut Microbiota-Dependent Metabolites and Cardiometabolic Health: A Bidirectional Mendelian Randomization Analysis. *Diabetes* (2019) 68(9):1747–55. doi: 10.2337/db19-0153
17. Zhang PP, Li LL, Han X, Li QW, Zhang XH, Liu JJ, et al. Fecal Microbiota Transplantation Improves Metabolism and Gut Microbiome Composition in Db/Db Mice. *Acta Pharmacol Sin* (2020) 41(5):678–85. doi: 10.1038/s41401-019-0330-9
18. Vangipurapu J, Fernandes Silva L, Kuulasmaa T, Smith U, Laakso M. Microbiota-Related Metabolites and the Risk of Type 2 Diabetes. *Diabetes Care* (2020) 43(6):1319–25. doi: 10.2337/dc19-2533
19. Hu N, Zhang Q, Wang H, Yang X, Jiang Y, Chen R, et al. Comparative Evaluation of the Effect of Metformin and Insulin on Gut Microbiota and Metabolome Profiles of Type 2 Diabetic Rats Induced by the Combination of Streptozotocin and High-Fat Diet. *Front Pharmacol* (2021) 12:794103. doi: 10.3389/fphar.2021.794103
20. Ng SC, Xu Z, Mak JWY, Yang K, Liu Q, Zuo T, et al. Microbiota Engraftment After Faecal Microbiota Transplantation in Obese Subjects With Type 2 Diabetes: A 24-Week, Double-Blind, Randomised Controlled Trial. *Gut* (2022) 71(4):716–23. doi: 10.1136/gutjnl-2020-323617
21. Hartstra AV, Bouter KE, Bäckhed F, Nieuwdorp M. Insights Into the Role of the Microbiome in Obesity and Type 2 Diabetes. *Diabetes Care* (2015) 38(1):159–65. doi: 10.2337/dc14-0769
22. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A Metagenome-Wide Association Study of Gut Microbiota in Type 2 Diabetes. *Nature* (2012) 490(7418):55–60. doi: 10.1038/nature11450
23. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut Metagenome in European Women With Normal, Impaired and Diabetic Glucose Control. *Nature* (2013) 498(7452):99–103. doi: 10.1038/nature12198
24. Medina-Vera I, Sanchez-Tapia M, Noriega-López L, Granados-Portillo O, Guevara-Cruz M, Flores-López A, et al. A Dietary Intervention With Functional Foods Reduces Metabolic Endotoxaemia and Attenuates Biochemical Abnormalities by Modifying Faecal Microbiota in People With Type 2 Diabetes. *Diabetes Metab* (2019) 45(2):122–31. doi: 10.1016/j.diabet.2018.09.004
25. Org E, Blum Y, Kasela S, Mehrabian M, Kuusisto J, Kangas AJ, et al. Relationships Between Gut Microbiota, Plasma Metabolites, and Metabolic Syndrome Traits in the METSIM Cohort. *Genome Biol* (2017) 18(1):70. doi: 10.1186/s13059-017-1194-2
26. Li SC, Xiao Y, Wu RT, Xie D, Zhao HH, Shen GY, et al. Comparative Analysis of Type 2 Diabetes-Associated Gut Microbiota Between Han and Mongolian People. *J Microbiol* (2021) 59(7):693–701. doi: 10.1007/s12275-021-0454-8
27. Wu H, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, et al. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based Cross-Sectional Study. *Cell Metab* (2020) 32(3):379–390.e3. doi: 10.1016/j.cmet.2020.06.011
28. Gou W, Ling CW, He Y, Jiang Z, Fu Y, Xu F, et al. Interpretable Machine Learning Framework Reveals Robust Gut Microbiome Features Associated

With Type 2 Diabetes. *Diabetes Care* (2021) 44(2):358–66. doi: 10.2337/dc20-1536
29. Agus A, Clément K, Sokol H. Gut Microbiota-Derived Metabolites as Central Regulators in Metabolic Disorders. *Gut* (2021) 70(6):1174–82. doi: 10.1136/gutjnl-2020-323071
30. Krautkramer KA, Fan J, Bäckhed F. Gut Microbial Metabolites as Multi-Kingdom Intermediates. *Nat Rev Microbiol* (2021) 19(2):77–94. doi: 10.1038/s41579-020-0438-4
31. Chen S, Henderson A, Petriello MC, Romano KA, Gearing M, Miao J, et al. Trimethylamine N-Oxide Binds and Activates PERK to Promote Metabolic Dysfunction. *Cell Metab* (2019) 30(6):1141–1151.e5. doi: 10.1016/j.cmet.2019.08.021
32. Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM, Reimann F. Bacterial Metabolite Indole Modulates Incretin Secretion From Intestinal Enteroendocrine L Cells. *Cell Rep* (2014) 9(4):1202–8. doi: 10.1016/j.celrep.2014.10.032
33. Galligan JJ. Beneficial Actions of Microbiota-Derived Tryptophan Metabolites. *Neurogastroenterol Motil* (2018) 30(2):e13283. doi: 10.1111/nmo.13283
34. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut Microbiota Metabolites and Risk of Major Adverse Cardiovascular Disease Events and Death: A Systematic Review and Meta-Analysis of Prospective Studies. *J Am Heart Assoc* (2017) 6(7):e004947. doi: 10.1161/jaha.116.004947
35. Saad MJ, Santos A, Prada PO. Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance. *Physiol (Bethesda)* (2016) 31(4):283–93. doi: 10.1152/physiol.00041.2015
36. Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, et al. Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-Like Receptor 4. *Immunity* (2014) 41(2):296–310. doi: 10.1016/j.immuni.2014.06.014
37. Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, et al. Intestinal Microbiota-Derived Short-Chain Fatty Acids Regulation of Immune Cell IL-22 Production and Gut Immunity. *Nat Commun* (2020) 11(1):4457. doi: 10.1038/s41467-020-18262-6
38. Ratajczak W, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczyńska M. Immunomodulatory Potential of Gut Microbiome-Derived Short-Chain Fatty Acids (SCFAs). *Acta Biochim Pol* (2019) 66(1):1–12. doi: 10.18388/abp.2018_2648
39. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, et al. The Gut Microbiota Suppresses Insulin-Mediated Fat Accumulation via the Short-Chain Fatty Acid Receptor GPR43. *Nat Commun* (2013) 4:1829. doi: 10.1038/ncomms2852
40. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia, and Nonalcoholic Fatty Liver Disease. *Gastroenterology* (2017) 152(7):1679–1694.e3. doi: 10.1053/j.gastro.2017.01.055
41. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The Role of Short-Chain Fatty Acids in Microbiota-Gut-Brain Communication. *Nat Rev Gastroenterol Hepatol* (2019) 16(8):461–78. doi: 10.1038/s41575-019-0157-3
42. Zhang Y, Peng Y, Zhao L, Zhou G, Li X. Regulating the Gut Microbiota and SCFAs in the Faeces of T2DM Rats Should be One of Antidiabetic Mechanisms of Mogrosides in the Fruits of Siraitia Grosvenorii. *J Ethnopharmacol* (2021) 274:114033. doi: 10.1016/j.jep.2021.114033
43. Yamaguchi Y, Adachi K, Sugiyama T, Shimozato A, Ebi M, Ogasawara N, et al. Association of Intestinal Microbiota With Metabolic Markers and Dietary Habits in Patients With Type 2 Diabetes. *Digestion* (2016) 94(2):66–72. doi: 10.1159/000447690
44. Ballan R, Saad SMI. Characteristics of the Gut Microbiota and Potential Effects of Probiotic Supplements in Individuals With Type 2 Diabetes Mellitus. *Foods* (2021) 10(11):2528. doi: 10.3390/foods10112528
45. Mueller NT, Differding MK, Zhang M, Maruthur NM, Juraschek SP, Miller ER, et al. Metformin Affects Gut Microbiome Composition and Function and Circulating Short-Chain Fatty Acids: A Randomized Trial. *Diabetes Care* (2021) 44(7):1462–71. doi: 10.2337/dc20-2257
46. Martin-Gallausiaux C, Marinelli L, Blottière HM, Larraufie P, Lapaque N. SCFA: Mechanisms and Functional Importance in the Gut. *Proc Nutr Soc* (2021) 80(1):37–49. doi: 10.1017/s0029665120006916
47. Yao Y, Yan L, Chen H, Wu N, Wang W, Wang D. Cyclocarya Paliurus Polysaccharides Alleviate Type 2 Diabetic Symptoms by Modulating Gut Microbiota and Short-Chain Fatty Acids. *Phytomedicine* (2020) 77:153268. doi: 10.1016/j.phymed.2020.153268
48. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites Produced by Commensal Bacteria Promote Peripheral Regulatory T-Cell Generation. *Nature* (2013) 504(7480):451–5. doi: 10.1038/nature12726
49. Simpson BW, Trent MS. Pushing the Envelope: LPS Modifications and Their Consequences. *Nat Rev Microbiol* (2019) 17(7):403–16. doi: 10.1038/s41579-019-0201-x
50. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut Microbiota in Human Adults With Type 2 Diabetes Differs From non-Diabetic Adults. *PloS One* (2010) 5(2):e9085. doi: 10.1371/journal.pone.0009085
51. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial Ecology: Human Gut Microbes Associated With Obesity. *Nature* (2006) 444(7122):1022–3. doi: 10.1038/4441022a
52. Denou E, Marcinko K, Surette MG, Steinberg GR, Schertzer JD. High-Intensity Exercise Training Increases the Diversity and Metabolic Capacity of the Mouse Distal Gut Microbiota During Diet-Induced Obesity. *Am J Physiol Endocrinol Metab* (2016) 310(11):E982–93. doi: 10.1152/ajpendo.00537.2015
53. Huang L, Thonusin C, Chattipakorn N, Chattipakorn SC. Impacts of Gut Microbiota on Gestational Diabetes Mellitus: A Comprehensive Review. *Eur J Nutr* (2021) 60(5):2343–60. doi: 10.1007/s00394-021-02483-6
54. Chen B, Wang Z, Wang J, Su X, Yang J, Zhang Q, et al. The Oral Microbiome Profile and Biomarker in Chinese Type 2 Diabetes Mellitus Patients. *Endocrine* (2020) 68(3):564–72. doi: 10.1007/s12020-020-02269-6
55. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, et al. The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients* (2020) 12(5):1474. doi: 10.3390/nu12051474
56. Gomes JMG, Costa JA, Alfenas RCG. Metabolic Endotoxemia and Diabetes Mellitus: A Systematic Review. *Metabolism* (2017) 68:133–44. doi: 10.1016/j.metabol.2016.12.009
57. Fang WY, Tseng YT, Lee TY, Fu YC, Chang WH, Lo WW, et al. Triptolide Prevents LPS-Induced Skeletal Muscle Atrophy via Inhibiting NF-κb/TNF-α and Regulating Protein Synthesis/Degradation Pathway. *Br J Pharmacol* (2021) 178(15):2998–3016. doi: 10.1111/bph.15472
58. Zhang H, Qi C, Zhao Y, Lu M, Li X, Zhou J, et al. Depletion of Gut Secretory Immunoglobulin A Coated Lactobacillus Reuteri is Associated With Gestational Diabetes Mellitus-Related Intestinal Mucosal Barrier Damage. *Food Funct* (2021) 12(21):10783–94. doi: 10.1039/d1fo02517a
59. Carvalho BM, Guadagnini D, Tsukumo DML, Schenka AA, Latuf-Filho P, Vassallo J, et al. Modulation of Gut Microbiota by Antibiotics Improves Insulin Signalling in High-Fat Fed Mice. *Diabetologia* (2012) 55(10):2823–34. doi: 10.1007/s00125-012-2648-4
60. Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, et al. Suppressed Hepatic Bile Acid Signalling Despite Elevated Production of Primary and Secondary Bile Acids in NAFLD. *Gut* (2018) 67(10):1881–91. doi: 10.1136/gutjnl-2017-314307
61. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-Mediated Bile Acid Sensing Controls Glucose Homeostasis. *Cell Metab* (2009) 10(3):167–77. doi: 10.1016/j.cmet.2009.08.001
62. Gu Y, Wang X, Li J, Zhang Y, Zhong H, Liu R, et al. Analyses of Gut Microbiota and Plasma Bile Acids Enable Stratification of Patients for Antidiabetic Treatment. *Nat Commun* (2017) 8(1):1785. doi: 10.1038/s41467-017-01682-2
63. Li W, Liu R, Li X, Tao B, Zhai N, Wang X, et al. Saxagliptin Alters Bile Acid Profiles and Yields Metabolic Benefits in Drug-Naïve Overweight or Obese Type 2 Diabetes Patient. *J Diabetes* (2019) 11(12):982–92. doi: 10.1111/1753-0407.12956
64. Haal S, Guman MSS, Boerlage TCC, Acherman YIZ, de Brauw LM, Bruin S, et al. Ursodeoxycholic Acid for the Prevention of Symptomatic Gallstone Disease After Bariatric Surgery (UPGRADE): A Multicentre, Double-Blind, Randomised, Placebo-Controlled Superiority Trial. *Lancet Gastroenterol Hepatol* (2021) 6(12):993–1001. doi: 10.1016/s2468-1253(21)00301-0

65. Velasquez MT, Ramezani A, Manal A, Raj DS. Trimethylamine N-Oxide: The Good, the Bad and the Unknown. *Toxins (Basel)* (2016) 8(11):326. doi: 10.3390/toxins8110326
66. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal Microbiota Composition Modulates Choline Bioavailability From Diet and Accumulation of the Proatherogenic Metabolite Trimethylamine-N-Oxide. *mBio* (2015) 6(2):e02481. doi: 10.1128/mBio.02481-14
67. Chen ML, Zhu XH, Ran L, Lang HD, Yi L, Mi MT. Trimethylamine-N-Oxide Induces Vascular Inflammation by Activating the NLRP3 Inflammasome Through the SIRT3-SOD2-mtROS Signaling Pathway. *J Am Heart Assoc* (2017) 6(9):e006347. doi: 10.1161/jaha.117.006347
68. Zhu Y, Zhao J, Luo L, Gao Y, Bao H, Li P, et al. Research Progress of Indole Compounds With Potential Antidiabetic Activity. *Eur J Med Chem* (2021) 223:113665. doi: 10.1016/j.ejmech.2021.113665
69. Abboud M, Rizk R, AlAnouti F, Papandreou D, Haidar S, Mahboub N. The Health Effects of Vitamin D and Probiotic Co-Supplementation: A Systematic Review of Randomized Controlled Trials. *Nutrients* (2020) 13 (1):111. doi: 10.3390/nu13010111
70. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human Gut Microbes Impact Host Serum Metabolome and Insulin Sensitivity. *Nature* (2016) 535(7612):376–81. doi: 10.1038/nature18646
71. He L. Metformin and Systemic Metabolism. *Trends Pharmacol Sci* (2020) 41 (11):868–81. doi: 10.1016/j.tips.2020.09.001
72. Lv Z, Guo Y. Metformin and Its Benefits for Various Diseases. *Front Endocrinol (Lausanne)* (2020) 11:191. doi: 10.3389/fendo.2020.00191
73. Wang Y, Xu W, Yan Z, Zhao W, Mi J, Li J, et al. Metformin Induces Autophagy and G0/G1 Phase Cell Cycle Arrest in Myeloma by Targeting the AMPK/mTORC1 and Mtorc2 Pathways. *J Exp Clin Cancer Res* (2018) 37 (1):63. doi: 10.1186/s13046-018-0731-5
74. Foretz M, Guigas B, Viollet B. Understanding the Glucoregulatory Mechanisms of Metformin in Type 2 Diabetes Mellitus. *Nat Rev Endocrinol* (2019) 15(10):569–89. doi: 10.1038/s41574-019-0242-2
75. Madiraju AK, Qiu Y, Perry RJ, Rahimi Y, Zhang XM, Zhang D, et al. Metformin Inhibits Gluconeogenesis via a Redox-Dependent Mechanism In Vivo. *Nat Med* (2018) 24(9):1384–94. doi: 10.1038/s41591-018-0125-4
76. 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(12):1919–29. doi: 10.1038/s41591-018-0222-4
77. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, et al. Disentangling Type 2 Diabetes and Metformin Treatment Signatures in the Human Gut Microbiota. *Nature* (2015) 528(7581):262–6. doi: 10.1038/nature15766
78. 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(3): e02392–17. doi: 10.1128/mBio.02392-17
79. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, 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(7):850–8. doi: 10.1038/nm.4345
80. Ahmadi S, Razazan A, Nagpal R, Jain S, Wang B, Mishra SP, et al. Metformin Reduces Aging-Related Leaky Gut and Improves Cognitive Function by Beneficially Modulating Gut Microbiome/Goblet Cell/Mucin Axis. *J Gerontol A Biol Sci Med Sci* (2020) 75(7):e9–e21. doi: 10.1093/gerona/glaa056
81. Huang F, Nilholm C, Roth B, Linninge C, Höglund 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(6):729–40. doi: 10.1080/09637486.2017.1408059
82. Palacios T, Vitetta L, Coulson S, Madigan CD, Lam YY, Manuel R, et al. Targeting the Intestinal Microbiota to Prevent Type 2 Diabetes and Enhance the Effect of Metformin on Glycaemia: A Randomised Controlled Pilot Study. *Nutrients* (2020) 12(7):2041. doi: 10.3390/nu12072041
83. 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(7):e100778. doi: 10.1371/journal.pone.0100778
84. de la Cuesta-Zuluaga J, Mueller NT, Corrales-Agudelo V, Velásquez-Mejía EP, Carmona JA, Abad JM, et al. Metformin Is Associated With Higher Relative Abundance of Mucin-Degrading Akkermansia Muciniphila and Several Short-Chain Fatty Acid-Producing Microbiota in the Gut. *Diabetes Care* (2017) 40(1):54–62. doi: 10.2337/dc16-1324
85. 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 (3):485–93. doi: 10.1007/s12020-019-02041-5
86. Barengolts E, Green SJ, Eisenberg Y, Akbar A, Reddivari B, Layden BT, et al. Gut microbiota varies by opioid use, circulating leptin and oxytocin in African American men with diabetes and high burden of chronic disease. *PloS one* (2018) 13(3):e0194171. doi: 10.1371/journal.pone.0194171
87. van Bommel EJM, Herrema H, Davids M, Kramer MHH, Nieuwdorp M, van Raalte DH. Effects of 12-Week Treatment With Dapagliflozin and Gliclazide on Faecal Microbiome: Results of a Double-Blind Randomized Trial in Patients With Type 2 Diabetes. *Diabetes Metab* (2020) 46(2):164–8. doi: 10.1016/j.diabet.2019.11.005
88. Deng X, Zhang C, Wang P, Wei W, Shi X, Wang P, et al. Cardiovascular Benefits of Empagliflozin are Associated With Gut Microbiota and Plasma Metabolites in Type 2 Diabetes. *J Clin Endocrinol Metab* (2022) 107 (7):1888–96. doi: 10.1210/clinem/dgac210
89. Smits MM, Fluitman KS, Herrema H, Davids M, Kramer MHH, Groen AK, et al. Liraglutide and Sitagliptin Have No Effect on Intestinal Microbiota Composition: A 12-Week Randomized Placebo-Controlled Trial in Adults With Type 2 Diabetes. *Diabetes Metab* (2021) 47(5):101223. doi: 10.1016/j.diabet.2021.101223
90. Smits MM, Tonneijck L, Muskiet MH, Hoekstra T, Kramer MH, Diamant M, et al. Biliary Effects of Liraglutide and Sitagliptin, a 12-Week Randomized Placebo-Controlled Trial in Type 2 Diabetes Patients. *Diabetes Obes Metab* (2016) 18(12):1217–25. doi: 10.1111/dom.12748
91. Wang Z, Wang J, Hu J, Chen Y, Dong B, Wang Y. A Comparative Study of Acarbose, Vildagliptin and Saxagliptin Intended for Better Efficacy and Safety on Type 2 Diabetes Mellitus Treatment. *Life Sci* (2021) 274:119069. doi: 10.1016/j.lfs.2021.119069
92. Takewaki F, Nakajima H, Takewaki D, Hashimoto Y, Majima S, Okada H, et al. Habitual Dietary Intake Affects the Altered Pattern of Gut Microbiome by Acarbose in Patients With Type 2 Diabetes. *Nutrients* (2021) 13(6):2107. doi: 10.3390/nu13062107
93. Su B, Liu H, Li J, Sunli Y, Liu B, Liu D, et al. Acarbose Treatment Affects the Serum Levels of Inflammatory Cytokines and the Gut Content of Bifidobacteria in Chinese Patients With Type 2 Diabetes Mellitus. *J Diabetes* (2015) 7(5):729–39. doi: 10.1111/1753-0407.12232
94. Zhang M, Feng R, Yang M, Qian C, Wang Z, Liu W, et al. Effects of Metformin, Acarbose, and Sitagliptin Monotherapy on Gut Microbiota in Zucker Diabetic Fatty Rats. *BMJ Open Diabetes Res Care* (2019) 7(1): e000717. doi: 10.1136/bmjdrc-2019-000717
95. Lee Y, Kim AH, Kim E, Lee S, Yu KS, Jang IJ, et al. Changes in the Gut Microbiome Influence the Hypoglycemic Effect of Metformin Through the Altered Metabolism of Branched-Chain and Nonessential Amino Acids. *Diabetes Res Clin Pract* (2021) 178:108985. doi: 10.1016/j.diabres.2021.108985
96. Zhang W, Xu JH, Yu T, Chen QK. Effects of Berberine and Metformin on Intestinal Inflammation and Gut Microbiome Composition in Db/Db Mice. *BioMed Pharmacother* (2019) 118:109131. doi: 10.1016/j.biopha.2019.109131
97. Liu Z, Liao W, Zhang Z, Sun R, Luo Y, Chen Q, et al. Metformin Affects Gut Microbiota Composition and Diversity Associated With Amelioration of Dextran Sulfate Sodium-Induced Colitis in Mice. *Front Pharmacol* (2021) 12:640347. doi: 10.3389/fphar.2021.640347
98. Schönfeld P, Wojtczak L. Short- and Medium-Chain Fatty Acids in Energy Metabolism: The Cellular Perspective. *J Lipid Res* (2016) 57(6):943–54. doi: 10.1194/jlr.R067629
99. 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 (5):727–35. doi: 10.1136/gutjnl-2012-303839

100. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the Gastrointestinal Tract. *Diabetologia* (2016) 59(3):426–35. doi: 10.1007/s00125-015-3844-9
101. Bennis Y, Bodeau S, Batteux B, Gras-Champel V, Masmoudi K, Maizel J, et al. A Study of Associations Between Plasma Metformin Concentration, Lactic Acidosis, and Mortality in an Emergency Hospitalization Context. *Crit Care Med* (2020) 48(12):e1194–202. doi: 10.1097/ccm.0000000000004589
102. Lazarus B, Wu A, Shin JI, Sang Y, Alexander GC, Secora A, et al. Association of Metformin Use With Risk of Lactic Acidosis Across the Range of Kidney Function: A Community-Based Cohort Study. *JAMA Intern Med* (2018) 178 (7):903–10. doi: 10.1001/jamainternmed.2018.0292
103. Bryrup 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. *Diabetologia* (2019) 62(6):1024–35. doi: 10.1007/s00125-019-4848-7
104. Cowie MR, Fisher M. SGLT2 Inhibitors: Mechanisms of Cardiovascular Benefit Beyond Glycaemic Control. *Nat Rev Cardiol* (2020) 17(12):761–72. doi: 10.1038/s41569-020-0406-8
105. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* (2019) 380(4):347–57. doi: 10.1056/NEJMoa1812389
106. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med* (2019) 380(24):2295–306. doi: 10.1056/NEJMoa1811744
107. Thirunavukarasu S, Jex N, Chowdhary A, Hassan IU, Straw S, Craven TP, et al. Empagliflozin Treatment Is Associated With Improvements in Cardiac Energetics and Function and Reductions in Myocardial Cellular Volume in Patients With Type 2 Diabetes. *Diabetes* (2021) 70(12):2810–22. doi: 10.2337/db21-0270
108. Yurista SR, Chong CR, Badimon JJ, Kelly DP, de Boer RA, Westenbrink BD. Therapeutic Potential of Ketone Bodies for Patients With Cardiovascular Disease: JACC State-Of-the-Art Review. *J Am Coll Cardiol* (2021) 77 (13):1660–9. doi: 10.1016/j.jacc.2020.12.065
109. Lee DM, Battson ML, Jarrell DK, Hou S, Ecton KE, Weir TL, et al. SGLT2 Inhibition via Dapagliflozin Improves Generalized Vascular Dysfunction and Alters the Gut Microbiota in Type 2 Diabetic Mice. *Cardiovasc Diabetol* (2018) 17(1):62. doi: 10.1186/s12933-018-0708-x
110. Li L, Xu S, Guo T, Gong S, Zhang C. Effect of Dapagliflozin on Intestinal Flora in MafA-Deficient Mice. *Curr Pharm Des* (2018) 24(27):3223–31. doi: 10.2174/1381612824666180912143434
111. Oh TJ, Sul WJ, Oh HN, Lee YK, Lim HL, Choi SH, et al. Butyrate Attenuated Fat Gain Through Gut Microbiota Modulation in Db/Db Mice Following Dapagliflozin Treatment. *Sci Rep* (2019) 9(1):20300. doi: 10.1038/s41598-019-56684-5
112. Yang M, Shi FH, Liu W, Zhang MC, Feng RL, Qian C, et al. Dapagliflozin Modulates the Fecal Microbiota in a Type 2 Diabetic Rat Model. *Front Endocrinol (Lausanne)* (2020) 11:635. doi: 10.3389/fendo.2020.00635
113. Mishima E, Fukuda S, Kanemitsu Y, Saigusa D, Mukawa C, Asaji K, et al. Canagliflozin Reduces Plasma Uremic Toxins and Alters the Intestinal Microbiota Composition in a Chronic Kidney Disease Mouse Model. *Am J Physiol Renal Physiol* (2018) 315(4):F824–f833. doi: 10.1152/ajprenal.00314.2017
114. Zhang Q, Yu H, Xiao X, Hu L, Xin F, Yu X. Inulin-Type Fructan Improves Diabetic Phenotype and Gut Microbiota Profiles in Rats. *PeerJ* (2018) 6: e4446. doi: 10.7717/peerj.4446
115. Just S, Mondot S, Ecker J, Wegner K, Rath E, Gau L, et al. The Gut Microbiota Drives the Impact of Bile Acids and Fat Source in Diet on Mouse Metabolism. *Microbiome* (2018) 6(1):134. doi: 10.1186/s40168-018-0510-8
116. Osataphan S, Macchi C, Singhal G, Chimene-Weiss J, Sales V, Kozuka C, et al. SGLT2 Inhibition Reprograms Systemic Metabolism via FGF21-Dependent and -Independent Mechanisms. *JCI Insight* (2019) 4(5): e123130. doi: 10.1172/jci.insight.123130
117. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPARγ Signaling and Metabolism: The Good, the Bad and the Future. *Nat Med* (2013) 19(5):557–66. doi: 10.1038/nm.3159
118. Yang H, Suh DH, Kim DH, Jung ES, Liu KH, Lee CH, et al. Metabolomic and Lipidomic Analysis of the Effect of Pioglitazone on Hepatic Steatosis in a Rat Model of Obese Type 2 Diabetes. *Br J Pharmacol* (2018) 175(17):3610–25. doi: 10.1111/bph.14434
119. Madsen MSA, Grønlund RV, Eid J, Christensen-Dalsgaard M, Sommer M, Rigbolt K, et al. Characterization of Local Gut Microbiome and Intestinal Transcriptome Responses to Rosiglitazone Treatment in Diabetic Db/Db Mice. *BioMed Pharmacother* (2021) 133:110966. doi: 10.1016/j.biopha.2020.110966
120. Lefebvre M, Paulweber B, Fajas L, Woods J, McCrary C, Colombel JF, et al. Peroxisome Proliferator-Activated Receptor Gamma is Induced During Differentiation of Colon Epithelium Cells. *J Endocrinol* (1999) 162(3):331–40. doi: 10.1677/joe.0.1620331
121. Li JM, Yu R, Zhang LP, Wen SY, Wang SJ, Zhang XY, et al. Dietary Fructose-Induced Gut Dysbiosis Promotes Mouse Hippocampal Neuroinflammation: A Benefit of Short-Chain Fatty Acids. *Microbiome* (2019) 7(1):98. doi: 10.1186/s40168-019-0713-7
122. Aggarwal H, Pathak P, Kumar Y, Jagavelu K, Dikshit M. Modulation of Insulin Resistance, Dyslipidemia and Serum Metabolome in iNOS Knockout Mice Following Treatment With Nitrite, Metformin, Pioglitazone, and a Combination of Ampicillin and Neomycin. *Int J Mol Sci* (2021) 23(1):195. doi: 10.3390/ijms23010195
123. Thornberry NA, Gallwitz B. Mechanism of Action of Inhibitors of Dipeptidyl-Peptidase-4 (DPP-4). *Best Pract Res Clin Endocrinol Metab* (2009) 23(4):479–86. doi: 10.1016/j.beem.2009.03.004
124. 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. *EBioMedicine* (2019) 44:665–74. doi: 10.1016/j.ebiom.2019.03.057
125. Ryan PM, Patterson E, Carafa I, Mandal R, Wishart DS, Dinan TG, et al. Metformin and Dipeptidyl Peptidase-4 Inhibitor Differentially Modulate the Intestinal Microbiota and Plasma Metabolome of Metabolically Dysfunctional Mice. *Can J Diabetes* (2020) 44(2):146–155.e2. doi: 10.1016/j.jcjd.2019.05.008
126. Silva-Veiga FM, Miranda CS, Martins FF, Daleprane JB, Mandarim-de-Lacerda CA, Souza-Mello V. Gut-Liver Axis Modulation in Fructose-Fed Mice: A Role for PPAR-Alpha and Linagliptin. *J Endocrinol* (2020) 247 (1):11–24. doi: 10.1530/joe-20-0139
127. Olivares M, Neyrinck AM, Pötgens SA, Beaumont M, Salazar N, Cani PD, et al. The DPP-4 Inhibitor Vildagliptin Impacts the Gut Microbiota and Prevents Disruption of Intestinal Homeostasis Induced by a Western Diet in Mice. *Diabetologia* (2018) 61(8):1838–48. doi: 10.1007/s00125-018-4647-6
128. Wang L, Li P, Tang Z, Yan X, Feng B. Structural Modulation of the Gut Microbiota and the Relationship With Body Weight: Compared Evaluation of Liraglutide and Saxagliptin Treatment. *Sci Rep* (2016) 6:33251. doi: 10.1038/srep33251
129. 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 (10):e0184735. doi: 10.1371/journal.pone.0184735
130. Yan X, Feng B, Li P, Tang Z, Wang L. Microflora Disturbance During Progression of Glucose Intolerance and Effect of Sitagliptin: An Animal Study. *J Diabetes Res* (2016) 2016:2093171. doi: 10.1155/2016/2093171
131. Dirir AM, Daou M, Yousef AF, Yousef LF. A Review of Alpha-Glucosidase Inhibitors From Plants as Potential Candidates for the Treatment of Type-2 Diabetes. *Phytochem Rev* (2021), 1–31. doi: 10.1007/s11101-021-09773-1
132. Balaich J, Estrella M, Wu G, Jeffrey PD, Biswas A, Zhao L, et al. The Human Microbiome Encodes Resistance to the Antidiabetic Drug Acarbose. *Nature* (2021) 600(7887):110–5. doi: 10.1038/s41586-021-04091-0
133. Tan K, Tesar C, Wilton R, Jedrzejczak RP, Joachimiak A. Interaction of Antidiabetic α-Glucosidase Inhibitors and Gut Bacteria α-Glucosidase. *Protein Sci* (2018) 27(8):1498–508. doi: 10.1002/pro.3444
134. 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(1):130. doi: 10.1186/s12866-019-1494-7
135. 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(8):691–702. doi: 10.1507/endocrj.EJ15-0747
136. Baxter NT, Lesniak 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(1):e00528–18. doi: 10.1128/mSphere.00528-18
137. Li ZR, Jia RB, Wu J, Lin L, Ou ZR, Liao B, et al. Sargassum Fusiforme Polysaccharide Partly Replaces Acarbose Against Type 2 Diabetes in Rats. *Int J Biol Macromol* (2021) 170:447–58. doi: 10.1016/j.ijbiomac.2020.12.126
138. Zhang L, Song P, Zhang X, Metea C, Schleisman M, Karstens L, et al. Alpha-Glucosidase Inhibitors Alter Gut Microbiota and Ameliorate Collagen-Induced Arthritis. *Front Pharmacol* (2019) 10:1684. doi: 10.3389/fphar.2019.01684
139. Kishida Y, Okubo H, Ohno H, Oki K, Yoneda M. Effect of Miglitol on the Suppression of Nonalcoholic Steatohepatitis Development and Improvement of the Gut Environment in a Rodent Model. *J Gastroenterol* (2017) 52 (11):1180–91. doi: 10.1007/s00535-017-1331-4
140. Kato T, Iizuka K, Takao K, Horikawa Y, Kitamura T, Takeda J. ChREBP-Knockout Mice Show Sucrose Intolerance and Fructose Malabsorption. *Nutrients* (2018) 10(3):340. doi: 10.3390/nu10030340
141. Walters WA, Xu Z, Knight R. Meta-Analyses of Human Gut Microbes Associated With Obesity and IBD. *FEBS Lett* (2014) 588(22):4223–33. doi: 10.1016/j.febslet.2014.09.039
142. Smith BJ, Miller RA, Schmidt TM. Muribaculaceae Genomes Assembled From Metagenomes Suggest Genetic Drivers of Differential Response to Acarbose Treatment in Mice. *mSphere* (2021) 6(6):e0085121. doi: 10.1128/msphere.00851-21
143. Nepal MR, Kang MJ, Kim GH, Cha DH, Kim JH, Jeong TC. Role of Intestinal Microbiota in Metabolism of Voglibose In Vitro and In Vivo. *Diabetes Metab J* (2020) 44(6):908–18. doi: 10.4093/dmj.2019.0147
144. Patnode ML, Beller ZW, Han ND, Cheng J, Peters SL, Terrapon N, et al. Interspecies Competition Impacts Targeted Manipulation of Human Gut Bacteria by Fiber-Derived Glycans. *Cell* (2019) 179(1):59–73.e13. doi: 10.1016/j.cell.2019.08.011
145. Huo T, Xiong Z, Lu X, Cai S. Metabonomic Study of Biochemical Changes in Urinary of Type 2 Diabetes Mellitus Patients After the Treatment of Sulfonylurea Antidiabetic Drugs Based on Ultra-Performance Liquid Chromatography/Mass Spectrometry. *BioMed Chromatogr* (2015) 29 (1):115–22. doi: 10.1002/bmc.3247
146. Đanić M, Pavlović N, Stanimirov B, Lazarević S, Vukmirović S, Al-Salami H, et al. PAMPA Model of Gliclazide Permeability: The Impact of Probiotic Bacteria and Bile Acids. *Eur J Pharm Sci* (2021) 158:105668. doi: 10.1016/j.ejps.2020.105668
147. Kondo Y, Hashimoto Y, Hamaguchi M, Ando S, Kaji A, Sakai R, et al. Unique Habitual Food Intakes in the Gut Microbiota Cluster Associated With Type 2 Diabetes Mellitus. *Nutrients* (2021) 13(11):3816. doi: 10.3390/nu13113816
148. Deeks ED. Chiglitazar: First Approval. *Drugs* (2022) 82(1):87–92. doi: 10.1007/s40265-021-01648-1
149. Hallakou-Bozec S, Vial G, Kergoat M, Fouqueray P, Bolze S, Borel AL, et al. Mechanism of Action of Imeglimin: A Novel Therapeutic Agent for Type 2 Diabetes. *Diabetes Obes Metab* (2021) 23(3):664–73. doi: 10.1111/dom.14277
150. Wang Y, Li H, Gao H, Xu X, Cai T, Wang H, et al. Effect of Chiglitazar and Sitagliptin on Glucose Variations, Insulin Resistance and Inflammatory-Related Biomarkers in Untreated Patients With Type 2 Diabetes. *Diabetes Res Clin Pract* (2022) 183:109171. doi: 10.1016/j.diabres.2021.109171
151. Li PP, Shan S, Chen YT, Ning ZQ, Sun SJ, Liu Q, et al. The PPARalpha/gamma Dual Agonist Chiglitazar Improves Insulin Resistance and Dyslipidemia in MSG Obese Rats. *Br J Pharmacol* (2006) 148(5):610–8. doi: 10.1038/sj.bjp.0706745
152. Dubourg J, Fouqueray P, Thang C, Grouin JM, Ueki K. Efficacy and Safety of Imeglimin Monotherapy Versus Placebo in Japanese Patients With Type 2 Diabetes (TIMES 1): A Double-Blind, Randomized, Placebo-Controlled, Parallel-Group, Multicenter Phase 3 Trial. *Diabetes Care* (2021) 44(4):952–9. doi: 10.2337/dc20-0763
153. Dubourg J, Fouqueray P, Quinslot D, Grouin JM, Kaku K. Long-Term Safety and Efficacy of Imeglimin as Monotherapy or in Combination With Existing Antidiabetic Agents in Japanese Patients With Type 2 Diabetes (TIMES 2): A 52-Week, Open-Label, Multicentre Phase 3 Trial. *Diabetes Obes Metab* (2022) 24(4):609–19. doi: 10.1111/dom.14613
154. Li J, Inoue R, Togashi Y, Okuyama T, Satoh A, Kyohara M, et al. Imeglimin Ameliorates β-Cell Apoptosis by Modulating the Endoplasmic Reticulum Homeostasis Pathway. *Diabetes* (2022) 71(3):424–39. doi: 10.2337/db21-0123
155. Toulis KA, Nirantharakumar K, Pourzitaki C, Barnett AH, Tahrani AA. Glucokinase Activators for Type 2 Diabetes: Challenges and Future Developments. *Drugs* (2020) 80(5):467–75. doi: 10.1007/s40265-020-01278-z
156. Chen X, Chen Z, Xu D, Lyu Y, Li Y, Li S, et al. De Novo Design of G Protein-Coupled Receptor 40 Peptide Agonists for Type 2 Diabetes Mellitus Based on Artificial Intelligence and Site-Directed Mutagenesis. *Front Bioeng Biotechnol* (2021) 9:694100. doi: 10.3389/fbioe.2021.694100
157. Scheen AJ. Could Metformin Modulate Cardiovascular Outcomes Differently With DPP-4 Inhibitors Compared With SGLT2 Inhibitors? *Diabetes Metab* (2021) 47(4):101209. doi: 10.1016/j.diabet.2020.11.001
158. Deeks ED, Scott LJ. Pioglitazone/Metformin. *Drugs* (2006) 66(14):1863–77; discussion 1878-80. doi: 10.2165/00003495-200666140-00007
159. Salgaço MK, Oliveira LGS, Costa GN, Bianchi F, Sivieri K. Relationship Between Gut Microbiota, Probiotics, and Type 2 Diabetes Mellitus. *Appl Microbiol Biotechnol* (2019) 103(23-24):9229–38. doi: 10.1007/s00253-019-10156-y
160. Schwientek P, Szczepanowski R, Rückert C, Kalinowski J, Klein A, Selber K, et al. The Complete Genome Sequence of the Acarbose Producer Actinoplanes Sp. SE50/110. *BMC Genomics* (2012) 13:112. doi: 10.1186/1471-2164-13-112
161. Panwar H, Calderwood D, Grant IR, Grover S, Green BD. Lactobacillus Strains Isolated From Infant Faeces Possess Potent Inhibitory Activity Against Intestinal Alpha- and Beta-Glucosidases Suggesting Anti-Diabetic Potential. *Eur J Nutr* (2014) 53(7):1465–74. doi: 10.1007/s00394-013-0649-9
162. Kattar SA, Jurjus R, Pinon A, Leger DY, Jurjus A, Boukarim C, et al. Metformin and Probiotics in the Crosstalk Between Colitis-Associated Colorectal Cancer and Diabetes in Mice. *Cancers (Basel)* (2020) 12 (7):1857. doi: 10.3390/cancers12071857
163. Snelson M, de Pasquale C, Ekinci EI, Coughlan MT. Gut Microbiome, Prebiotics, Intestinal Permeability and Diabetes Complications. *Best Pract Res Clin Endocrinol Metab* (2021) 35(3):101507. doi: 10.1016/j.beem.2021.101507
164. Guo Y, Yu Y, Li H, Ding X, Li X, Jing X, et al. Inulin Supplementation Ameliorates Hyperuricemia and Modulates Gut Microbiota in Uox-Knockout Mice. *Eur J Nutr* (2021) 60(4):2217–30. doi: 10.1007/s00394-020-02414-x
165. Pedersen C, Gallagher E, Horton F, Ellis RJ, Ijaz UZ, Wu H, et al. Host-Microbiome Interactions in Human Type 2 Diabetes Following Prebiotic Fibre (Galacto-Oligosaccharide) Intake. *Br J Nutr* (2016) 116(11):1869–77. doi: 10.1017/s0007114516004086
166. Burton JH, Johnson M, Johnson J, Hsia DS, Greenway FL, Heiman ML. Addition of a Gastrointestinal Microbiome Modulator to Metformin Improves Metformin Tolerance and Fasting Glucose Levels. *J Diabetes Sci Technol* (2015) 9(4):808–14. doi: 10.1177/1932296815577425
167. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational Diabetes Mellitus. *Nat Rev Dis Primers* (2019) 5(1):47. doi: 10.1038/s41572-019-0098-8
168. Molina-Vega M, Picón-César MJ, Gutiérrez-Repiso C, Fernández-Valero A, Lima-Rubio F, González-Romero S, et al. Metformin Action Over Gut Microbiota is Related to Weight and Glycemic Control in Gestational Diabetes Mellitus: A Randomized Trial. *BioMed Pharmacother* (2022) 145:112465. doi: 10.1016/j.biopha.2021.112465
169. Taylor BL, Woodfall GE, Sheedy KE, O'Riley ML, Rainbow KA, Bramwell EL, et al. Effect of Probiotics on Metabolic Outcomes in Pregnant Women With Gestational Diabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients* (2017) 9(5):461. doi: 10.3390/nu9050461
170. Miao M, Wang Q, Wang X, Fan C, Luan T, Yan L, et al. The Protective Effects of Inulin-Type Fructans Against High-Fat/Sucrose Diet-Induced Gestational Diabetes Mice in Association With Gut Microbiota Regulation. *Front Microbiol* (2022) 13:832151. doi: 10.3389/fmicb.2022.832151
171. Callaway LK, McIntyre HD, Barrett HL, Foxcroft K, Tremellen A, Lingwood BE, et al. Probiotics for the Prevention of Gestational Diabetes Mellitus in Overweight and Obese Women: Findings From the SPRING Double-Blind Randomized Controlled Trial. *Diabetes Care* (2019) 42(3):364–71. doi: 10.2337/dc18-2248

172. Hanssen NMJ, de Vos WM, Nieuwdorp M. Fecal Microbiota Transplantation in Human Metabolic Diseases: From a Murky Past to a Bright Future? *Cell Metab* (2021) 33(6):1098–110. doi: 10.1016/j.cmet.2021.05.005
173. Su L, Hong Z, Zhou T, Jian Y, Xu M, Zhang X, et al. Health Improvements of Type 2 Diabetic Patients Through Diet and Diet Plus Fecal Microbiota Transplantation. *Sci Rep* (2022) 12(1):1152. doi: 10.1038/s41598-022-05127-9
174. Liu Y, Wang Y, Ni Y, Cheung CKY, Lam KSL, Wang Y, et al. Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. *Cell Metab* (2020) 31(1):77–91.e5. doi: 10.1016/j.cmet.2019.11.001
175. Ding D, Yong H, You N, Lu W, Yang X, Ye X, et al. Prospective Study Reveals Host Microbial Determinants of Clinical Response to Fecal Microbiota Transplant Therapy in Type 2 Diabetes Patients. *Front Cell Infect Microbiol* (2022) 12:820367. doi: 10.3389/fcimb.2022.820367

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Gut microbiota is associated with differential metabolic characteristics: A study on a defined cohort of Africans and Chinese

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Objective: This study intended to determine the associations between gut microbiota and glucose response in healthy individuals and analyze the connection between the gut microbiome and glucose-metabolism-related parameters.

Methods: Fecal bacterial composition and anthropometric, body composition, body fat distribution, and biochemical measures were analyzed. A 75-g oral glucose tolerance test (OGTT) was given to each participant to investigate changes in glucagon-like peptide 1 (GLP-1), insulin, and glucose. The whole body fat and the regions of interest of local body composition were analyzed using dual-energy X-ray absorptiometry (DEXA), and gut microbiota composition was assessed through variable regions (V3–V4) of the bacterial 16s ribosomal RNA gene using high-throughput sequencing techniques. Spearman correlation analysis was used to evaluate the association between gut microbiota and clinical and metabolic changes.

Results: The number of operational taxonomic units (OTUs) demonstrated a reduction in the diversity and composition of gut microbiota associated with enhanced adiposity, dyslipidemia, insulin resistance, and hyperglycemia. The alpha diversity revealed that microbiota diversity, richness, and composition were higher in the African group and lower in the Chinese group. Principal coordinates analysis (PCoA) plots of beta diversity showed significant variability in gut microbial community structure between the two groups (p = 0.0009). LEfSe analysis showed that phylum Bacteroidetes was significantly more abundant in the Chinese group, and this group also harbored members of the order Bacteroidales, family Bacteroidaceae, and genus *Bacteroides*. In contrast, the phylum Verrucomicrobia was significantly more prevalent in the African group (all $p < 0.05$). Concerning species, metastats analysis revealed 8

species in the Chinese group and 18 species in the African group that were significantly abundant. Spearman's correlation analysis demonstrated that gut microbiota correlated with the factors that related to glucose metabolism.

Conclusion: Our data suggest that there is an interaction between gut microbiota, host physiology, and glucometabolic pathways, and this could contribute to adiposity and pathophysiology of hyperlipidemia, insulin resistance, and hyperglycemia. These findings provide an important basis for determining the relation between the gut microbiota and the pathogenesis of various metabolic disorders.

KEYWORDS

Chinese, Africans, healthy, gut microbiota, 16S rRNA gene sequencing, bacterial communities

1 Introduction

Obesity and T2DM are the most prevalent metabolic disorders and worldwide major health challenges today. The incidence of T2DM is rising globally (1, 2) with a high increase in Asian countries (1, 3, 4). Plenty of genomic studies have reported connections between gut microbiota and metabolic disorders such as obesity, insulin resistance, and T2DM, and this gives an idea that a causal relationship could exist. The pathogenesis of obesity, insulin resistance, and T2DM in Asians and Africans is very different (5–9). It is unclear whether these pathophysiological differences may be related to differences in the gut microbiota. The gut microbiota is the intestinal microbial community that performs an important role in maintaining the host physiology, sustaining health, and disease pathogenesis. Currently, the gut microbiota is increasingly recognized as an endocrine organ that maintains host energy homeostasis and contributes to host immunity (10, 11). Earlier studies on gut microbiota have suggested that the composition of gut microbiota can contribute to health and is closely associated with metabolic disorders. For example, alteration (dysbiosis) of gut microbiota can lead to a dramatically altered symbiotic relationship between gastrointestinal microbiota (gut bacteria) and the host, which contributes to the development of obesity, metabolic syndrome, T2DM (12, 13), and cardiometabolic disease (12), and non-alcoholic fatty liver disease (12, 14). Moreover, this dysbiosis of the gut microbiota may give rise to a pathophysiological mechanism underlying systemic inflammation in insulin resistance (15, 16). Despite the immense contributions of gut flora in multiple disorders, gut microbiota characteristics in healthy Chinese and Africans are poorly understood. It has been suggested that the gut microbiota can involve in the modulation of host energy metabolism, fat storage, glucose control, and insulin sensitivity by regulating certain factors such as fats, lipids, bile acids, and glucagon-like peptide 1 (GLP-1) that participate in metabolic pathways of glucose metabolism (12, 17, 18). Current studies have highlighted gut microbiota as a new therapeutic target to improve metabolic health (12), although many factors (e.g., diets, lifestyle changes, urbanization, environmental conditions, and genetic factors) have been reported to shape the gut microbiota community (19–25), making it difficult to perform vital functions like nutrition, physiology, metabolism, and immune function. A growing understanding of the risk factors that impact the incidence of metabolic disorders such as obesity, insulin resistance, and diabetes can disseminate advanced knowledge on the pathophysiology of these disorders and can facilitate adopting treatment or prevention strategies and/or measures to delay their occurrence. This study evaluated the associations between gut microbiota and glucose response in healthy individuals and analyzed the connection between gut microbiota and factors related to glucose metabolism.

2 Materials and methods

2.1 Subject enrollment criteria

This study recruited 27 Han-Chinese and 29 African citizens (university students) living in Changsha, China *via* on-campus advertisements. These African citizens were born in Africa (Burundi, Rwanda, Uganda, and Tanzania) and have no known recent non-African ancestry. Participants were male and female with age 18–35 years (Table 1) who were metabolically stable within the last 6 months and had a long stay in China for at least 1 year, with no participation in any clinical trial until the day of the study

TABLE 1 Anthropometric profiles, body composition and body fat distribution, and biochemical and clinical measurements in Chinese and African subjects.

Characteristic	Chinese (n=27)	Africans (n=29)	*p*-value
Gender:			
Male, n (%)	19 (70.4%)	21 (72.4%)	–
Female, n (%)	8 (29.6%)	8 (27.6%)	–
Age (year)	25.07 ± 1.49	26.1 ± 4.24	0.895
Height (cm)	171.63 ± 7.41	173.07 ± 8.15	0.493
Weight (kg)	66.33 ± 9.80	67.45 ± 11.53	0.699
BMI (kg/m^2)	22.46 ± 2.48	22.53 ± 3.56	0.932
Waist circumference (cm)	76.06 ± 10.71	79.66 ± 9.16	0.182
Hip circumference (cm)	90.66 ± 9.10	95.82 ± 9.26	**0.040**
Waist–hip ratio	0.84 ± 0.07	0.83 ± 0.04	0.621
Arm circumference (cm)	27.72 ± 3.77	27.31 ± 3.43	0.673
Systolic BP (mmHg)	112.74 ± 9.49	112.1 ± 10.43	0.812
Diastolic BP (mmHg)	73.03 ± 8.62	69.76 ± 6.32	0.109
SMI (kg/m^2)	15.51 ± 2.08	15.17 ± 2.36	0.568
LS-BMD (g/cm^2)	0.98 ± 0.09	1.01 ± 0.10	0.081
PF-BMD (g/cm^2)	0.98 ± 0.12	0.99 ± 0.14	0.699
Total body BMD (g/cm^2)	1.17 ± 0.06	1.15 ± 0.09	0.360
Body fat (%)	26.77 ± 5.57	27.05 ± 8.82	0.885
A/G ratio	1.06 ± 0.19	0.87 ± 0.17	**<0.001**
$FMR_{trunk\text{-}to\text{-}limb}$	1.08 ± 0.23	0.85 ± 0.17	**<0.0001**
Trunk/leg fat ratio	1.02 ± 0.22	0.87 ± 0.13	**0.003**
Total cholesterol (mmol/L)	4.22 ± 0.70	4.36 ± 0.88	0.222
LDL cholesterol (mmo/L)	2.42 ± 0.66	2.53 ± 0.81	0.549
HDL cholesterol (mmol/L)	1.44 ± 0.28	1.53 ± 0.34	0.275
Triglycerides (mmol/L)	1.03 ± 0.59	0.83 ± 52	**0.039**
Total bile acids (μmol/L)	5.37 ± 5.56	3.24 ± 2.93	**0.049**
Total bilirubin (μmol/L)	14.60 ± 5.32	11.44 ± 5.49	**0.022**
Direct bilirubin (μmol/L)	6.54 ± 2.26	4.88 ± 2.30	**0.009**
Fasting GLP-1 (ng/ml)	0.28 ± 0.33	0.19 ± 0.08	0.652
Fasting insulin (μU/ml)	6.97 ± 2.25	6.56 ± 2.42	0.513
Fasting glucose (mmol/L)	4.58 ± 0.40	4.61 ± 0.46	0.774
Δglucose (30–0 min)	2.68± 1.08	1.94± 1.17	**0.017**
DI_{180}	95.83 ± 28.87	115.10 ± 42.87	0.056
Matsuda index	7.08 ± 2.47	8.36 ± 4.10	0.314
$HOMA\text{-}IR_{30min}$	20.36 ± 8.66	16.36 ± 10.64	**0.026**

Data are reported as means and standard deviations (X ± SD).
BMI, body mass index; BP, blood pressure; SMI, skeletal muscle mass index; LS-BMD, lumbar spine bone mineral density; PF-BMD, proximal femur bone mineral density; A/G ratio, android/gynoid ratio; $FMR_{trunk\text{-}to\text{-}limb}$, trunk/limb fat mass ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; GLP-1, glucagon-like peptide 1; Δ Glucose (30–0 min), incremental glucose level at 30 min; $HOMA\text{-}IR_{30min}$, homeostatic model assessment for insulin resistance at 30 min; DI_{180}, a disposition index obtained from the product of $AUCins_{0-180}$/$AUCglu_{0-180}$ ×Matsuda index. The bold for values was just to emphasize the statistical significance.

and had no special food habits. Exclusion criteria included pregnancy, lactation, smoking, and history of chronic metabolic diseases and/or neurological, autoimmune, and gastrointestinal diseases. Subjects taking any medication that interferes with insulin, glucose, and GLP-1 or with treatments affecting gut permeability, motility, or microbiota were also excluded. We confirmed health status through blood pressure measurements and lipid and biochemical profiles (Table 1) and by the absence of glucose intolerance (26).

2.2 Anthropometric measurements and biochemical analysis

All subjects were refrained from doing vigorous exercise and underwent overnight fasting of 12 h. Height, weight, blood pressure, and circumferences of arm, waist, and hip were measured, and a standard 75-g oral glucose tolerance test (OGTT) was given to each participant early in the morning at 8 a.m., and venous blood samples were drawn at the time points

of 0, 30, 60, 120, and 180 min for the measurements of glucose, insulin, and GLP-1. The samples for the determination of GLP-1 levels were collected in tubes free of aprotinin or DPP-IV. Sample tubes were centrifuged at 1,000×*g* for 15 min at 4°C, and the resulting supernatants were collected and stored at −80° C until analysis of plasma total GLP-1 and insulin. The levels of triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose at fasting and glucose during an OGTT, and total bile acids were measured by a Beckman-AU680 automatic biochemistry analyzer with Beckman Coulter kits and Leadman kit, respectively. The hexokinase method was applied for glucose measurements, while automated enzymatic methods were used for lipid profiles and total bile acids. Direct and total bilirubin levels were detected using the diazo method, Azobirubin (Beckman Coulter, USA). Insulin concentrations were detected using chemiluminescent microparticle immunoassay with ARCHITECT kits (DENKA Seiken Co., Ltd., Tokyo, Japan), and the levels of total GLP-1 were measured using ELISA with Elabscience kits (Elabscience Biotechnology Co., Ltd., Wuhan, China).

2.3 Body composition assessment and determination of fat distribution

Subjects were asked to empty their bladder and remove any metallic objects before the scan. Subjects were also instructed to breath normally and not talk or move (lie still) during the entire scan for approximately 7 min. Dual-energy X-ray absorptiometry (Hologic QDR 4500A, Hologic Corporation, USA) was used to evaluate fat and bone mineral density (BMD) in the whole body and BMD in the lumbar spine (L1–L4) and proximal femoral. Fat distribution patterns were automatically calculated by performing and executing the analysis (Hologic APEX for Windows software version 5.5.3) according to the operator's standard analysis protocol.

2.4 Gut microbiota analyses

2.4.1 DNA extraction, library preparation, and high-throughput sequencing (16s rRNA gene sequencing)

Fecal samples were collected at baseline, and DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). Based on the preliminary quantitative results of agarose gel electrophoresis, the concentration and purity of sample libraries were assessed by an Invitrogen Qubit 3.0 spectrophotometer (Thermo Fisher Scientific, USA). The quantity and size distribution of DNA fragment libraries and validation of biological replicates were determined using an Agilent 2100 bioanalyzer (Agilent Technologies, USA). The V3–V4 region of the rRNA gene was amplified and then subjected to high-throughput profiling of microbial communities using the MiSeq platform (Illumina, USA). Simply, the 16S rRNA V3–V4 region was amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-CCTACGGGNGGCWGCAG-3′), and 250-bp paired-end reads were generated.

2.4.2 Sequencing quality control, data processing, and gut microbial community analysis

The mothur version 1.41.1 was used to generate the reads for further analysis. USEARCH was applied to conduct filtering of the duplicated sequences and chimera removal. The lasting sequences were grouped into operational taxonomic units (OTUs) with a 97% threshold of similarity using the UPARSE and then categorized against the SILVA database.

2.4.3 Calculations and statistical analyses

We determined the presence of insulin resistance by applying the transformed homeostasis model assessment (HOMA-My) (27) for insulin resistance (IR). The transformed HOMA-My indices were obtained using the following formula: Iy (μIU/ml) × Gy (mmol/L)/22.5, where y indicates 30, 60, 120, or 180 min insulin (I) and glucose (G) values from the OGTT. Insulin secretion derived from the OGTT was obtained from the product of the insulin/glucose 0–180 min total areas under the curve ratio and the Matsuda index ($AUCins_{0-180}/AUCglu_{0-180}$ × Matsuda index) to assess beta cell function (28) and was expressed as disposition index (DI_{180}). The areas under the curves (AUCs) for insulin and glucose were calculated using the trapezium rule (29). The SPSS software (v.18.0.0) was used to perform analyses for baseline anthropometric profiles, body composition and body fat distribution, biochemical and clinical measurements, and data from OGTT. Independent *t*-tests, Mann–Whitney U test, and χ^2 test were used for parametric, non-parametric, and categorical data to assess differences in measurements between groups, respectively. Alpha diversity was determined based on biodiversity metrics (observed species, Chao1, and Shannon index) to analyze the disparity in the gut microbiota richness and diversity using Wilcoxon tests. Venn diagram was generated using the R package (v1.6.20) and rarefaction and Shannon–Wiener curves were plotted using

ggplot2 in R (v3.3.0). Beta diversity was determined based on OTU counts in line with the Bray–Curtis distance metric (30). Principal coordinates analysis (PCoA) was conducted to visualize similarities or differences between data, and permutational multivariate analysis of variance (PERMANOVA) with Adonis was used to evaluate the significant variation in microbial communities between groups. Metastats analysis was performed at multiple taxonomic levels (phylum, class, order, family, genus, and species) to identify differentially abundant taxa between the two groups. Additionally, Benjamin and Hochberg's false discovery rate (FDR) method was applied to correct and adjust p-values. Linear discriminant analysis (LDA) effect size (LEfSe) with the default alpha value of 0.05 was carried out using the available website "http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=PICRUSt_normalize" to screen taxa that serve mostly as biomarkers between the two groups of participants. Spearman's rank correlation coefficient was applied to determine the relationships between the sequencing data and other data.

3 Results

3.1 Study population

The anthropometrical, clinical, biochemical, and body composition characteristics of the participants are given in Table 1 and Supplementary Table S1. We further analyzed these characteristics based on sex-specific classification, and data from this analysis are summarized in Supplementary Table S2.

3.2 Microbiota profiles in Chinese and African subjects

We obtained a total of 4,470,507 reads from 56 samples, with 79,830 reads estimated as an average for each sample. The statistics and quality score of sequencing reads used in this study are detailed in Supplementary Table S3. The reads were clustered into 1,022 OTUs based on a similarity score of 97% at the 16S rRNA gene. A Venn diagram demonstrated that 636 OTUs were shared by both groups, whereas 66 OTUs and 320 OTUs were unique for the Chinese and Africans, respectively (Figure 1A).

3.3 Alpha diversity and beta diversity in Chinese and African subjects

The OTUs evaluation in respect of diversity indices exhibited pronounced variations between groups. The rarefaction curves demonstrated that all samples were detected for OTUs and approached a plateau, which evidences the adequacy of our sequencing data for this study (Supplementary Figure S1A), and the Shannon diversity index indicates that the microbial diversity of the gut flora in the African group was the highest and that in the Chinese group was the lowest (Supplementary Figure S1B). Indeed, rank-abundance curves indicated that the African group stands out for species abundance, richness, and evenness (Supplementary Figure S2). To better understand the distribution and diversity of microbial communities in these two groups, we evaluated the overall community heterogeneity by measuring ecological indices based on Alpha diversity using Wilcoxon tests. The finding revealed that the Alpha diversity decreased significantly in the Chinese group (all $p<0.05$, Figure 1B). To determine the dissimilarities in microbial community structures between the Chinese and African group, we calculated beta diversity based on Bray–Curtis distances. The PCoA plot with Bray–Curtis distance matrix revealed that the gut microbiota samples from the African group were clustered separately from the Chinese group, with 27.52% (X-axis) and 8.56% (Y-axis) of the total variance in microbiota composition (Adonis, p = 0.0009, Figure 1C).

3.4 Composition and abundance of gut microbiota in Chinese and African subjects

The statistics of the OTUs indicated that they were grouped in 12 phyla (Figure 2A). To better understand how the gut microbial community composition differs between the Chinese and African group, we examined which microbial groups were present at multiple taxonomic levels together with their relative abundance. Metastats analysis showed that the taxonomic compositions differed between the two groups. At the phylum level, Bacteroidetes was the most widely represented in both groups, with a relative abundance of 65.14% in the Chinese group and 53.54% in the African group. Firmicutes was the second most widely abundant, accounting for the relative abundance of 20.45% and 23.54%, respectively. The next widely abundant phyla were Proteobacteria, Verrucomicrobia, and Spirochaetes (Figure 2B and Table 2). At the class level, the Chinese group showed significantly greater relative abundance of Bacteroidia (64.91% vs. 52.71%, $p<0.001$) than the African group. We also observed that the relative abundance of Spirochaetia was absent in Chinese ($p>0.05$). There were no differences in abundances of Clostridia, Negativicutes, Betaproteobacteria, and Gammaproteobacteria (all $p>0.05$, Table 2). At the order level, the Chinese group had a significant increase in relative abundance of Bacteroidales (64.91% vs. 52.71%, $p<0.001$) than the African group, whereas the relative abundance of Aeromonadales (0% vs. 1.82%, $p<0.05$)

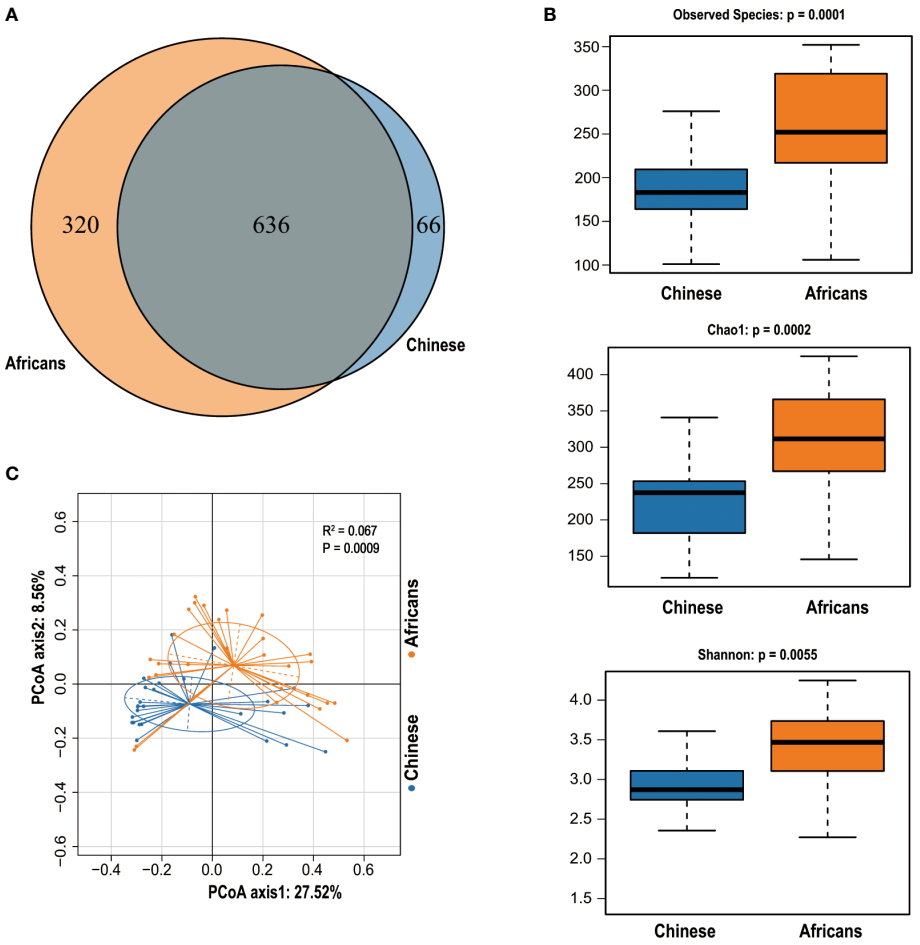

FIGURE 1
The Venn diagram and community diversity analysis. The Venn diagram depicts the overlapping OTUs between Chinese and Africans **(A)**. The Alpha diversity metrics used to estimate microbial richness and diversity **(B)**. PCoA was computed to display the variability of gut communities among all samples from Chinese and Africans **(C)**. A permutational multivariate analysis of variance (PERMANOVA) with Adonis on Bray–Curtis distances confirmed these differences (R^2 = 0.067, p = 0.0009).

together with Spirochaetales (p>0.05) was completely absent in the Chinese group (Table 2). On the other hand, the relative abundances of Selenomonadales and Acidaminococcales were predominant in the Chinese group but absent in the African group (p>0.05). There were no differences in abundances of Clostridiales, Burkholderiales, and Enterobacterales (all p>0.05, Table 2). At the family level, the members of the family Oscillospiraceae and Succinivibrionaceae were significantly absent in the Chinese group (all, p<0.05, Table 2). In addition, the member of the family Spirochaetaceae was completely absent in this group (p>0.05). On the other hand, the Chinese group had a significant increase in relative abundance of Bacteroidaceae (43.22% vs. 22.24%, p<0.01) than the African group and the member of the family Clostridiaceae was significantly absent in the African group (p<0.05, Table 2). In addition, members of the family Selenomonadaceae and Acidaminococcaceae were also predominant in the Chinese group but absent in the African group (all p>0.05, Table 2). There were no differences in abundances of members of the family Porphyromonadaceae, Rikenellaceae, Prevotellaceae, Lachnospiraceae, Ruminococcaceae, Sutterellaceae, and Enterobacteriaceae (all p>0.05, Table 2). At the genus level, the genera *Parasutterella* (p<0.01, Table 2) and *Succinivibrio* (p<0.05, Table 2) were significantly absent in the Chinese group, and also the relative abundance of *Treponema* was completely absent in this group (p>0.05). However, the Chinese group had a significant increase in relative abundance of *Bacteroides* (42.83% vs. 22.17%, p<0.01, Table 2) than the African group. The genera *Megamonas* and *Phascolarctobacterium* were predominant in the Chinese group but absent in the African group (p>0.05). There were no differences in abundances of

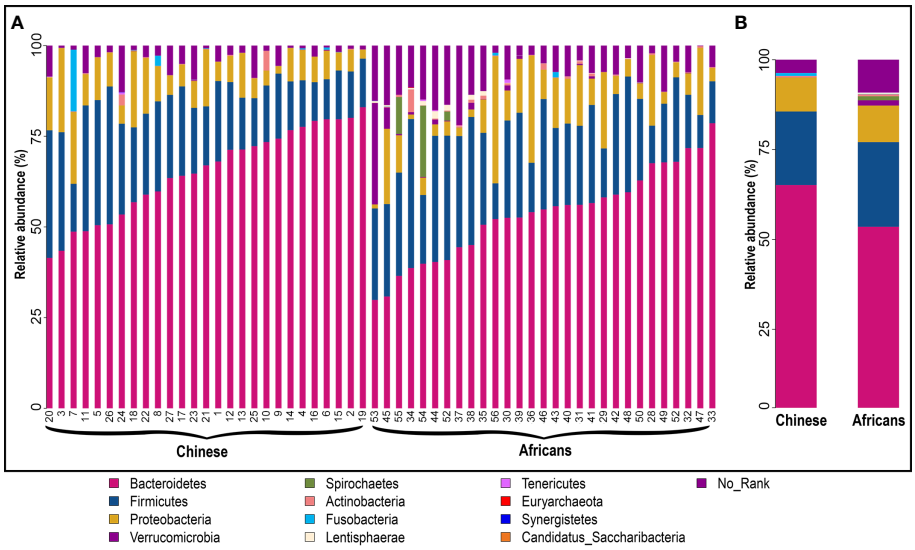

FIGURE 2
Structural composition of gut microbiota community in the two groups. Microbial composition and their relative abundance in each sample **(A)** and between groups **(B)** at phylum level. The taxa with relative abundance ≥1% are presented. The remaining unmapped taxa are grouped as "No_Rank." Each bar denotes a single sample or group, and each color represents the relative abundance in percentage for each OTU.

genera *Parabacteroides*, *Alistipes*, *Prevotella*, *Ruminococcus*, *Faecalibacterium*, *Sutterella*, and *Escherichia* (all $p>0.05$, Table 2). The number and proportion of unmapped reads (No_Rank) at each taxonomic level are presented in Supplementary Table S4.

3.5 Differences in the gut microbiome between Chinese and Africans

Analysis of 16S rRNA sequence data using metastats revealed that 26 species, excluding the uncultured forms, differed significantly between the two groups (all, $p<0.05$, Figure 3A). Species such as *Bacteroides massiliensis*, *Bacteroides stercoris*, *Bacteroides coprocola*, *Bacteroides ovatus*, *Parasutterella excrementihominis*, *Phascolarctobacterium faecium*, *Bacteroides coprophilus*, and *Clostridium* sp. AT5 were significantly enriched in the Chinese group, whereas species such as *Akkermansia muciniphila*, *Prevotella colorans*, *Prevotella* sp. Marseille-P2439, *Prevotella stercorea*, *Phascolarctobacterium succinatutens*, *Succinivibrio dextrinosolvens*, *Sutterellaceae bacterium* Marseille-P2968, *Coprococcus comes*, *Holdemanella biformis*, *Dorea longicatena*, *Marseillibacter massiliensis*, *Oscillibacter* sp. ER4, *Veillonella dispar*, *Eubacterium coprostanoligenes*, *Butyricicoccus* sp. K4410.MGS-46, *Butyrivibrio crossotus*, *Bifidobacterium adolescentis*, and *Collinsella aerofaciens* were significantly enriched in the African group (all, $p<0.05$, Figure 3A). Wilcoxon rank-sum test revealed that the ratio of Firmicutes/Bacteroidetes increased significantly in the African group ($p = 0.0209$, Figure 3B). We further performed a metagenomic study based on linear discriminant analysis effect size (LEfSe) to identify the core taxa contributing to the differences between the two groups. The cladogram obtained from the LEfSe analysis showed that the Chinese group had a significant increase in the phylum Bacteroidetes, class Bacteroidia, order Bacteroidales, family Bacteroidaceae, and genus *Bacteroides*, whereas it had a significant decrease in the phylum Verrucomicrobia compared with the African group (Figure 3C).

3.6 Correlations between gut microbiota and parameters related to glucose metabolism

To evaluate the correlation between gut microbiota and parameters related to glucose metabolism, we performed a Spearman correlation analysis of parameters related to glucose metabolism and microbiota abundance. In this regard, only data that showed significant differences were subjected to this analysis, and a heat map was used to depict these associations. The results demonstrated that the total bile acids positively correlated with phylum Bacteroidetes and species *B. coprophilus* and negatively associated with Firmicutes/Bacteroidetes ratio and *D. longicatena*. The levels of triglycerides positively correlated with *Clostridium* sp. AT5 and negatively correlated with *P. colorans*, *P. succinatutens*,

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TABLE 2 Most abundant bacterial taxa in the Africans (n=29) and Chinese (n=27).

Taxon	Annotation	Mean relative abundance		*p*-value	
		Chinese	Africans	*p*-value	FDR
Bacteroidetes	**Phylum**	65.14%	53.54%	**0.000999**	0.0025974
Bacteroidia	Class	64.91%	52.71%	**0.000999**	0.007659
Bacteroidales	Order	64.91%	52.71%	**0.000999**	0.013986
Bacteroidaceae	Family	43.22%	22.24%	**0.001998**	0.021312
Bacteroides	Genus	42.83%	22.17%	**0.001998**	0.040245
Porphyromonadaceae	Family	3.74%	3.75%	0.993007	1
Parabacteroides	Genus	2.99%	2.91%	0.895105	1
Rikenellaceae	Family	2.09%	1.7%	0.462537	1
Alistipes	Genus	2.09%	1.7%	0.462537	1
Prevotellaceae	Family	14.88%	22.8%	0.208791	0.668131
Prevotella	Genus	11.08%	15.54%	0.348651	0.927543
Firmicutes	**Phylum**	20.45%	23.54%	0.167832	0.311688
Clostridia	Class	16.67%	19.23%	0.206793	0.475624
Clostridiales	Order	16.66%	19.2%	0.208791	0.584615
Clostridiaceae	Family	1.34%	0%	**0.030969**	0.198202
Lachnospiraceae	Family	4.01%	4.65%	0.381618	0.939367
Oscillospiraceae	Family	0%	1.42%	**0.041958**	0.223776
Negativicutes	Class	3.01%	2.28%	0.371628	0.610532
Ruminococcus	Genus	1.82%	2.27%	0.498501	1
Ruminococcaceae	Family	5.48%	5.58%	0.918082	1
Faecalibacterium	Genus	3.15%	2.46%	0.293706	0.898680
Selenomonadales	Order	1.08%	0%	0.519481	1
Selenomonadaceae	Family	1.06%	0%	0.519481	1
Megamonas	Genus	1.06%	0%	0.356643	0.931235
Acidaminococcales	Order	1.35%	0%	0.117882	0.380850
Acidaminococcaceae	Family	1.35%	0%	0.117882	0.443791
Phascolarctobacterium	Genus	1.35%	0%	0.110889	0.488605
Proteobacteria	**Phylum**	9.47%	10.17%	0.712288	0.841795
Betaproteobacteria	Class	4%	3%	0.090909	0.232323
Burkholderiales	Order	4%	3%	0.090909	0.318182
Sutterellaceae	Family	3.35%	2.62%	0.24975	0.729452
Sutterella	Genus	1.62%	1.13%	0.426573	1
Parasutterella	Genus	1.73%	0%	**0.003996**	0.070430
Gammaproteobacteria	Class	4.62%	6.27%	0.356643	0.610532
Enterobacterales	Order	4.23%	4.09%	0.927073	1
Enterobacteriaceae	Family	4.23%	4.09%	0.927073	1
Escherichia	Genus	2.94%	3.24%	0.824176	1
Aeromonadales	Order	0%	1.82%	**0.048951**	0.256993
Succinivibrionaceae	Family	0%	1.82%	**0.046953**	0.231153
Succinivibrio	Genus	0%	1.82%	**0.046953**	0.293967
Verrucomicrobia	**Phylum**	0%	1.45%	**0.000999**	0.002597
Spirochaetes	**Phylum**	0%	1.12%	0.077922	0.168831
Spirochaetia	Class	0%	1.12%	0.077922	0.232323
Spirochaetales	Order	0%	1.12%	0.077922	0.316592
Spirochaetaceae	Family	0%	1.12%	0.077922	0.331668
Treponema	Genus	0%	1.12%	0.077922	0.41736

The bold for values emphasizes the statistical significance.

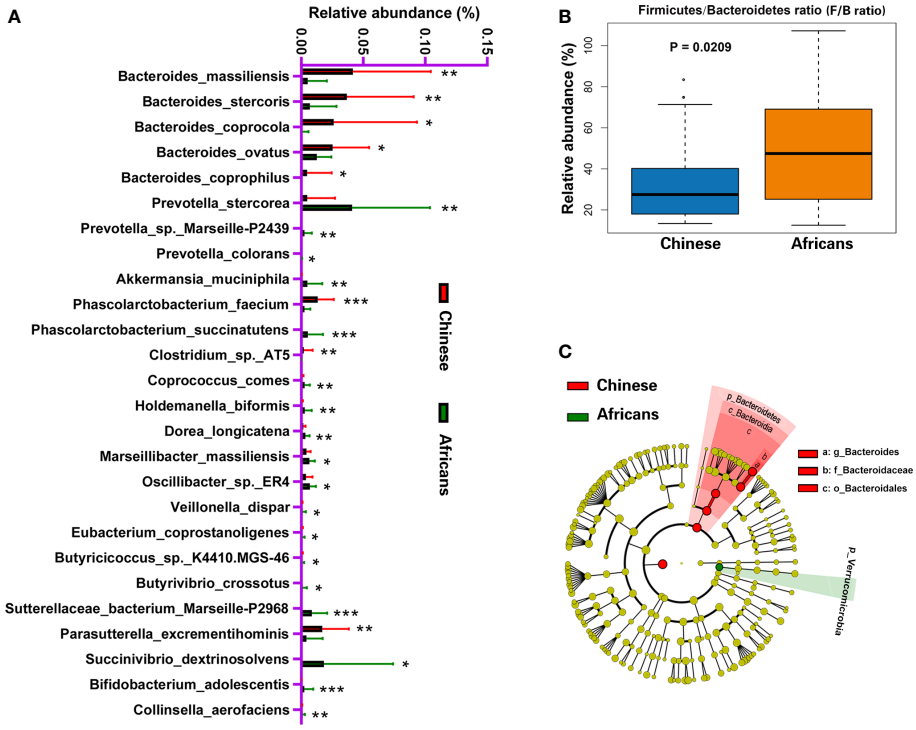

FIGURE 3
Distinct gut microbiota in Chinese and African groups. Bacterial species commonly and rarely present in either Chinese or Africans **(A)**. Boxplot showing the Firmicutes/Bacteroidetes ratio of community for the two groups **(B)**. The cladogram **(C)** was generated to depict the key and most differentially abundant taxa associated with ethnicity in Chinese (red) and Africans (green). Logarithmic LDA score = 3.8, and α-value = 0.05. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

and *M. massiliensis*. Measures of body fat distribution, A/G ratio, trunk/leg fat ratio, and $FMR_{trunk\text{-}to\text{-}limb}$ positively correlated with *B. massiliensis* and *Clostridium* sp. AT5 and negatively associated with *M. massiliensis*, *Oscillibacter* sp. ER4 and *B. crossotus*. In addition, trunk/leg fat ratio and $FMR_{trunk\text{-}to\text{-}limb}$ showed a negative association with Firmicutes/Bacteroidetes ratio and *A. muciniphila*, and $FMR_{trunk\text{-}to\text{-}limb}$ also showed a negative association with phylum Verrucomicrobia and species *C. comes* and *B. adolescentis*, and A/G ratio was positively associated with *S. dextrinosolvens* and negatively correlated with *Butyricicoccus* sp. K4410.MGS-46. Another indicator of fat distribution, hip circumference, was positively associated with *V. dispar*. Total and direct bilirubin were positively associated with *B. massiliensis* and negatively correlated with *P. stercorea*, *H. biformis*, and *S. bacterium* Marseille-P2968, and total bilirubin was also correlated negatively with *E. coprostanoligenes*. Further associations were observed: lower levels of insulin sensitivity (HOMA-IR 30 min) and levels of plasma glucose at 30 min were positively associated with *B. massiliensis* and negatively correlated with Firmicutes/Bacteroidetes ratio, *Prevotella* sp. Marseille-P2439, *P. succinatutens*, *Oscillibacter* sp. ER4, and *Collinsella aerofaciens*; HOMA-IR 30 min was also positively correlated with phylum Bacteroidetes and negatively correlated with species *B. adolescentis*; and glucose 30 min positively correlated with species *V. dispar* (all $p < 0.05$, Figures 4A, B).

4 Discussion

This study is the first study to directly characterize the diversity and profile of gut microbiota in a group of adults and healthy Chinese and African subjects with NGT.

Accumulating studies have reported that gut microbiota acts as a crucial modulator of fat storage, glucose, and energy metabolism (12, 13). Currently, there is evidence that gut microbiota plays a causative role in the pathogenesis of metabolic disorders such as obesity, insulin resistance, and T2DM (10–12, 17, 18, 31), although many factors such as genetics and environment-related factors, including diets, lifestyle changes, geographical location, and migration, can shape the human gut microbiota community (19–25), leading to microbiota dysbiosis. This dysbiosis is associated with metabolic disorders (12, 13, 15, 16). Our study demonstrated host physiology–microbiota interactions in healthy individuals and also characterized specific bacteria associated with

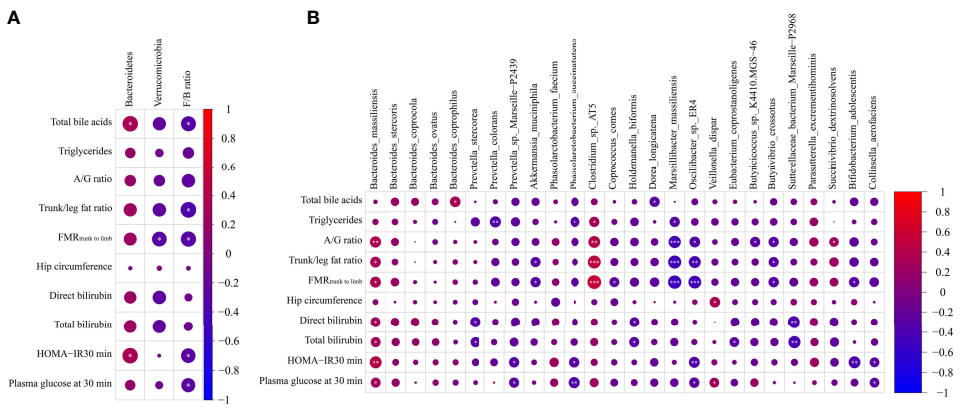

FIGURE 4
Heatmap spearman correlation analysis between parameters related to glucose metabolism and relative abundance of gut microbiome at both phylum **(A)** and species **(B)** levels in the Chinese group (n = 27) and African group (n = 29). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

glucometabolic pathways. The metabolic disorders identified in our study included adiposity, hyperlipidemia, insulin resistance, and hyperglycemia. These metabolic disorders significantly increased as microbiota diversity, richness, and composition decreased in Chinese group with a body mass index (BMI) of 22.46 kg/m^2 than in their African counterparts. These findings indicate that gut microbiota is associated with glucose regulation and utilization *in vivo*. The human microbiota is classified as the second genome due to its capability of carrying more than 98% of the genetic activity (32). Metagenomics is the study used to assess genetic material directly from environmental samples. In our study, we sequenced the V3–V4 region of the 16S rRNA gene to analyze the microbial community in the feces of Chinese and African groups. Rarefaction curves indicated that the diversity and abundance of gut microbiota in the Chinese group were relatively lower than those in the African group. Alpha diversity is a measure indicating various microbial species in stool samples. The higher Alpha diversity is an indicator of high abundance in a sample (33). In our study, the Alpha diversity measures included Chao 1 and observed species (indicator of microbiome richness), and Shannon index (indicator of microbiome richness and diversity). These indicators indicated lower microbiome richness and diversity in the Chinese group. Beta diversity is an indicator of gut microbiota heterogeneity between samples within each group. A higher beta diversity indicates greater differences in the composition of gut microbiota between samples in a specific group (33). We used the Bray–Curtis distance matrix to compare the heterogeneity in gut microbial communities and detected segregated clustering patterns in the Chinese and African groups, suggesting that the gut microbial community of the Chinese group is relatively unique from that of the African group. Collectively, these data indicate that the two groups were dissimilar to each other in the context of gut microbiota composition, richness, and diversity, despite the similar BMI. A high composition, high diversity, and microbiome stability are key indicators of healthy gut microbial communities (12). Recent findings demonstrate that a decline in the gut microbiota composition and diversity is linked with the prevalence of metabolic disorders (12). In both lean and obese individuals, low gut microbiome richness and diversity are linked with an increase in body adiposity, dyslipidemia, insulin resistance, and inflammation (34). Additionally, a defect in microbial diversity has been recently reported to reduce ecosystem functions and services (35). The human gut microbiota is a complex and diversified ecosystem with diverse bacteria that are dominated by the five major bacterial phyla, including Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, and Actinobacteria (36). Among these, Firmicutes and Bacteroidetes account up to 90% of all gut bacteria (36). In contrast, our study detected that the top 5 phyla of the gut microbiota in adults were Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicobia, and Spirochaetes. However, our study agrees with the aforementioned findings that Bacteroidetes and Firmicutes are the most prevalent in the microbiota. These differences may be attributed to the subject's characteristics, various environmental factors, and genetic factors (21, 23), although high-throughput sequencing technology demonstrated that the most prevalent bacterial phyla are Bacteroidetes and Firmicutes both in the Chinese and African groups. However, their abundance differed between groups. Indeed, we observed several different species belonging to the Firmicutes phylum such as *P. succinatutens*, *C. comes*, *H. biformis*, *D. longicatena*, *M. massiliensis*, *Oscillibacter* sp. ER4, *V. dispar*, *E. coprostanoligenes*, *Butyricicoccus* sp. K4410.MGS-46, and *B. crossotus*, and species belonging to Bacteroidetes phylum such as *P. stercorea*, *Prevotella* sp. Marseille-P2439, and *P. colorans* were significantly abundant in the gut microbiome of

the African group, whereas species belonging to phylum Firmicutes such as *P. faecium* and *Clostridium* sp. AT5 and species belonging to phylum Bacteroidetes such as *B. massiliensis*, *B. stercoris*, *B. coprocola*, *B. ovatus*, and *B. coprophilus* were enriched in the Chinese group. Both Firmicutes and Bacteroidetes are responsible for the metabolism of the carbohydrates (37). Firmicutes and Bacteroidetes are also involved in energy generation and conversion, transport and metabolism of amino acids, and production of short-chain fatty acids (SCFAs) (37). A great number of studies have extensively emphasized the contributions of microbiota to health and disease. In the gut, SCFAs hold a protective role against enteric/bacterial pathogens, thereby playing antimicrobial and anti-inflammatory activities (38). The SCFAs also augment energy expenditure and increase glucose tolerance by fostering gut motility and hormone secretion (39). We identified *Bacteroides* and *Prevotella* enterotypes whose functions are opposite. In humans, *Bacteroides* has been associated with high fat and protein intake (40), whereas *Prevotella* has been associated with high intake of fiber-rich diets (40). In addition, the genus *Prevotella* is known to produce great amounts of SCFAs (41), indicating the role of *Prevotella* in gut health status. Depletion in *Prevotella* may suggest a decrease in SCFAs, which are the major source of energy for enterocytes. This reduction in SCFAs increases mucosa permeability, resulting in bacterial translocation to the blood flow and extraintestinal organs (42) and, thus, metabolic disorders (43). Our results indicated that *Bacteroides* had significantly increased abundance in the Chinese group, while *Prevotella* had increased abundance in the African group. *Bacteroides* may become a member of the human flora immediately after birth, and species of this genus are genetic (44). *Bacteroides* is a genus that belongs to the phylum Bacteroidetes and has the capability to deconjugate and desiccate the primary bile acids and control their conversion into secondary bile acids (45). In addition, *Bacteroides* is known as an enterotoxin and can reduce insulin sensitivity by producing proinflammatory cytokines and lipopolysaccharides (4). These findings support our results regarding the effect of gut microbiota on host metabolism, and this could have resulted in several metabolic disorders observed, including excessive fat accumulation, hyperlipidemia, insulin resistance, and hyperglycemia. To date, *Bacteroides* is a recognized independent risk factor for T2DM (4). It is still completely unclear why Asians increase the incidence of diabetes at an early age for any given BMI than Africans and other ethnic groups, including Europeans (6, 7). It is noteworthy to mention that the Verrucomicrobia, a hallmark of glucose homeostasis and healthy gut, was more significantly prevalent in the African group only. This finding is of great importance considering these properties that are attributed to this microorganism, and these results represent a milestone baseline that will allow characterizing dysbiosis in the major diseases affecting the African population. *Akkermansia muciniphila*, a typical verrucomicrobia is the only detected species of this phylum in our study and enriched its abundance in favor of the African group. *Akkermansia muciniphila* is a Gram-negative, mucus-degrading bacterium with anti-inflammatory and immunostimulant functions and has probiotic properties (12). Previous studies have demonstrated that *A. muciniphila* can ameliorate obesity, insulin sensitivity, and endotoxinemia (46, 47). *Akkermansia muciniphila* can also regulate lipid metabolism, control adipocytes distribution, maintain glucose homeostasis, and restore gut barrier function (46, 47). *Akkermansia muciniphila* depletion is linked with obesity, insulin resistance, T2DM, and cardiometabolic disorders both in rodents and humans (11, 12), which suggests that the decline in this bacterium may also have significantly contributed to these metabolic disorders observed in our study. Accumulating evidence from animal studies shows that *A. muciniphila* can delay the onset of diabetes by promoting gut microbiota remodeling (48) and can also decrease fat mass development and alleviate dyslipidemia and insulin resistance (49). People with a higher abundance of *A. muciniphila* are characterized by a healthier metabolic status, especially in body fat distribution, triglycerides, and glucose levels, and have greater insulin sensitivity (46). This indicates that gut microbiome stability plays a prominent role in sustaining the host's metabolic integrity, thereby contributing to energy harvest and metabolic regulation. It is surprising that Africans are more insulin resistant, while they have lower adiposity and good ability to secrete insulin (5, 8, 9). There is evidence suggesting the sex-specific pathways or responses to metabolic disorders, especially in Africans (50, 51). Indeed, our gender-specific analysis revealed that the African women were more likely to have aberrant glucose homeostasis, while men were more likely to have dyslipidemia that is characterized with abnormal LDL cholesterol. The present study has other important observation such as the detection of exclusive bacterial taxa in the Chinese and African groups. The relative abundances of Clostridiaceae and *Parasutterella* were significant and present in the Chinese group only, whereas the relative abundances of Aeromonadales, Oscillospiraceae, Succinivibrionaceae, and *Succinivibrio* were significantly in the African group and absent in Chinese group, suggesting the distinct microbiota signatures associated with these groups. Moreover, our study found phylum Spirochaetes that was previously reported in hunter–gatherer populations to be enriched in the African group and absent in the Chinese group (21). The presence and role of these taxa in the gut microbiota of Chinese and Africans should be examined in more detailed large-scale studies to confirm the present findings. The gut microbiota regulates various host metabolic pathways, which physiologically link the gut, pancreas, liver, adipose tissue, skeletal muscle, and brain *via* multiple mechanisms, including (1) energy extraction by absorption and digestion of monosaccharides and fibers into SCFAs, (2)

modulation of fat storage *via* SCFAs, and (3) translocation of bacteria and their products by binding to G-protein-coupled receptors (GPCRs) that are expressed by enteroendocrine cells (12). The gut-microbiota-mediated pathways further interacted with the production of gut hormones such as GLP-1, leading to enhanced energy expenditure, decreased food intake, and improved lipid and glucose metabolism and insulin biosynthesis (secretion) and sensitivity (12). Gut microbiota also affects host metabolism by modulating various metabolites including bile acids (52). Bile acids are important signaling molecules and act as metabolic regulators that support digestion by facilitating intestinal absorption and transport of lipids (53). Excessive accumulation of bile acids in the liver or circulation results in malabsorption of fat and deposition of toxic xenobiotics and endobiotics, and this can damage cells and organs in the gastrointestinal tract (53). Gut dysbiosis is the term commonly used to refer to unbalanced gut microbiota, which is associated with an unhealthy outcome (54). Dysbiosis of the gut microbiota leads to improper microbial-derived metabolite signaling, intestinal barrier dysfunction, oxidative stress, and immune dysregulation (12). This dysbiosis can also cause abnormal aryl hydrocarbon receptor (AHR) and GLP-1 resistance and decrease G-protein receptor expression, which result in the development of obesity, insulin resistance, and T2DM (Zhang, 12, 13, 43, 55). The Firmicutes/Bacteroidetes ratio (F/B ratio) has been proposed as an important marker for gut microbial dysbiosis (56). A recent study comparing insulin-sensitive and insulin-resistant obese subjects found that the Firmicutes/Bacteroidetes ratio increased as insulin sensitivity increased (57), suggesting its role in glucose–insulin homeostasis. Our study found that the Chinese group had a significant decrease in Firmicutes content and Firmicutes/Bacteroidetes ratio compared with African group. The change in (F/B ratio) is associated with various metabolic disorders in humans (56, 58) and has negative correlations with glucose levels (58), which is in agreement with our study findings. Further correlations between gut microbiota and factors that are involved in glucose metabolism were observed. For example, we found that the phylum Bacteroidetes was positively correlated with total bile acids and $HOMA_{\text{-IR30min}}$, and the species *B. massiliensis* belonging to Bacteroidetes was positively associated with A/G ratio, trunk/leg fat ratio, $FMR_{\text{trunk-to-limb}}$, $HOMA_{\text{-IR30min}}$, and levels of bilirubin and glucose (glucose 30 min). Verrucomicrobia was negatively correlated with $FMR_{\text{trunk-to-limb}}$, and its species *A. muciniphila* was negatively associated with both trunk/leg fat ratio and $FMR_{\text{trunk-to-limb}}$. These results indicate that the gut microbiome composition may be implicated in the modulation of glucose metabolism in non-obese conditions. Our study combined both data from the two groups to analyze associations between the gut microbiome and parameters of glucose metabolism; this should be regarded as a confounding factor. An individual's genetic makeup affects the composition of the key microbiome (20). For example, the microbiota of identical twins living separately is significantly more alike than those of uncoupled individuals (20). Contrarily, the environment appears to have minor significance, since married couples did not have a significantly higher similarity of microbial communities than uncoupled individuals, even though these couples lived in the same environment with similar dietary practices (19). In the same manner, in a 16S rRNA sequencing study comparing the gut microbiota of 2,084 subjects from many different countries who live in the same city, the genetic background explained the dissimilarities in microbiome composition (23). However, a recent study by Vengay and collaborators investigated the impact of migration on microbiome composition and showed that migration to the United States profoundly affects the microbiome in the long term even after several generations (24), indicating that migrancy has an important impact on health. Overall, our results showed that there were associations between the gut microbiota, host physiology, and glucometabolic pathways, which can play a significant role in the occurrence and evolution of metabolic disorders. Despite the importance of understanding the connection between environmental factors, host genetics, microbiota, and health disparities, there are no findings on how the baseline gut microbiotas of Chinese and African healthy individuals are linked with their metabolic phenotypes. We observed differences in the gut microbiome that were associated with the metabolic phenotypes of the two groups. Although the microbiome status at the group level is very different, there were some overlaps, too. These differences in microbiome composition may be explained by the factors such as genetic background, current diet and lifestyle, and even more by migrancy. The present study revealed diverse gut microbiome and metabolic phenotypes in two closely matched healthy groups of people who have lived in the same city for at least a year and characterized specific microbiome associated with glucometabolic pathways. Our LEfSe and metastats analyses found differentially abundant and core bacterial taxa in the Chinese and African groups, and these taxa could be potential biomarkers. This study has some limitations. We did not collect information about the lifestyle and dietary nutrition of participants to evaluate if there was any association between nutrient intake or physical activity level (lifestyle) and differences in the composition of the gut microbiota. Another limitation is that the sample size was too small and included only healthy subjects. Further large-scale longitudinal studies wherein the subjects are followed over a long period of time (evaluation from insulin sensitive to obesity to insulin resistance and T2DM) would confirm a potential and dynamic change in microbiome status, genetic diversity, and general metabolic response with

diverse statuses of glucose metabolism, and could determine causality.

5 Conclusion

This study gives evidence of an interaction between the gut microbiome, host physiology, and glucometabolic pathways, and this could contribute to adiposity and pathophysiology of dyslipidemia, insulin resistance, and hyperglycemia. Interestingly, the gut microbiota reveals a high abundance of the phylum Bacteroidetes in the Chinese group and phylum Verrucomicrobia and Firmicutes/Bacteroidetes ratio (F/B ratio) in the African group. Furthermore, the abundance of some bacteria related to metabolism was associated with glucose-metabolism-related parameters. These findings provide an important basis for determining the relation between the gut microbiome and the pathogenesis of various metabolic disorders and constitute the road map to examine further mechanisms related to gut dysbiosis in the disease conditions.

Data availability statement

The data presented in this study are deposited in the NCBI repository, accession number PRJNA853567.

Ethics statement

This study was reviewed and approved by Medical Ethics Committee of Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MXL and PN conceived this study. PN, MXL, and BYX established the analysis design. MXL, PN, XHL, CJL, and ZHL contributed to the statistical analysis plan. PN, BYX, LRL, LPL, TTL, and MJ were responsible for data collection and statistical analyses. PN wrote the manuscript. All authors contributed to the interpretation of the findings and the manuscript's critical revision. All authors have read and approved the final version of the manuscript. MXL and PN had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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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.

Data are presented as percentage (%), and were calculated between the relative abundance of bacterial taxa at multiple taxonomic levels.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.942383/full#supplementary-material

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9 (th) edition." *Diabetes Res Clin Pract* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843

2. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. "Epidemiology of type 2 diabetes - global burden of disease and forecasted trends." *J Epidemiol Glob. Health* (2020) 10(1):107–11. doi: 10.1016/j.diabres.2019.107843

3. Xue H, Wang C, Li Y, Chen J, Yu L, Liu X, et al. "Incidence of type 2 diabetes and number of events attributable to abdominal obesity in China: A cohort study." *J Diabetes* (2016) 8(2):190–8. doi: 10.1111/1753-0407.12273

4. Wang J, Li W, Wang C, Wang L, He T, Hu H, et al. "Enterotype bacteroides is associated with a high risk in patients with diabetes: A pilot study." *J Diabetes Res* (2020) 2020:6047145. doi: 10.1155/2020/6047145

5. Kodama K, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. "Ethnic differences in the relationship between insulin sensitivity and insulin response: A systematic review and meta-analysis." *Diabetes Care* (2013) 36(6):1789–96. doi: 10.2337/dc12-1235

6. Yabe D, Seino Y, Fukushima M, Seino S. "β cell dysfunction versus insulin resistance in the pathogenesis of type 2 diabetes in East asians." *Curr Diabetes Rep* (2015) 15(6):602. doi: 10.1007/s11892-015-0602-9

7. Hu C, Jia W. "Diabetes in China: Epidemiology and genetic risk factors and their clinical utility in personalized medication." *Diabetes* (2018) 67(1):3–11. doi: 10.2337/dbi17-0013

8. Bello O, Mohandas C, Shojee-Moradie F, Jackson N, Hakim O, Alberti K, et al. "Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat." *Diabetologia* (2019) 62(5):835–44. doi: 10.1007/s00125-019-4820-6

9. Goedecke JH, Olsson T. "Pathogenesis of type 2 diabetes risk in black africans: A south African perspective." *J Intern Med* (2020) 288(3):284–94. doi: 10.1111/joim.13083

10. Rastelli M, Cani PD, Knauf C. "The gut microbiome influences host endocrine functions." *Endocr Rev* (2019) 40(5):1271–84. doi: 10.1210/er.2018-00280

11. Cani PD, Van Hul M, Lefort C, Depommier C, Rastelli M, Everard A. "Microbial regulation of organismal energy homeostasis." *Nat Metab* (2019) 1 (1):34–46. doi: 10.1038/s42255-018-0017-4

12. Fan Y, Pedersen O. "Gut microbiota in human metabolic health and disease." *Nat Rev Microbiol* (2021) 19(1):55–71. doi: 10.1038/s41579-020-0433-9

13. Singh R, Zogg H, Wei L, Bartlett A, Ghoshal UC, Rajender S, et al. "Gut microbial dysbiosis in the pathogenesis of gastrointestinal dysmotility and metabolic disorders." *J Neurogastro. Motil.* (2021) 27(1):19–34. doi: 10.5056/jnm20149

14. Wei L, Yue F, Xing L, Wu S, Shi Y, Li J, et al. "Constant light exposure alters gut microbiota and promotes the progression of steatohepatitis in high fat diet rats." *Front Microbiol* (2020) 11:1975. doi: 10.3389/fmicb.2020.01975

15. Winer DA, Luck H, Tsai S, Winer S. "The intestinal immune system in obesity and insulin resistance." *Cell Metab* (2016) 23(3):413–26. doi: 10.1016/j.cmet.2016.01.003

16. He FF, Li YM. "Role of gut microbiota in the development of insulin resistance and the mechanism underlying polycystic ovary syndrome: a review." *J Ovarian Res* (2020) 13(1):73. doi: 10.1186/s13048-020-00670-3

17. Schoeler M, Caesar R. "Dietary lipids, gut microbiota and lipid metabolism." *Rev Endocr Metab Disord* (2019) 20(4):461–72. doi: 10.1007/s11154-019-09512-0

18. Basak S, Banerjee A, Pathak S, Duttaroy AK. "Dietary fats and the gut microbiota: Their impacts on lipid-induced metabolic syndrome." *J Funct Foods* (2022) 91:105026. doi: 10.1016/j.jff.2022.105026

19. Zoetendal EG, Akkermans ADL, Vliet WMA-v, Visser JA, Vos W. "The host genotype affects the bacterial community in the human gastrointestinal tract." *Microbial. Ecol Health Dis* (2001) 13:129–34. doi: 10.1016/j.cell.2018.10.029

20. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. "A core gut microbiome in obese and lean twins." *Nature* (2009) 457 (7228):480–4. doi: 10.1038/nature07540

21. Gupta VK, Paul S, Dutta C. "Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity." *Front Microbiol* (2017) 8:1162. doi: 10.3389/fmicb.2017.01162

22. Winglee K, Howard AG, Sha W, Gharaibeh RZ, Liu J, Jin D, et al. "Recent urbanization in China is correlated with a westernized microbiome encoding increased virulence and antibiotic resistance genes." *Microbiome* (2017) 5(1):121. doi: 10.1186/s40168-017-0338-7

23. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, et al. "Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography." *Nat Med* (2018) 24(10):1526–31. doi: 10.1038/s41591-018-0160-1

24. Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, et al. "US immigration westernizes the human gut microbiome." *Cell* (2018) 175(4):962–972.e910. doi: 10.1016/j.cell.2018.10.029

25. Lu J, Zhang L, Zhai Q, Zhao J, Zhang H, Lee YK, et al. "Chinese gut microbiota and its associations with staple food type, ethnicity, and urbanization." *NPJ Biofilms Microbiomes.* (2021) 7(1):71. doi: 10.1038/s41522-021-00245-0

26. World Health O, International Diabetes F. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation*. Geneva: World Health Organization (2006).

27. Morciano A, Romani F, Sagnella F, Scarinci E, Palla C, Moro F, et al. "Assessment of insulin resistance in lean women with polycystic ovary syndrome." *Fertil. Steril.* (2014) 102(1):250–256.e253. doi: 10.1016/j.fertnstert.2014.04.004

28. Stancáková A, Javorský M, Kuulasmaa T, Haffner SM, Kuusisto J, Laakso M. "Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 Finnish men." *Diabetes* (2009) 58(5):1212–21. doi: 10.2337/db08-1607

29. Matthews JN, Altman DG, Campbell MJ, Royston P. "Analysis of serial measurements in medical research." *Bmj* (1990) 300(6719):230–5. doi: 10.1136/bmj.300.6719.230

30. Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y, Zhou HW, et al. "Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland." *Microb Ecol* (2013) 66(1):96–104. doi: 10.1007/s00248-013-0238-8

31. Zaky A, Glastras SJ, Wong MYW, Pollock CA, Saad S. "The role of the gut microbiome in diabetes and obesity-related kidney disease." *Int J Mol Sci* (2021) 22 (17):151–70. doi: 10.3390/ijms22179641

32. Grice EA, Segre JA. "The human microbiome: our second genome." *Annu Rev Genomics Hum Genet* (2012) 13:151–70. doi: 10.1146/annurev-genom-090711-163814

33. Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, et al. "Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention." *Nat Med* (2017) 23(7):859–68. doi: 10.1038/nm.4358

34. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. "Richness of human gut microbiome correlates with metabolic markers." *Nature* (2013) 500(7464):541–6. doi: 10.1038/nature12506

35. Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, et al. "Microbial diversity drives multifunctionality in terrestrial ecosystems." *Nat Commun* (2016) 7:10541. doi: 10.1038/ncomms10541

36. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. "Diversity of the human intestinal microbial flora." *Science* (2005) 308 (5728):1635–8. doi: 10.1126/science.1110591

37. Kumar Singh A, Cabral C, Kumar R, Ganguly R, Kumar Rana H, Gupta A, et al. "Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficiency." *Nutrients* (2019) 11(9):S100–105. doi: 10.3390/nu11092216

38. Kles KA, Chang EB. "Short-chain fatty acids impact on intestinal adaptation, inflammation, carcinoma, and failure." *Gastroenterology* (2006) 130 (2 Suppl 1):S100–105. doi: 10.1053/j.gastro.2005.11.048

39. Kimura I, Inoue D, Hirano K, Tsujimoto G. "The SCFA receptor GPR43 and energy metabolism." *Front Endocrinol (Lausanne)* (2014) 5:85. doi: 10.3389/fendo.2014.00085

40. Fujio-Vejar S, Vasquez Y, Morales P, Magne F, Vera-Wolf P, Ugalde JA, et al. "The gut microbiota of healthy Chilean subjects reveals a high abundance of the phylum verrucomicrobia." *Front Microbiol* (2017) 8:1221. doi: 10.3389/fmicb.2017.01221

41. Chen T, Long W, Zhang C, Liu S, Zhao L, Hamaker BR. "Fiber-utilizing capacity varies in prevotella- versus bacteroides-dominated gut microbiota." *Sci Rep* (2017) 7(1):2594. doi: 10.1038/s41598-017-02995-4

42. Parada Venegas D, de la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, et al. "Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases." *Front Immunol* (2019) 10:277. doi: 10.3389/fimmu.2019.00277

43. Zhang D, Zhang L, Yue F, Zheng Y, Russell R. "Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation." *Eur J Endocrinol* (2015) 172(1):29–36. doi: 10.1530/EJE-14-0589

44. Wexler HM. "Bacteroides: the good, the bad, and the nitty-gritty." *Clin Microbiol Rev* (2007) 20(4):593–621. doi: 10.1128/CMR.00008-07

45. Fukiya S, Arata M, Kawashima H, Yoshida D, Kaneko M, Minamida K, et al. "Conversion of cholic acid and chenodeoxycholic acid into their 7-oxo derivatives by bacteroides intestinalis AM-1 isolated from human feces." *FEMS Microbiol Lett* (2009) 293(2):263–70. doi: 10.1111/j.1574-6968.2009.01531.x

46. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, et al. "Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology." *Gut* (2016) 65(3):426–36. doi: 10.1136/gutjnl-2014-308778

47. Xu Y, Wang N, Tan HY, Li S, Zhang C, Feng Y. "Function of akkermansia muciniphila in obesity: Interactions with lipid metabolism, immune response and gut systems." *Front Microbiol* (2020) 11:219. doi: 10.3389/fmicb.2020.00219

48. Hänninen A, Toivonen R, Pöysti S, Belzer C, Plovier H, Ouwerkerk JP, et al. "Akkermansia muciniphila induces gut microbiota remodelling and controls islet autoimmunity in NOD mice." *Gut* (2018) 67(8):1445–53. doi: 10.1136/gutjnl-2017-314508

49. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al. "A purified membrane protein from akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice." *Nat Med* (2017) 23 (1):107–13. doi: 10.1038/nm.4236

50. Moore JX, Chaudhary N , Akinyemiju T. "Metabolic syndrome prevalence by Race/Ethnicity and sex in the united states, national health and nutrition examination survey 1988-2012." *Prev Chronic Dis* (2017) 14:E24. doi: 10.1136/gutjnl-2017-314508

51. Jaspers Faijer-Westerink H, Kengne AP, Meeks KAC, Agyemang C. "Prevalence of metabolic syndrome in sub-Saharan Africa: A systematic review and meta-analysis." *Nutr Metab Cardiovasc Dis* (2020) 30(4):547–65. doi: 10.1016/j.numecd.2019.12.012

52. Schroeder BO, Bäckhed F. "Signals from the gut microbiota to distant organs in physiology and disease." *Nat Med* (2016) 22(10):1079–89. doi: 10.1038/nm.4185

53. Chiang JY. "Bile acid metabolism and signaling." *Compr Physiol* (2013) 3 (3):1191–212. doi: 10.1002/cphy.c120023

54. Hooks KB, O'Malley MA. "Dysbiosis and its discontents." *mBio.* (2017) 8 (5):1079–89. doi: 10.1128/mBio.01492-17

55. 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

56. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. "Microbial ecology: human gut microbes associated with obesity." *Nature* (2006) 444(7122):1022–3. doi: 10.1038/4441022a

57. Yuan X, Chen R, Zhang Y, Lin X, Yang X, McCormick KL. "Gut microbiota of Chinese obese children and adolescents with and without insulin resistance." *Front Endocrinol (Lausanne)* (2021) 12:636272. doi: 10.3389/fendo.2021.636272

58. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. "Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults." *PloS One* (2010) 5(2):e9085. doi: 10.1371/journal.pone.0009085

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Gut microbiota is correlated with gastrointestinal adverse events of metformin in patients with type 2 diabetes

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Aim: Gastrointestinal discomfort is the most common adverse event in metformin treatment for type 2 diabetes. The mechanism of action of metformin is associated with gut microbiota. However, the gut microbial community structure related to metformin-induced gastrointestinal adverse events remains unclear. This study aimed to investigate it.

Methods: 50 patients with newly diagnosed diabetes were treated with metformin 1500mg/d for 12 weeks. The patients were divided into two groups according to whether gastrointestinal adverse events occurred (group B) or did not occur (group A) after treatment. The fecal bacterial communities and short-chain fatty acids (SCFAs) were sequenced and compared. 70 diabetes mice were randomly divided into 8 groups and treated with metformin (Met), clindamycin (Clin) and/or SCFA, which were the Met+/Clin+, Met+/Clin-, Met-/Clin+, Met-/Clin-, Met+/SCFA+, Met+/SCFA-, Met-/SCFA+ and Met-/SCFA- group. After 4 weeks of metformin treatment, blood glucose, food intake, fecal SCFAs, gut microbiota and gut hormones were measured.

Results: Metformin increased the abundance of *Phascolarctobacterium*, *Intestinimonas* and *Clostridium III*. Functional prediction analysis showed that the propanoate metabolism pathway was significantly up-regulated. The concentrations of acetic acid and propanoic acid in feces were significantly increased. The abundance of *Clostridium sensu stricto, Streptococcus* and *Akkermansia* induced by metformin in group B was higher than that in group A. The propanoate metabolism pathway and propanoic acid in feces were significantly up-regulated in group B. In the animal experiments, the food intake decreased and glucose control increased in metformin groups compared with those in the control groups. The total GLP-1 level in the Met+/Clin- group was significantly higher than that in the Met-/Clin- group, while there was no statistical difference between the Met-/Clin- and Met+/Clin+ group. The total GLP-1 level in the Met-/SCFA+ group was significantly higher than that in the Met-/SCFA-group, while the levels of total GLP-1 and active GLP-1 in the Met+/SCFA- group and the Met+/SCFA+ group were significantly higher than those in the Met-/SCFA-group.

Conclusions: Our data suggest that metformin promotes the secretion of intestinal hormones such as GLP-1 by increasing the abundance of SCFA-producing bacteria, which not only plays an anti-diabetic role, but also may causes gastrointestinal adverse events.

KEYWORDS

type 2 diabetes, metformin, gastrointestinal adverse events, gut microbiota, short-chain fatty acids (SCFAs)

Introduction

Type 2 diabetes is a metabolic syndrome that is primarily induced by β-cell dysfunction and insulin resistance (1). Insulin sensitivity and diabetes progression are closely related to the modulation of gut microbial composition (2). The insulin sensitizer metformin is recommended as the preferred, initial medication for the treatment of type 2 diabetes among numerous antidiabetic agents (3). It is effective and inexpensive, does not stimulate insulin secretion and does not cause hypoglycemia, and may reduce the risk of cardiovascular outcomes in diabetic people (4). However, the principal side effects of metformin are gastrointestinal adverse events due to abdominal pain, abdominal distention, diarrhea, nausea and inappetence. The high frequency (20-35%) of gastrointestinal adverse events was the most prevalent problem with metformin (5, 6), especially in elderly patients (about 54%) (7) and patients with Helicobacter pylori infection (about 62%) (8).

Two elegant studies by Forslund et al. in 2015 (6) and de la Cuesta-Zuluaga et al. in 2017 (9) addressed the drug signatures in the gut microbiome of diabetic patients. They indicated that metformin functioned by increasing short-chain fatty acids (SCFAs) production and elevating *Akkermansia* and *Escherichia*. The enteric nervous system may be directly or indirectly affected by the gut microbiota and its metabolites or signals (such as SCFAs, neurotransmitters, etc.), regulating the process of glucose and lipid metabolism in fat, liver, and brain. Both duodenal total glucagon-like peptide-1 receptor (GLP-1R)-protein kinase A signaling and a neuronal-mediated gut-brain-liver pathway were required for metformin to lower hepatic glucose production and plasma glucose levels (10). However, the mechanism of gastrointestinal adverse effects of metformin remains not fully understood until now. Metformin is highly water-soluble, which can irritate the gastrointestinal mucosa after entering the gastrointestinal tract. In addition, metformin increases the bile acid pool within the intestine predominantly through reduced ileal absorption (11). This disruption of the enterohepatic circulation of bile salts has potential consequences for diarrhea. The alteration in bile acid absorption may increasing the concentration of intestinal peptide GLP-1 and causing upper digestive tract discomfort (12).

Our hypothesis suggests that metformin may cause gastrointestinal adverse events by regulating gut microbiota. This study aimed to illustrate the gut microbial community structure underlying metformin-induced gastrointestinal adverse events in elderly patients with type 2 diabetes and in diabetic mice. The difference in gut microbial community structure between diabetic patients with and without gastrointestinal adverse events was compared and illustrated. Also, the bacteria and clinical factors that might be associated with the incidence of metformin-induced adverse events were identified. Furthermore, we aimed to explore the relationship between gut microbiota, SCFAs, gut hormones and metformin-induced gastrointestinal adverse events in animal experiments.

Material and methods

Patients enrollment and study protocol

50 antidiabetic agents treatment-naïve patients with newly diagnosed type 2 diabetes, aged≥60 years, with body mass index≥18.5 kg/m^2 and Hemoglobin A1c (HbA1c) of 7.0%-9.0% were enrolled from a previous study (7). The exclusion criteria included the following: (a) confirmed or suspected type 1 diabetes; (b) previous treatment with insulin or other antidiabetic drugs for more than 14 days; (c) a history of known peptic ulcers, Helicobacter pylori infection, gastrointestinal surgery, chronic gastritis, gastrointestinal tumor or severe gastrointestinal discomfort; (d) current (within 3 months of screening) diabetic ketoacidosis or hyperosmolar coma; (e) current cardiovascular disease or other serious disease; (f) a creatinine clearance rate <60 mL/min; (g) liver enzymes more than 2 times the upper limit of normal at screening; (h) use of unknown combination drugs; and (i) poor drug compliance. This study was approved by the Ethics Committee of Huadong Hospital Affiliated to Fudan University, Shanghai, China (Ethics Number: 2018K065). Written informed consent was obtained from all subjects prior to the study.

Eligible people were randomized 1:1:1 to receive 1000 mg/d, 1500 mg/d or 2000 mg/d of metformin (Sino-American Shanghai Squibb Pharmaceuticals Ltd., Shanghai, China). This study has a 12 weeks treatment period. Biochemical measurements of plasma glucose, insulin and HbA1c were performed in a central laboratory at baseline and after 12 weeks. Body mass index (BMI, kg/m^2) was calculated by dividing body weight by the square of height. HbA1c was determined by high pressure liquid phase method (Bio-Rad variant II, USA), insulin was detected by chemiluminescence (Snibe MAGLUMI 4000, China); glucose was measured by hexokinase Colorimetry (HITACHI 7600 Series, Japan). Insulin sensitivity was calculated as the homeostasis model assessment of insulin resistance index (HOMA-IR) by using the HOMA Calculator (Headington, Oxford, UK; http://www.dtu.ox.ac.uk). The fecal samples were randomly collected from 50 patients in the metformin 1500m/d group before and after treatment. The patients were divided into two groups according to whether gastrointestinal adverse events occurred (group B) or did not occur (group A) after treatment. The fecal bacterial communities and SCFAs were tested and compared (Figure 1A). The SCFAs (including acetic acid, propanoic acid and butyric acid) in feces were determined by gas chromatography/mass spectrometry. Fecal samples were homogenized and diluted with distilled-deionized water in a ratio 1:1. An aliquot of 1 g was spiked with a combined standard solution of SCFAs diluted in water (organic acid kit ref. 47264, Supelco, Bellefonte, PA) to obtain curves in the range 25–750 ng/mL. The analytes were injected in the splitless mode into a gas chromatography system (Agilent GC6890, USA). The chromatographic peaks were checked for homogeneity using the extracted ions of the characteristic fragments to optimize the resolution and peak symmetry (13).

DNA extraction, gut microbiota analysis and data processing

Three DNA samples were extracted from each fecal sample collected from patients in the group A (n=27) and the group B (n=23) before and after treatment using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany). DNA samples at a dilution of 1 ng/μL were used as templates for amplification targeting the V4 variable region of the 16S rRNA with the barcoded primers (515F/806R). The high-fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA) and Phusion® High-Fidelity PCR Master Mix kit (New England Biolabs) was used for the amplification. PCR products were used for the construction of the DNA libraries using the TruSeq® DNA PCR-Free sample preparation kit (Illumina Inc., San Diego, CA, USA). The 16s rDNA sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). The sequencing data in the format of FASTQ was processed using the FLASH software (14). After data filtering using the Usearch and Uchime software, raw tags were quality-filtered using the Qiime software (15). The clustering of operational taxonomic units (OTU) was performed using the Usearch software (16). Bacterial taxonomies at the levels of phylum, family, and genus were annotated using the RDP classifier based on the Bergey's taxonomy and Naïve Bayesian assignment. The ribosomal

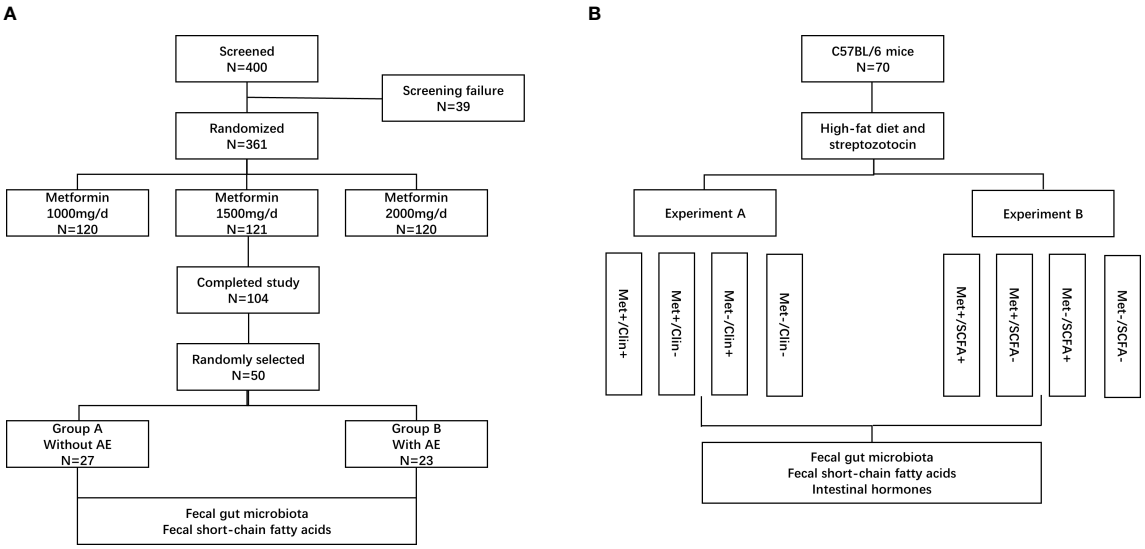

FIGURE 1
Patient disposition and study protocol. AE, adverse events; Met, metformin; Clin, clindamycin; SCFA, short-chain fatty acid. **(A)** clinical trial; **(B)** animal experiments.

RNA sequences were annotated in the SILVA ribosomal RNA gene database based on the same criteria. The alpha diversity indexes were analyzed using the Qiime (15) and the beta-diversity metrics were calculated using the weighted UniFrac algorithm (Vegan package R, version 2.0-10). Principal coordinate analysis (PCoA) was performed for all samples. The iconic bacteria in each group were identified using the linear discriminant analysis (LEfSe, version 1.1.0). The statistical significance among groups was assessed using the analysis of similarities (ANOSIM) of weighted UniFrac distances. The functional profiles of prokaryotic communities were predicted using Tax4Fun2 based on 16S rRNA sequencing data (17).

Animal experiments

The animal study was reviewed and approved by the Ethics Committee of Fudan University (Ethics Number: 202101004S). A total of 70 male 8-week-old C57BL/6 mice [Beijing Vital River Laboratory Animal Technology Co., Ltd., China, animal production license number: SCXK(Beijing) 2021-0011] were selected and treated with high-fat diet (Trophic Animal Feed High-Tech Co., Ltd., China) combined with low dose of streptozotocin (Merck KGaA, Darmstadt, Germany) to establish a type 2 diabetic mouse model. After four weeks on the high fat diet, the mice were treated with 75 mg/kg streptozotocin followed three days later with a second dose of streptozotocin (50 mg/kg) as needed. Mice with blood glucose ≥13.8 mmol/L were considered diabetic (18). It has been reported that intake of clindamycin reduced the total concentration of SCFAs in faeces (19). The mice were randomly divided into two experiments and treated with metformin (Sino-American Shanghai Squibb Pharmaceuticals Ltd., Shanghai, China, 200mg/kg/d), clindamycin (Shandong Fangming Co., Ltd., China, 15mg/kg/d) and/or propanoate (MedChemExpress LLC., USA, 300mg/kg/d). There were four groups in experiment A: 9 mice in the metformin and clindamycin group (Met+/Clin+), 8 mice in the metformin group (Met+/Clin-), 9 mice in the clindamycin group (Met-/Clin+), and 8 mice in the control group (Met-/Clin-). There were four groups in experiment B: 9 mice in the metformin and propanoate group (Met+/SCFA+), 9 mice in the metformin group (Met+/SCFA-), 9 mice in the propanoate group (Met-/SCFA+), and 9 mice in the control group (Met-/SCFA-) (Figure 1B).

After 4 weeks of metformin treatment, the body weight, blood glucose, food intake, intestinal hormones, fecal SCFAs and gut microbiota were measured. Glucose was measured by portable blood glucose meter (Roche ACCU-CHEK Performa). Intestinal tissue of mice was immersed in phosphate buffer solution (PBS). Dipeptidyl peptidase-4 (DPP-IV) inhibitor was rapidly added. Then it was homogenized, centrifuged and diluted to an appropriate concentration. The levels of total glucagon-like peptide-1 (GLP-1), active GLP-1 and peptide YY (PYY) in intestinal tissue were determined by ELISA according to the kit instructions (Millipore ELISA kits, USA) (20). The expression levels of GLP-1, GLP-1 receptor (GLP-1R) and PYY in intestinal tissue were detected by immunofluorescence. The intestinal tissues were paraffin embedded and sectioned. After baking overnight in the oven, xylene dewaxing was performed the next day. Then it was soaked with gradient alcohol (100%, 95%, 70%, 50%). They were incubated with primary antibody to GLP-1, GLP-1R and PYY (abcam, UK) at 4° C overnight. Sections were stained by 4',6-diamidino-2-phenylindole (DAPI, abcam, UK) and washed for 3 times with PBS. GLP-1, GLP-1R, PYY and DAPI were observed by a fluorescence microscope (Olympus, Japan) after triggered at 594 nm and 358 nm respectively (21). Image was analyzed quantitatively by Image J 1.8.0 (National Institutes of Health, USA). The SCFAs (including acetic acid, propanoic acid and butyric acid) in feces were determined by gas chromatography/mass spectrometry and according to the method described by de la Cuesta-Zuluaga et al. (13). Fecal bacterial communities in experiment A were determined using the method described above.

Statistical analyses

The demographic data was analyzed using the SPSS 23.0 software (SPSS Inc, Chicago, USA). Based on the result of the Kolmogorov-Smirnov test, normally and non-normally distributed continuous variables were expressed as mean ± standard deviation and median (interquartile range), respectively. The differences in dichotomous variables between groups were analyzed using the $\chi 2$ test, and the differences in continuous variables between groups were analyzed using the Mann-Whitney U test or t-test. Logistic regression analysis was performed to identify the bacteria or factors associated with metformin-induced gastrointestinal adverse effects and to calculate the 95% confident interval (CI) and odds ratio (OR). For all analyses, P values < 0.05 were considered statistically significant. The statistical diagrams were plotted using GraphPad Prism 5 (Graphpad software, Inc., La Jolla, CA, USA).

Results

Characteristics of patients

A total of 50 patients (27 males and 23 females) were included according to the inclusion and exclusion criteria. The average age of all patients was 68.52 ± 6.45 years and the average baseline fasting glucose was 8.13 ± 1.89 mmol/L. 23 patients (46.00%) developed gastrointestinal adverse events after metformin treatment. Accordingly, all patients were assigned into two groups: patients with adverse events (n = 23) in group B and patients without adverse events (n = 27) in group A. The

baseline characteristics of patients assigned to the two groups are shown in Table 1. Patients with metformin-induced adverse events had a lower body height (161.61 ± 8.94 cm) compared with patients without adverse events (167.50 ± 8.22 cm; P= 0.019). There was no difference in the other variables between patients with and without adverse events, including the age, gender, body mass index, blood pressure, fasting glucose (before and after treatment), HbA1c (before and after treatment), HbA1c reduction, and fecal SCFAs (before treatment, including acetic acid, propanoic acid, butyric acid). After metformin treatment for 12 weeks, the concentrations of acetic acid and propanoic acid were significantly increased in both group A and group B (all $P<0.05$). The concentration of propanoic acid in feces in group B was significantly higher than that in group A after treatment (P<0.05).

Summary of the illumina sequencing data

After Illumina sequencing and data processing, 53,968 - 95,508 clean reads were obtained in each sample. After OTU annotation, 8,312 OTUs were identified, including 4,303 OTUs common to the stool samples collected from patients before and after treatment (Figure 2A). There was no difference in the alpha diversity indexes (ACE, Shannon, Chao1, and Simpson) between samples collected before and after treatment or among samples collected from patients with and without adverse events. The fact that the sample rank-abundance curve (Figure 2B) and species accumulation curve (Figure 2C) reached a plateau showed deeper sequencing will not increase bacterial diversity. Also, the PCoA analysis based on the weighted UniFrac distances and Bray-Curtis dissimilarity of OTUs showed that there was no distinct group boundary among the samples sequenced (Figure 2D).

Metformin-induced differences in bacteria composition

The differences in the abundance of OTUs at different levels were compared between samples collected from patients before and after metformin treatment. Taxonomy annotation showed that the dominant phyla were *Firmicutes* (52.24% and 56.46%), *Bacteroidetes* (19.00% and 15.80%), *Proteobacteria* (12.37% and 14.42%), and *Actinobacteria* (11.04% and 9.94%; Figure 3A) in diabetic patients. At the family level, *Ruminococcaceae* (20.99% and 15.80%), *Lachnospiraceae* (18.17% and 15.89%), *Bacteroidaceae* (11.38% and 14.21%), *Enterobacteriaceae* (13.48% and 11.14%), and *Bifidobacteriaceae* (7.92% and 8.63%; Figure 3B) had a relatively high abundance before and after metformin treatment. We found that 12-week metformin treatment increased the abundance of the family *Prevotellaceae* (P=0.0467) and *Xanthomonadaceae* (P=0.0454), but decreased

TABLE 1 Characteristics of patients with type 2 diabetes.

Variables	Group A (without AE, n=27)	Group B (with AE, n=23)	*P* value
Age (years)	68.20 ± 7.03	68.74 ± 5.77	0.436[a]
Height (cm)	167.50 ± 8.22	161.61 ± 8.94	***0.019***[a]
Male/female	18/9	9/14	0.087[b]
Body mass index (kg/m^2)	24.79 ± 2.89	25.82 ± 2.88	0.217 [a]
Systolic BP (mmHg)	134.00 ± 10.83	133.78 ± 16.01	0.470 [a]
Diastolic BP (mmHg)	74.82 ± 6.72	77.70 ± 8.58	0.189 [a]
FBG (mmol/L; before T)	7.96 ± 1.91	8.20 ± 1.93	0.225 [a]
FBG (mmol/L; post T)	7.52 ± 1.46	7.71 ± 1.71	0.267 [a]
Fasting insulin (mIU/L)	9.02 ± 4.67	8.89 ± 4.54	0.932 [a]
HOMA-IR (before T)	3.23 ± 1.89	3.22 ± 2.01	0.798[a]
HbA1c (%; before T)	8.09 ± 0.47	8.1 ± 0.5	0.934 [a]
HbA1c (%; post T)	7.22 ± 0.62	7.16 ± 0.56	0.840 [a]
HbA1c reduction	1.03 ± 0.29	0.93 ± 0.44	0.218 [a]
Acetic acid (mmol/L, before T)	39.9 ± 4.3	38.0 ± 5.1	0.552 [a]
Propanoic (mmol/L, before T)	2.6 ± 0.7	2.4 ± 0.8	0.347 [a]
Butyric (mmol/L, before T)	1.5 ± 0.5	1.7 ± 0.4	0.377 [a]
Acetic acid (mmol/L, post T)	45.6 ± 7.3	44.2 ± 8.1	0.533 [a]
Propanoic (mmol/L, post T)	3.0 ± 0.8	3.5 ± 0.7	***0.022*** [a]
Butyric (mmol/L, post T)	1.6 ± 0.5	1.9 ± 0.7	0.265 [a]

a and b, statistical analysis by t-test and χ^2 test, respectively.
Normally distributed data were expressed as mean ± standard deviation.
AE, adverse events; FBG, fasting blood glucose; T, treatment;
HbA1c, hemoglobin Alc; HOMA-IR, homeostasis model assessment of insulin resistance.

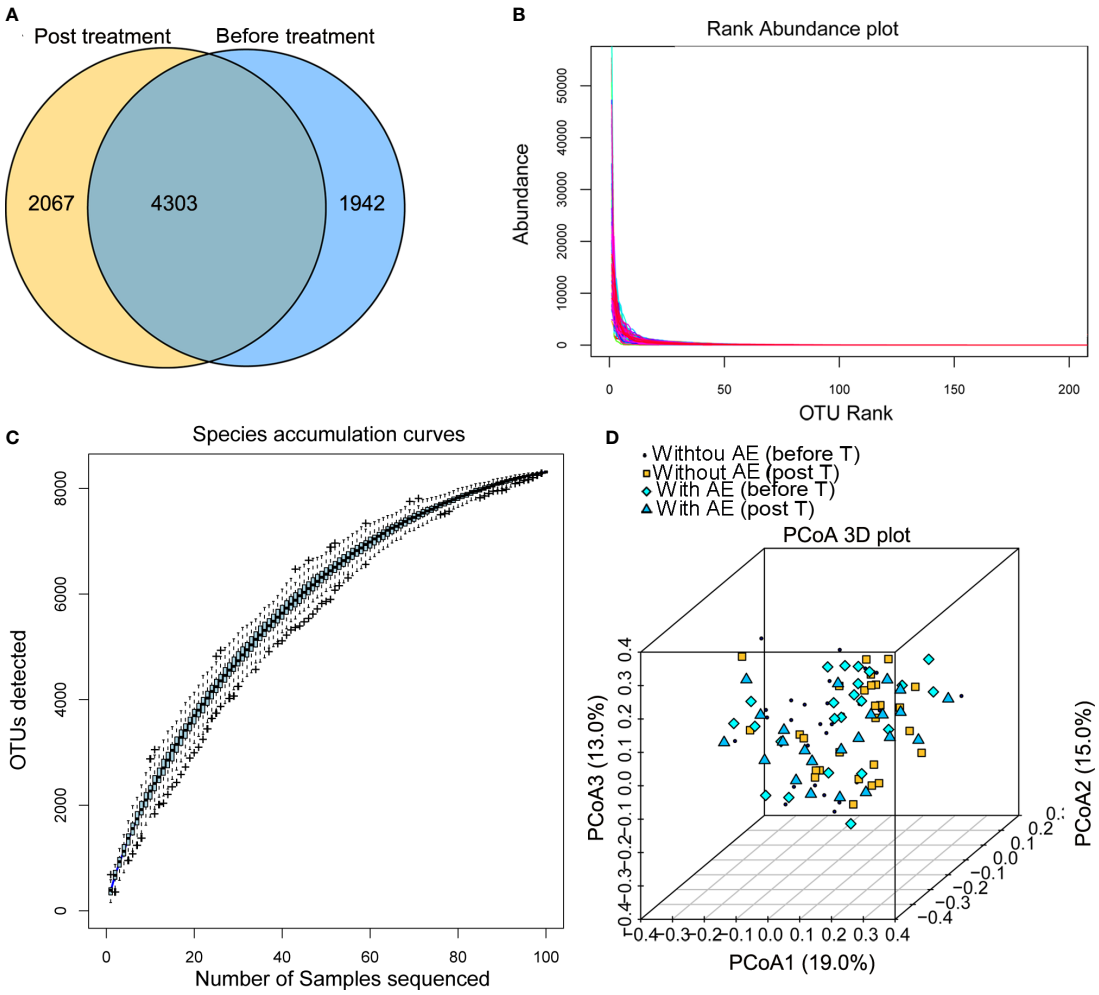

FIGURE 2
Summary of the Illumina sequencing data. **(A)** OTUs of samples collected from patients before and after treatment; **(B)** the sample rank-abundance curve; **(C)** species accumulation curve; **(D)** the PCoA analysis. AE, adverse events; T, treatment.

the abundance of the family *Peptostreptococcaceae* (P=0.0011), *Clostridiaceae* 1 (P = 0.0014), and *Saprospiraceae* (P=0.0122) significantly in patients (Figure 3C). Besides, the dominant genera in diabetic patients were *Bacteroides* (11.38% and 14.21%), *Escherichia/Shigella* (11.08% and 8.47%), and *Bifidobacterium* (7.92% and 8.61%; Figure 3D). Metformin treatment induced significantly differences in the abundance of 12 genera, including *Holdemania* (P=0.0021), *Anaerofustis* (P=0.0085), *Coriobacterium* (P=0.016), *Phascolarctobacterium* (P=0.026), *Actinomyces* (P=0.038), *Clostridium III* (P=0.049), *Intestinimonas* (P=0.035) and *Paraprevotella* (P=0.035, Figure 3E). Functional prediction analysis showed that pathways such as the pantothenate and CoA biosynthesis, protein export, necroptosis, propanoate metabolism were significantly up-regulated after metformin treatment.

Differences in bacterial abundance between patients with and without adverse events

Figure 4A shows the group similarity of samples at the genus level. There was not significant distance in the genus composition between patients with and without adverse events before treatment. We identified that the abundance of five genera were significantly different in patients without and with gastrointestinal adverse events after treatment, including *Clostridium sensu stricto* (P=0.031), *Akkermansia* (P=0.045), *Streptococcus* (P=0.047), *Rhizobium* (P=0.019) and *Phascolarctobacterium* (P =0.050; Figure 4B). The STAMP analysis showed that the abundance of *Clostridium sensu stricto* (P=0.031), *Akkermansia* (P=0.046) and *Streptococcus* (P=0.047)

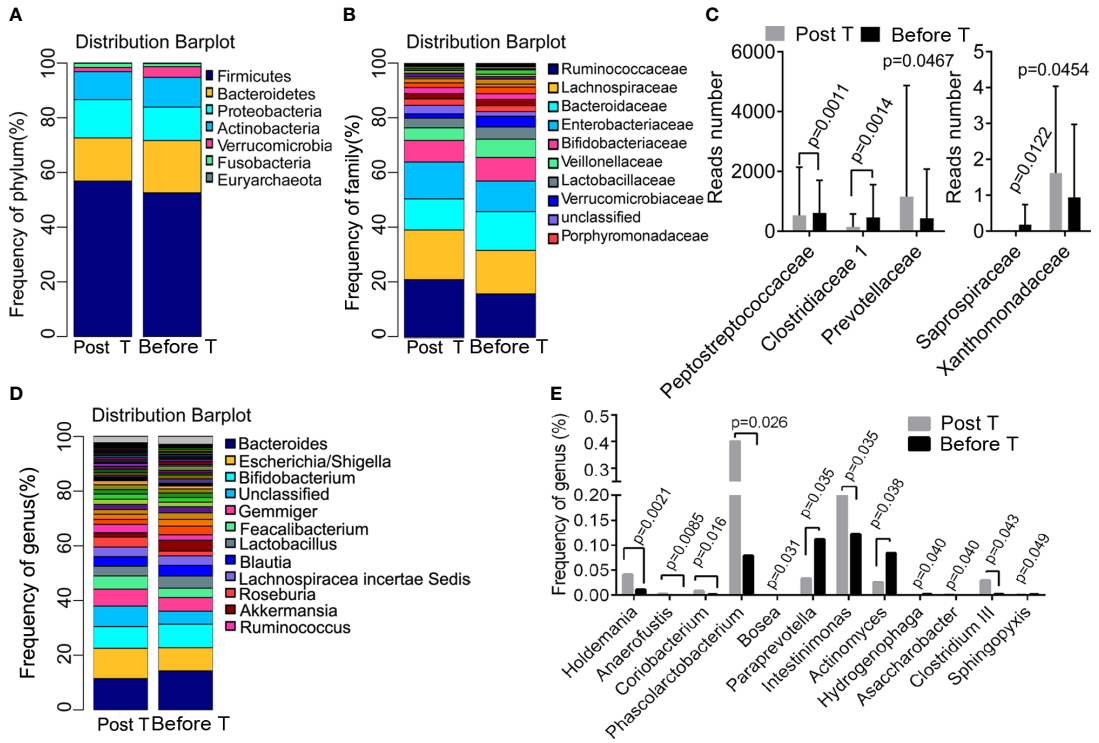

FIGURE 3
Metformin-induced differences in bacteria composition. **(A)** the dominant phyla before and after treatment; **(B)** the dominant family before and after treatment; **(C)** the different family before and after treatment; **(D)** the dominant genera before and after treatment; **(E)** the different genera before and after treatment. T, treatment.

was significantly higher in group B than that in group A after treatment. Besides, the LEfSe analysis showed that *Romboutsia, Erysipelotrichaceae, Erysipelotrichales, Erysipelotrichia*, and *Peptostreptococcaceae* were dominant bacteria in patients with metformin-induced gastrointestinal adverse events (Figure 4C). Functional prediction analysis showed that pathways such as pentose and glucuronate interconversions, pantothenate and CoA biosynthesis, biosynthesis of secondary metabolites, phenylalanine, tyrosine and tryptophan biosynthesis, aminoacyl-tRNA biosynthesis, biofilm formation of *Escherichia coli*, biosynthesis of amino acids, propanoate metabolism in feces were significantly up-regulated in patients with adverse events after metformin treatment.

The results of logistic regression analysis showed that body height was a protective factor against metformin-related gastrointestinal adverse events (OR=0.913, 95%CI 0.844-0.986, P=0.021). *Akkermansia* (OR=1.045, 95%CI 1.007-1.112, P=0.032) and *Streptococcus* (OR=1.121, 95%CI 1.033-1.262, P=0.046) were risk factors for metformin-related upper gastrointestinal adverse events (including abdominal pain, nausea, vomiting, bloating and anorexia).

Animal experiment A

After four weeks of treatment, the food intake of mice in the Met-/Clin+ group, the Met+/Clin- or the Met+/Clin+ group decreased compared with that in the Met-/Clin- group (P<0.05). The fasting glucose and 2h glucose of mice in the Met+/Clin- group and the Met+/Clin+ group were lower than those in the Met-/Clin- group (P<0.05). The levels of fecal acetic acid and propanoic acid of mice in the Met-/Clin+ and the Met +/Clin+ group were significantly decreased (P<0.01), while the fecal propanoic acid and butyric acid in the Met+/Clin- group were significantly increased (P<0.05). The total GLP-1 level in the Met+/Clin- group was significantly higher than that in the Met-/Clin- group (P<0.01), and there was no statistical difference in the levels of the total GLP-1, active GLP-1 and PYY of mice between the Met-/Clin- group and the Met+/Clin+ group (P>0.05; Table 2A). The abundance of *Akkemansia* (P<0.01), *Clostridium* (P<0.05), *Phascolarctobacterium* (P<0.01) and *Escherichia* (P<0.05) in the Met+/Clin- group increased significantly, while the abundance of *Staphylococcus* (P<0.05), *Akkemansia*

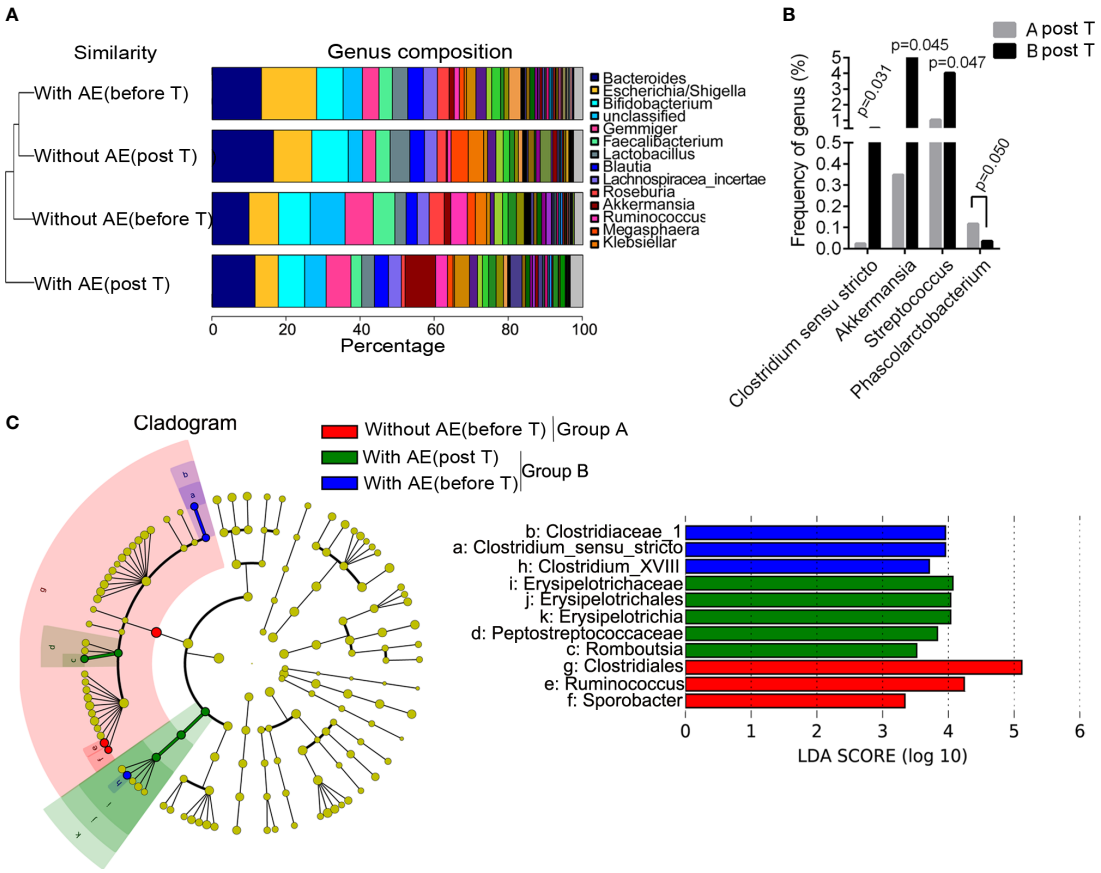

FIGURE 4
Differences in bacterial abundance between patients with and without adverse events. **(A)** the group similarity of samples at the genus level; **(B)** the different genera in patients without and with gastrointestinal adverse events after treatment; **(C)** the LEfSe analysis. AE, adverse events; T, treatment.

(P<0.05), *Clostridium* (P<0.01), *Bilophila* (P<0.05), *Bifidobacterium* (P<0.05), *Lachnospiraceae* (P<0.05) and *Peptostreptococcaceae* (P<0.05) in the Met-/Clin+ group and the Met+/Clin+ group decreased significantly, compared with those in the Met-/Clin- group (Figure 5).

Animal experiment B

After four weeks of treatment, the food intake of mice in the Met-/SCFA+ group and the Met+/SCFA- group decreased compared with that in the Met-/SCFA- group (P<0.05), while the food intake in the Met+/SCFA+ group decreased more significantly (P<0.01). The fasting glucose and 2h glucose of mice in the Met+/SCFA- group and the Met+/SCFA+ group were lower than those in the Met-/SCFA- group (P<0.05). The levels of fecal propanoic acid of mice in the Met-/SCFA+ group, the Met+/SCFA- group and the Met+/SCFA+ group were significantly higher than those in the Met-/SCFA- group (P<0.05). The total GLP-1 level in the Met-/SCFA+ group (P<0.05), and the levels of total GLP-1 and active GLP-1 in the Met+/SCFA- group and the Met+/SCFA+ group were significantly higher (P<0.05; Table 2B) than those in the Met-/SCFA- group. The expression levels of GLP-1 in the Met+/SCFA- group and the Met-/SCFA+ group (P<0.05), and GLP-1, GLP-1R and PYY in the Met+/SCFA+ group increased significantly (P<0.01; Figure 6) compared with those in the Met-/SCFA- group.

Discussion

The gut microbiome has prevalent roles in human health through modulating intestinal ecology and physiology and the metabolic and immune system of the host (22–24). Hypoglycemic drugs like α-glucosidase inhibitors, DPP-4i, and metformin influence the structure of the human gut microbial community (6, 25). Previous studies showed that the abundance of *Ruminococcus*, *Phascolarctobacterium*, *Intestinimonas*, *Corynebacterium*, *Megasphaera*, and *Akkermansia* were changed by metformin in animal models and humans (6, 26–29). These

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TABLE 2A Characteristics of mice in experiment A (after treatment).

Variables	Met-/Clin-	Met-/Clin+	Met+/Clin-	Met+/Clin+
Food intake (g/d)	4.3 ± 0.6	3.6 ± 0.6*	3.6 ± 0.5*	3.5 ± 0.4*
Weight (g)	42.1 ± 2.8	41.4 ± 3.3	42.5 ± 4.6	40.8 ± 5.0
Fasting glucose (mmol/L)	25.0 ± 5.7	25.2 ± 4.5	16.4 ± 4.4**	16.2 ± 5.2**
2h glucose (mmol/L)	30.4 ± 6.2	30.8 ± 7.0	20.4 ± 5.6**	21.8 ± 7.3**
Acetic acid (mmol/L)	35.6 ± 3.3	23.6 ± 4.5**	35.1 ± 2.5	26.7 ± 3.7**
Propanoic acid (mmol/L)	14.7 ± 1.5	10.0 ± 1.5**	16.9 ± 1.7*	9.6 ± 1.9**
Butyric acid (mmol/L)	3.9 ± 0.8	3.3 ± 0.8	5.9 ± 0.8**	4.5 ± 1.0
Total GLP-1 (pmol/L)	28.4 ± 4.4	30.2 ± 4.2	37.4 ± 6.3**	29.2 ± 7.0
Active GLP-1 (pmol/L)	3.5 ± 0.5	3.3 ± 0.6	3.7 ± 0.6	3.6 ± 0.7
PYY (pmol/L)	12.0 ± 2.1	10.8 ± 1.1	10.3 ± 1.4	11.8 ± 3.1

*Compared with the Met-/Clin- group, P<0.05; **Compared with the Met-/Clin- group, P<0.01.

TABLE 2B Characteristics of mice in experiment B (after treatment).

Variables	Met-/SCFA-	Met-/SCFA+	Met+/SCFA-	Met+/SCFA+
Food intake (g/d)	4.5 ± 0.4	3.8 ± 0.6*	3.9 ± 0.6*	3.5 ± 0.6**
Weight (g)	43.5 ± 3.8	42.0 ± 5.3	44.5 ± 5.7	40.7 ± 5.6
Fasting glucose (mmol/L)	24.2 ± 5.0	24.2 ± 4.5	17.5 ± 4.7**	18.8 ± 5.2*
2h glucose (mmol/L)	29.3 ± 5.2	30.7 ± 4.0	20.6 ± 3.9**	21.2 ± 5.0**
Acetic acid (mmol/L)	35.9 ± 3.0	35.0 ± 3.1	36.0 ± 2.9	36.8 ± 3.7
Propanoic acid (mmol/L)	15.4 ± 2.0	19.4 ± 1.8**	17.0 ± 1.6*	19.1 ± 1.7**
Butyric acid (mmol/L)	4.5 ± 0.7	4.3 ± 0.4	5.9 ± 0.7**	5.3 ± 0.8*
Total GLP-1 (pmol/L)	29.9 ± 5.0	35.3 ± 3.4*	44.5 ± 5.9**	49.8 ± 10.1**
Active GLP-1 (pmol/L)	3.3 ± 0.6	3.5 ± 0.5	4.1 ± 0.7*	4.9 ± 0.9**
PYY (pmol/L)	13.4 ± 1.8	12.1 ± 1.9	13.2 ± 1.7	15.0 ± 2.5

*Compared with the Met-/SCFA- group, P<0.05; **Compared with the Met-/SCFA- group, P<0.01.

results confirmed the influence of metformin treatment on the structure of gut microbial community in diabetic patients.

Metformin is an old drug with a lot of history and still much more to tell us. With the advance in clinical and experimental science, always newer pleiotropic effects come out, making it one of the most valuable drugs available. Metformin has good effects on lowering glucose, improving insulin sensitivity and lipid profile, reducing cardiovascular events and mortality, and preventing progression to heart failure in patients with diabetes (30, 31). Endothelial dysfunction is a well-known important risk factor for the development of diabetes cardiovascular complications. Adenosine 5'-monophosphate-activated protein kinase pharmacological activation plays a key role, with metformin inhibiting inflammation and improving endothelial dysfunction. The effects of metformin on endothelial dysfunction seem to be among the main factors responsible for the cardiovascular prevention (32). Metformin also displays significant growth inhibitory and proapoptotic effects in several cancer models. More preclinical data support the role of metformin as an adjuvant drug in the treatment of lung cancer (33). Metformin can decrease insulin resistance, increase the utilization of glucose and the production of lactic acid, and promote the secretion of postprandial GLP-1 in the intestine (34–36). A previous report has shown that the SCFA-producing bacteria decrease in patients with diabetes and increase after metformin treatment (37). Bacterial SCFAs influence innate and adaptive immunity of the host and play an anti-inflammatory role (38). The decreased abundance of SCFA-producing bacteria in fecal has been associated with an increased incidence of diabetes (37, 38). The metformin treatment for diabetic animals and patients significantly increased the abundance of *Akkermansia* (9), and other SCFA-producing bacteria like *Ruminococcus*, *Phascolarctobacterium*, and *Intestinimonas* (39, 40). It has been reported that the increased abundance of SCFA-producing bacteria was associated with improved insulin sensitivity (39). Therefore, even the increased prevalence in gastrointestinal adverse events, which are surely detrimental for patients' quality of life and adherence, metformin should be provided as the first-choice drug and changed only in case of intolerance (41, 42).

Our present study demonstrated that the 12-week metformin treatment decreased the abundance of *Actinomyces* and *Paraprevotella*, but increased the abundance of SCFA-producing gut bacteria, including *Phascolarctobacterium*, *Clostridium III*, and *Intestinimonas*. Functional prediction analysis showed that

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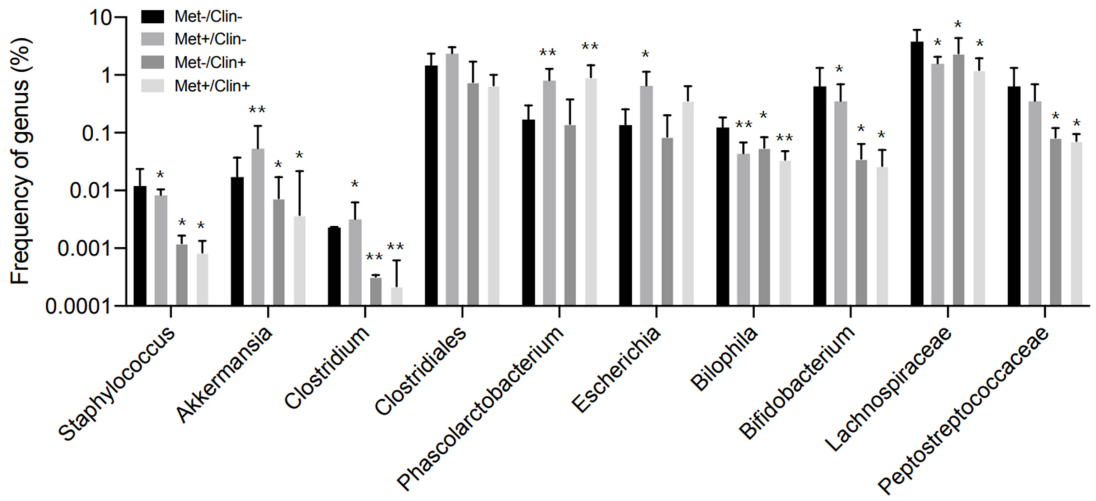

FIGURE 5
Frequency of genus in experiment A. Met, metformin; Clin, clindamycin. *Compared with the Met-/Clin- group, $P<0.05$; **Compared with the Met-/Clin- group, $P<0.01$.

pathways such as propanoate metabolism were significantly up-regulated after metformin treatment. The concentrations of acetic acid and propanoic acid increased significantly after metformin treatment. However, the concentrations of acetic acid did not increase in diabetic mice after metformin treatment. This may be due to the species difference between human and mice. These results showed that metformin-mediated glucose control in diabetic patients were mediated by modulating the composition of SCFA-producing bacteria.

We further analyzed and compared the gut microbiota between diabetic patients with and without gastrointestinal adverse events after taking metformin. Comparison analysis indicated that metformin also increased the abundance of *Clostridium sensu stricto*, *Akkermansia* and *Streptococcus* in patients with gastrointestinal adverse events compared with that in patients without adverse events. *Akkermansia* and *Streptococcus* were both risk factors for metformin-related upper gastrointestinal adverse events in patients aged > 60 years old. Propanoate metabolism were significantly up-regulated and the concentration of propanoic acid in feces was significantly higher in patients with gastrointestinal adverse events after treatment. These results are somewhat regrettable because the increase in the abundance of SCFA-producing bacteria is very important in the hypoglycemic mechanism of metformin. However, our results showed that the increased abundance of some SCFA-producing bacteria might also be related to the gastrointestinal adverse effects of metformin.

Interestingly, here we for the first time showed that body height might be a protective factor against metformin-induced gastrointestinal adverse effects, as patients (>60 years old) without adverse events had a higher body height compared with patients with adverse events. Lower body height correlates with a high risk of diabetes, especially in women (43, 44). Higher height means larger body surface area. A previous study presented that metformin $AUC_{0\text{-}48h}$ was inversely associated with body surface area (45). This present study showed that diabetic patients (>60 years old) who had a relatively high body height might be at a low risk of gastrointestinal adverse events following the metformin treatment.

The mechanism by which gastrointestinal adverse events are induced by metformin is not fully understood. The reported gastrointestinal adverse events may be caused by bile salt reabsorption (46), gut microbiota alteration (6), organic cation transporter (OCT) polymorphism (34, 47), OCT-1 inhibiting agents, age, female (48), chronic gastritis (49) and H.pylori infection (8). It is generally believed that metformin may cause diarrhea by reducing ileal bile salt reabsorption leading to elevated colonic bile salt concentration (46). Metformin can also increase the abundance of *Escherichia* and cause bloating by promoting the production of hydrogen (6). In addition to the above mechanisms, it is speculated that metformin may cause gastrointestinal adverse events by regulating SCFA-producing bacteria (50). Therefore, we designed animal experiments to confirm the relationship between metformin, SCFA-producing bacteria and gut hormones.

The food intake decreased and glucose control increased in metformin groups compared with those in the control group. In experiment A, the abundance of SCFA-producing bacteria

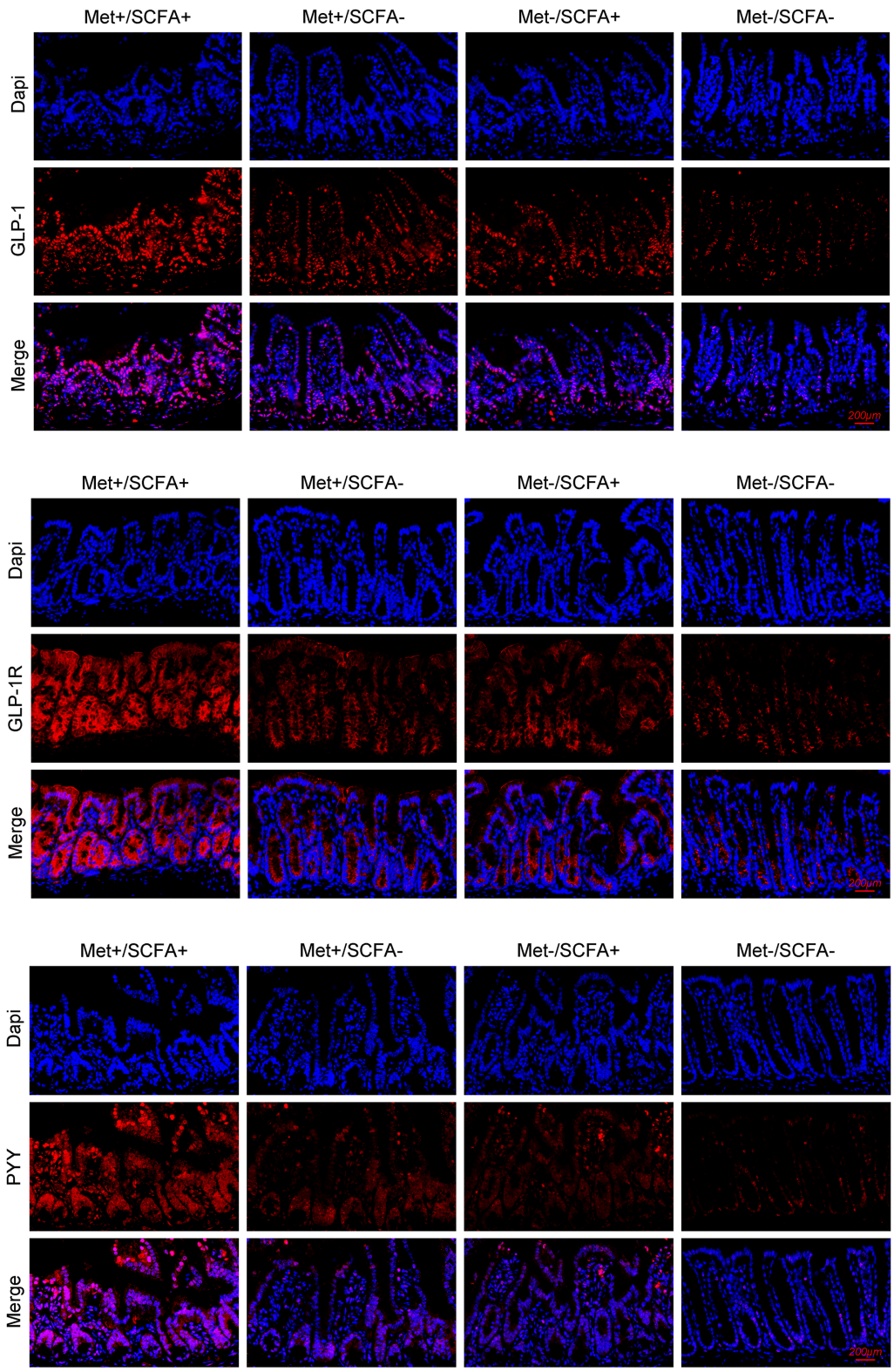

FIGURE 6
The expression levels of GLP-1, GLP-1R and PYY. Met, metformin; Clin, clindamycin; SCFA, short-chain fatty acid.

(including *Akkemansia*, *Clostridium*, *Phascolarctobacterium*) and fecal SCFAs increased significantly in the Met+/Clin- group after treatment. The total GLP-1 level in the Met+/Clin- group was significantly higher than that in the control group. It has been reported that clindamycin induced pronounced changes in fecal SCFAs (19). In our study, the use of clindamycin reduced SCFA-producing bacteria such as *Akkemansia*, *Clostridium* and *Streptococcus*, and the levels of fecal acetic acid and propanoic acid. More interestingly, the simultaneous use of metformin and clindamycin could inhibit the promoting effect of metformin on SCFAs and GLP-1. In experiment B, the expression levels of GLP-1 in the Met+/SCFA- group and the Met-/SCFA+ group, and GLP-1, GLP-1R and PYY in the Met+/SCFA+ group increased compared with those in the control group. It has been well reported that the SCFA receptor exists on the colonic enteroendocrine L cells (51, 52). SCFAs can stimulate the secretion of both PYY and GLP-1 from wild-type primary murine colonic crypt cultures (53, 54). PYY and GLP-1 can acutely suppress appetite by inhibiting gastric emptying and reducing intestinal peristalsis (55). Therefore, we speculated that metformin can not only increase the abundance of SCFA-producing bacteria but also promote the secretion of GLP-1, therefore resulting in an increased risk of gastrointestinal adverse events and better glucose control.

There are still limitations in our study that need to be noted. This is a monocentric study, so results seem difficult to generalize. The sample size of human experimented patients is low. Moreover, this is a prospective study, and it is unable to assess a cause-effect relationship between gut microbiota and metformin-induced gastrointestinal adverse events. Although the animal experiments were grouped according to the presence or absence of Met, Clin and SCFA, the mice could not be examined that they are undergoing gastrointestinal adverse events such as abdominal pain, abdominal distention, nausea and inappetence after treatment. We can only observe that almost all mice have reduced food intake after metformin treatment. Therefore, it only speculated that metformin causes gastrointestinal adverse events may through increasing GLP-1 with the abundance of SCFA-producing bacteria.

In summary, we identified that metformin induced a significant difference in gut microbial community structure in diabetic patients. Also, there was a difference in the gut microbial community between patients with and without gastrointestinal adverse effects. We speculated that metformin promotes the secretion of intestinal hormones such as GLP-1 by increasing the abundance of SCFA-producing bacteria, which not only plays an anti-diabetic role, but also causes gastrointestinal adverse events.

Data availability statement

The data presented in the study are deposited in the GenBank repository, accession number OP649987-OP649993.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Huadong Hospital Affiliated to Fudan University (Ethics Number: 2018K065). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Ethics Committee of Fudan University (Ethics Number: 202101004S).

Author contributions

YH and XL: Conceptualization, methodology, writing manuscript, funding acquisition. CJ: Investigation. XT and XJ: Investigation, data curation, supervision, funding acquisition. JS: Conceptualization. ZB: Conceptualization, methodology. All authors contributed to the article and approved the submitted version.

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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.

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References

1. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. *Nat Med* (2017) 23(7):804–14. doi: 10.1038/nm.4350

2. Haro C, Montes-Borrego M, Rangel-Zuniga OA, Alcala-Diaz JF, Gomez-Delgado F, Perez-Martinez P, et al. Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population. *J Clin Endocrinol Metab* (2016) 101(1):233–42. doi: 10.1210/jc.2015-3351

3. American Diabetes, A. 9. pharmacologic approaches to glycemic treatment: Standards of medical care in diabetes-2019. *Diabetes Care* (2019) 42(Suppl 1):S90–S102. doi: 10.2337/dc19-S009

4. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* (2008) 359(15):1577–89. doi: 10.1056/NEJMoa0806470

5. Bouchoucha M, Uzzan B, Cohen R. Metformin and digestive disorders. *Diabetes Metab* (2011) 37(2):90–6. doi: 10.1016/j.diabet.2010.11.002

6. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* (2015) 528(7581):262–6. doi: 10.1038/nature15766

7. Yuxin H, Cuiping J, Wen T, Jieyuzhen Q, Xiaoming T, Qin G, et al. Comparison of gastrointestinal adverse events with different doses of metformin in the treatment of elderly people with type 2 diabetes. *J Clin Pharm Ther* (2020) 45 (3):470–6. doi: 10.1111/jcpt.13087

8. Huang Y, Sun J, Wang X, Tao X, Wang H, Tan W. Helicobacter pylori infection decreases metformin tolerance in patients with type 2 diabetes mellitus. *Diabetes Technol Ther* (2015) 17(2):128–33. doi: 10.1089/dia.2014.0203

9. de la Cuesta-Zuluaga J, Mueller NT, Corrales-Agudelo V, Velasquez-Mejia EP, Carmona JA, Abad JM, et al. Metformin is associated with higher relative abundance of mucin-degrading akkermansia muciniphila and several short-chain fatty acid-producing microbiota in the gut. *Diabetes Care* (2017) 40(1):54–62. doi: 10.2337/dc16-1324

10. Duca FA, Cote CD, Rasmussen BA, Zadeh-Tahmasebi M, Rutter GA, Filippi BM, et al. Metformin activates a duodenal ampk-dependent pathway to lower hepatic glucose production in rats. *Nat Med* (2015) 21(5):506–11. doi: 10.1038/nm.3787

11. Lien F, Berthier A, Bouchaert E, Gheeraert C, Alexandre J, Porez G, et al. Metformin interferes with bile acid homeostasis through AMPK-FXR crosstalk. *J Clin Invest* (2014) 124(3):1037–51. doi: 10.1172/JCI68815

12. Beysen C, Murphy EJ, Deines K, Chan M, Tsang E, Glass A, et al. Effect of bile acid sequestrants on glucose metabolism, hepatic *de novo* lipogenesis, and cholesterol and bile acid kinetics in type 2 diabetes: a randomised controlled study. *Diabetologia* (2012) 55(2):432–42. doi: 10.1007/s00125-011-2382-3

13. de la Cuesta-Zuluaga J, Mueller NT, Alvarez-Quintero R, Velasquez-Mejia EP, Sierra JA, Corrales-Agudelo V, et al. Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. *Nutrients* (2018) 11(1). doi: 10.3390/nu11010051

14. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* (2011) 27(21):2957–63. doi: 10.1093/bioinformatics/btr507

15. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* (2011) 27(16):2194–200. doi: 10.1093/bioinformatics/btr381

16. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* (2010) 26(19):2460–1. doi: 10.1093/bioinformatics/btq461

17. Wemheuer F, Taylor JA, Daniel R, Johnston E, Meinicke P, Thomas T, et al. Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. *Environ Microbiome* (2020) 15 (1):11. doi: 10.1186/s40793-020-00358-7

18. Yorek MS, Obrosov A, Shevalye H, Coppey LJ, Kardon RH, Yorek MA. Early vs. late intervention of high fat/low dose streptozotocin treated C57Bl/6J mice with enalapril, alpha-lipoic acid, menhaden oil or their combination: Effect on diabetic neuropathy related endpoints. *Neuropharmacology* (2017) 116:122–31. doi: 10.1016/j.neuropharm.2016.12.022

19. Hoverstad T, Carlstedt-Duke B, Lingaas E, Midtvedt T, Norin KE, Saxerholt H, et al. Influence of ampicillin, clindamycin, and metronidazole on faecal excretion of short-chain fatty acids in healthy subjects. *Scand J Gastroenterol* (1986) 21(5):621–6. doi: 10.3109/00365528609003109

20. Zhang Y, An H, Pan SY, Zhao DD, Zuo JC, Li XK, et al. Jiang tang xiao ke granule, a classic Chinese herbal formula, improves the effect of metformin on lipid and glucose metabolism in diabetic mice. *Evid Based Complement Alternat Med* (2016) 2016:1592731. doi: 10.1155/2016/1592731

21. Zhang H, Song B, Zhu W, Liu L, He X, Wang Z, et al. Glucagon-like peptide-1 attenuated carboxymethyl lysine induced neuronal apoptosis *via* peroxisome proliferation activated receptor-gamma. *Aging (Albany NY)* (2021) 13(14):19013–27. doi: 10.18632/aging.203351

22. Montandon SA, Jornayvaz FR. Effects of antidiabetic drugs on gut microbiota composition. *Genes (Basel)* (2017) 8(10). doi: 10.3390/genes8100250

23. Valeriano VD, Balolong MP, Kang DK. Probiotic roles of lactobacillus sp. in swine: insights from gut microbiota. *J Appl Microbiol* (2017) 122(3):554–67. doi: 10.1111/jam.13364

24. Sethi V, Kurtom S, Tarique M, Lavania S, Malchiodi Z, Hellmund L, et al. Gut microbiota promotes tumor growth in mice by modulating immune response. *Gastroenterology* (2018) 155(1):33–37.e36. doi: 10.1053/j.gastro.2018.04.001

25. Tan K, Tesar C, Wilton R, Jedrzejczak RP, Joachimiak A. Interaction of antidiabetic alpha-glucosidase inhibitors and gut bacteria alpha-glucosidase. *Protein Sci* (2018) 27(8):1498–508. doi: 10.1002/pro.3444

26. 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/srep14405

27. Cui HX, Zhang LS, Luo Y, Yuan K, Huang ZY, Guo Y. A purified anthraquinone-glycoside preparation from rhubarb ameliorates type 2 diabetes mellitus by modulating the gut microbiota and reducing inflammation. *Front Microbiol* (2019) 10:1423. doi: 10.3389/fmicb.2019.01423

28. Li Q, He R, Zhang F, Zhang J, Lian S, Liu H. Combination of oligofructose and metformin alters the gut microbiota and improves metabolic profiles, contributing to the potentiated therapeutic effects on diet-induced obese animals. *Front Endocrinol (Lausanne)* (2019) 10:939. doi: 10.3389/fendo.2019.00939

29. Hiel S, Gianfrancesco MA, Rodriguez J, Portheault D, Leyrolle Q, Bindels LB, et al. Link between gut microbiota and health outcomes in inulin -treated obese patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial. *Clin Nutr* (2020) 39(12):3618–28. doi: 10.1016/j.clnu.2020.04.005

30. Nesti L, Natali A. Metformin effects on the heart and the cardiovascular system: A review of experimental and clinical data. *Nutr Metab Cardiovasc Dis* (2017) 27(8):657–69. doi: 10.1016/j.numecd.2017.04.009

31. Petrie JR, Chaturvedi N, Ford I, Brouwers M, Greenlaw N, Tillin T, et al. Cardiovascular and metabolic effects of metformin in patients with type 1 diabetes (REMOVAL): a double-blind, randomised, placebo-controlled trial. *Lancet Diabetes Endocrinol* (2017) 5(8):597–609. doi: 10.1016/S2213-8587(17)30194-8

32. Salvatore T, Pafundi PC, Galiero R, Rinaldi L, Caturano A, Vetrano E, et al. Can metformin exert as an active drug on endothelial dysfunction in diabetic subjects? *Biomedicines* (2020) 9(1). doi: 10.3390/biomedicines9010003

33. Morgillo F, Sasso FC, Della Corte CM, Festino L, Manzo A, Martinelli E, et al. Metformin in lung cancer: rationale for a combination therapy. *Expert Opin Investig Drugs* (2013) 22(11):1401–9. doi: 10.1517/13543784.2013.828691

34. Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER. Association of organic cation transporter 1 with intolerance to metformin in type 2 diabetes: A GoDARTS study. *Diabetes* (2015) 64(5):1786–93. doi: 10.2337/db14-1388

35. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. *Diabetologia* (2016) 59(3):426–35. doi: 10.1007/s00125-015-3844-9

36. Wu T, Xie C, Wu H, Jones KL, Horowitz M, Rayner CK. Metformin reduces the rate of small intestinal glucose absorption in type 2 diabetes. *Diabetes Obes Metab* (2017) 19(2):290–3. doi: 10.1111/dom.12812

37. Xiao L, Van't Land B, Engen PA, Naqib A, Green SJ, Nato A, et al. Human milk oligosaccharides protect against the development of autoimmune diabetes in NOD-mice. *Sci Rep* (2018) 8(1):3829. doi: 10.1038/s41598-018-22052-y

38. Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PloS One* (2011) 6(10):e25792. doi: 10.1371/journal.pone.0025792

39. Panasevich MR, Morris EM, Chintapalli SV, Wankhade UD, Shankar K, Britton SL, et al. Gut microbiota are linked to increased susceptibility to hepatic steatosis in low-aerobic-capacity rats fed an acute high-fat diet. *Am J Physiol Gastrointest Liver Physiol* (2016) 311(1):G166–179. doi: 10.1152/ajpgi.00065.2016

40. Li L, Guo WL, Zhang W, Xu JX, Qian M, Bai WD, et al. Grifola frondosa polysaccharides ameliorate lipid metabolic disorders and gut microbiota dysbiosis in high-fat diet fed rats. *Food Funct* (2019) 10(5):2560–72. doi: 10.1039/c9fo00075e

41. Caturano A, Galiero R, Pafundi PC. Metformin for type 2 diabetes. *JAMA* (2019) 322(13):1312. doi: 10.1001/jama.2019.11489

42. Salvatore T, Pafundi PC, Morgillo F, Di Liello R, Galiero R, Nevola R, et al. Metformin: An old drug against old age and associated morbidities. *Diabetes Res Clin Pract* (2020) 160:108025. doi: 10.1016/j.diabres.2020.108025

43. Rudra CB, Sorensen TK, Leisenring WM, Dashow E, Williams MA. Weight characteristics and height in relation to risk of gestational diabetes mellitus. *Am J Epidemiol* (2007) 165(3):302–8. doi: 10.1093/aje/kwk007

44. Koncz V, Geldsetzer P, Manne-Goehler J, Wendt AS, Teufel F, Subramanian SV, et al. Shorter height is associated with diabetes in women but not in men: Nationally representative evidence from Namibia. *Obes (Silver Spring)* (2019) 27 (3):505–12. doi: 10.1002/oby.22394

45. Santoro AB, Botton MR, Struchiner CJ, Suarez-Kurtz G. Influence of pharmacogenetic polymorphisms and demographic variables on metformin pharmacokinetics in an admixed Brazilian cohort. *Br J Clin Pharmacol* (2018) 84 (5):987–96. doi: 10.1111/bcp.13522

46. Scarpello JH, Hodgson E, Howlett HC. Effect of metformin on bile salt circulation and intestinal motility in type 2 diabetes mellitus. *Diabetes Med* (1998) 15(8):651–6. doi: 10.1002/(SICI)1096-9136(199808)15:8<651::AID-DIA628>3.0.CO;2-A

47. Dujic T, Zhou K, Tavendale R, Palmer CN, Pearson ER. Effect of serotonin transporter 5-HTTLPR polymorphism on gastrointestinal intolerance to metformin: A GoDARTS study. *Diabetes Care* (2016) 39(11):1896–901. doi: 10.2337/dc16-0706

48. Dawed AY, Zhou K, van Leeuwen N, Mahajan A, Robertson N, Koivula R, et al. Variation in the plasma membrane monoamine transporter (PMAT) (Encoded by SLC29A4) and organic cation transporter 1 (OCT1) (Encoded by SLC22A1) and gastrointestinal intolerance to metformin in type 2 diabetes: An IMI DIRECT study. *Diabetes Care* (2019) 42(6):1027–33. doi: 10.2337/dc18-2182

49. Huang Y, Sun J, Wang X, Tao X, Wang H, Tan W. Asymptomatic chronic gastritis decreases metformin tolerance in patients with type 2 diabetes. *J Clin Pharm Ther* (2015) 40(4):461–5. doi: 10.1111/jcpt.12290

50. Bahne E, Hansen M, Bronden A, Sonne DP, Vilsboll T, Knop FK. Involvement of glucagon-like peptide-1 in the glucose-lowering effect of metformin. *Diabetes Obes Metab* (2016) 18(10):955–61. doi: 10.1111/dom.12697

51. Nohr MK, Pedersen MH, Gille A, Egerod KL, Engelstoft MS, Husted AS, et al. GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* (2013) 154(10):3552–64. doi: 10.1210/en.2013-1142

52. Shackley M, Ma Y, Tate EW, Brown AJH, Frost G, Hanyaloglu AC. Short chain fatty acids enhance expression and activity of the umami taste receptor in enteroendocrine cells *via* a galphai/o pathway. *Front Nutr* (2020) 7:568991. doi: 10.3389/fnut.2020.568991

53. Freeland KR, Wolever TM. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-alpha. *Br J Nutr* (2010) 103(3):460–6. doi: 10.1017/S0007114509991863

54. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion *via* the G-protein-coupled receptor FFAR2. *Diabetes* (2012) 61(2):364–71. doi: 10.2337/db11-1019

55. Psichas A, Sleeth ML, Murphy KG, Brooks L, Bewick GA, Hanyaloglu AC, et al. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion *via* free fatty acid receptor 2 in rodents. *Int J Obes (Lond)* (2015) 39(3):424–9. doi: 10.1038/ijo.2014.153

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Evidence for proton-pump inhibitor (PPI)-associated dysbiosis in metabolically unhealthy obesity

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Obesity adversely impacts millions of American adults by predisposing them to significant health risks and further complications. Obesity is differentiated into two groups: metabolically healthy and metabolically unhealthy. In contrast to metabolically healthy counterparts, obese individuals who are metabolically unhealthy display hallmark symptoms of metabolic syndrome (*e.g.*, hypertension, dyslipidemia, hyperglycemia, abdominal obesity). Gastroesophageal reflux disease (GERD) commonly occurs in all obese populations, as do poor dietary habits. Proton-pump inhibitors (PPIs), due to their wide availability, are most often used to treat GERD-related heartburn and other symptoms. Here, we review the evidence on how poor diet as well as short- and long-term use of PPIs adversely affect the gastrointestinal microbiota to cause dysbiosis. Key components of dysbiosis-induced metabolically unhealthy obesity (MUO) associated with PPI use include "leaky gut," systemic low-grade inflammation, and reduced amounts of short-chain fatty acids (SCFAs) such as butyrate that promote metabolic health. The benefit of using probiotics to mitigate PPI-induced dysbiosis and MUO is also discussed.

KEYWORDS

proton-pump inhibitor (PPI), metabolically unhealthy obesity (MUO), dysbiosis, inflammation, butyrate, short-chain fatty acids (SCFAs), probiotics

Introduction

Obesity is a chronic, progressive disease with significant adverse health effects and is clinically defined by a body mass index (BMI) >30 kg/m^2 (1). According to the 2022 National Health and Nutrition Examination Survey, the obesity rate in American adults is 42% (2). Obesity is a significant risk factor for a myriad of comorbidities including type 2 diabetes mellitus (T2DM), cardiovascular disease, metabolic syndrome, gastrointestinal (GI) tract diseases, kidney damage, liver dysfunction, mental illness, and several cancers. Obesity imparts a significant healthcare burden. Healthcare costs are estimated at $172

billion, with heightened costs in severely obese individuals (BMI >35) that increase with age (3).

While most obese individuals exhibit one or more additional metabolic complications, some lack any overt sign of coinciding disease. To differentiate between these two conditions, the medical community coined the terms metabolically unhealthy obesity (MUO) and metabolically healthy obesity (MHO) (4, 5). Obesity is oftentimes accompanied by gastroesophageal reflux disease (GERD), prompting the use of proton-pump inhibitors (PPIs), among other medications, to manage acid reflux and related symptoms (6–8). Mounting evidence indicate that several oral medications including antibiotics and PPIs unfavorably alter the gut microbiota; the resultant dysbiosis is implicated in the etiology and pathogenesis of obesity. Many findings about diet composition, obesity, and PPI use come from preclinical research in animals. Here, we explore the relationships between poor diet, GERD, PPI use, metabolic disease, immune dysfunction, and dysbiosis as well as their associative and potentially causal roles in MUO.

Metabolically healthy obesity (MHO) vs. metabolically unhealthy obesity (MUO)

MHO is clinical obesity without any comorbidities associated with metabolic syndrome. MHO is characterized by preserved insulin sensitivity, reduced systemic inflammation, less visceral fat, and more favorable hepatic function than MUO counterparts (5, 9). The following MHO criteria are proposed: fasting triglycerides ≤150 mg/dL; high density lipoprotein serum concentration >40 mg/dL in men or >50 mg/dL in women; systolic blood pressure <130 mmHg; diastolic blood pressure <85 mmHg; and fasting blood glucose <100 mg/dL (4, 10). Since MHO individuals have no cardiometabolic disorder, medications for dyslipidemia, hypertension, or diabetes are not required (4, 10). Lack of concrete MHO criteria has led to a large degree of heterogeneity amongst research participants, generating debate about whether to classify MHO as a distinct phenotype or place it on a spectrum that incorporates a devolution to MUO (4, 5). Factors promoting MHO status include healthy diet; regular physical activity; genetic predisposition towards more subcutaneous (vs. visceral) fat; and gut microbiome diversity (5, 10). Metabolic heterogeneity amongst obese individuals is partly governed by differences in adipose tissue physiology, whereby genetic determinants of body fat distribution, depot-specific fat metabolism, adipose tissue plasticity, and adipogenesis predispose some individuals to adiposopathy and MUO (5). Adverse changes in body weight, body composition (*i.e.*, lean vs. fat mass), metabolism (*i.e.*, food intake, energy expenditure (EE), glucose clearance, glucose-stimulated insulin secretion), and fecal microbiota richness are observed in mice fed a high calorie diet and treated with the PPI omeprazole; results varied depending on sex and genetic background (11).

Gastroesophageal reflux disease (GERD) and proton-pump inhibitors (PPIs)

Individuals with MHO or MUO are equally susceptible to developing GERD (12). Obese individuals who experience GERD commonly use PPIs to relieve heartburn and other discomfort (*e.g.*, chest or upper abdominal pain, dysphagia, globus sensation, food regurgitation) caused by acid reflux. PPIs reduce stomach acid production by inhibiting the H^+,K^+-ATPase, an ion pump located on the luminal surface of gastric parietal cells, and blocking hydrochloric acid secretion (13). Through irreversible inhibition of the proton pump, PPIs yield greater acid suppression and have a longer duration of action than other acid-controlling medications such as histamine-2 receptor antagonists or antacids (13). Thus, PPIs are more favorable for reducing gastric acid secretion and relieving pain. PPIs are the medication of choice not only for GERD but also peptic ulcer disease and associated bleeding, *Helicobacter pylori* infection (in combination with antibiotics), NSAID-induced ulcers, erosive esophagitis, Zollinger-Ellison syndrome, and functional dyspepsia (14).

Fueled by over-the-counter availability, PPI usage has steadily increased since 2003, when omeprazole (Prilosec) was FDA-approved for purchase without a prescription (15). Approximately 15 million Americans use PPIs annually (16). The number of documented indications for PPI use has also increased (17). PPIs are commonly administered in the outpatient, ambulatory care setting for GERD-related symptoms and in the inpatient, critical care setting for stress ulcer prophylaxis. Shortly following OTC availability, many PPI users continued to take these medications, even without documented GI complaints and/or diagnoses or other indications for use (17). Individuals still frequently remain on PPIs long-term (clinically defined as >8 weeks) after either being initiated on therapy in non-outpatient settings or self-prescribing (17, 18). The long-term use of PPIs is especially concerning due to numerous possible adverse side effects, including T2DM, dysbiosis, *Clostridium difficile infection* (CDI)-associated diarrhea, enteric infections, increased risk of community-acquired pneumonia, magnesium and vitamin B_{12} deficiency, osteoporosis, bone fractures, and dementia (14, 19–22).

Dysbiosis and metabolic disease

Gut microbiota are a core participant in host metabolic health by modulating digestion and absorption, whereby foodstuffs are converted into essential nutrients and minerals. A diet enriched in prebiotic and probiotic foods including plant-derived protein while limited in processed foods and animal-derived protein, healthy lifestyle, and environmental and genetic factors all support a diverse and optimal gut microbiota (23–25). The healthy human microbiota exhibits a balance of the phyla Firmicutes and Bacteroidetes, which represent 90% of gut microbiota (26, 27). The remaining dominant phyla include Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. A rich microbiota contributes to health by facilitating drug metabolism, synthesis of essential vitamins B and K, and physical and chemical protection against colonization by pathogens (7, 23, 26). These microbiota also ferment fiber and other indigestible polysaccharides, yielding short-chain fatty acids (SCFAs) that beneficially impact body weight, inflammatory status, insulin sensitivity, and glucose and lipid homeostasis (28).

Reduced biodiversity of gut microbiota, coupled with subsequent expansion of disease-promoting pathogens, is referred

to as dysbiosis (23). Dysbiosis is a hallmark of inflammatory bowel disease (IBD) and is also associated with several autoimmune, neurological, and metabolic disorders, with causal evidence emerging (23, 29–35). Variations in the composition and abundance of oral and/or gut microbiota, especially at the phylum level, are implicated in metabolic disease (7, 9, 36, 37). Namely, an increase in the Firmicutes to Bacteroidetes (F/B) ratio occurs in overweight and obese individuals (38). High fat diet (HFD)-fed mice show an increase in Firmicutes and decrease in Bacteroidetes proportions, leading to a higher F/B ratio vs. lean mice (36). In obese, human, metabolic syndrome recipients, allogenic fecal microbiota transfer (FMT) using metabolic syndrome donors (vs. post-gastric bypass donors) decreases insulin sensitivity, suggesting that dysbiosis can trigger MUO (39). Conversely, FMT using normal diet-fed and exercised donor mice improves metabolism and inflammatory status in HFD-fed recipients (40). However, FMT using healthy lean donors fails to potentiate the improved insulin sensitivity imparted by consumption of a healthy diet in MUO individuals (41, 42).

The F/B ratio's validity as a reliable biomarker has been challenged by various confounding factors in study populations and lack of clear correlation between its numerical value and BMI. This discrepancy suggests that dysbiotic gut events impacting metabolic health are more nuanced (9, 27, 43, 44). Compared to MHO individuals, intestinal levels of inflammatory-associated microbiota are elevated in MUO, accompanied by lower bacterial diversity and reduced potential for butyrate production (45–47). Alpha diversity, an index of taxa richness and abundance, is lower in MUO vs. MHO adults and children (9, 47). The genera *Oscillospira* and *Clostridum*, microbial sources of beneficial SCFAs, are more abundant in MHO individuals (9). Butyrate, a key SCFA, exhibits anti-inflammatory properties by reducing pro-inflammatory cytokines and GI mucosal permeability, thereby preventing inflammation mediated by the bacterial endotoxin lipopolysaccharide (LPS) (9, 28). Butyrate-producing bacteria are significantly decreased in T2DM, suggesting that this SCFA confers protection against the development of insulin resistance (9). Family members of Firmicutes and Actinobacteria associated with beneficial metabolic effects are also more abundant in MHO vs. MUO individuals (48).

In contrast, Fusobacteria is more abundant in MUO individuals (9). Despite increased abundance in T2DM individuals, elevated Fusobacteria levels do not significantly correlate with increased BMI (49). Differing from most other microorganisms, Fusobacteria is abundant with intestinal inflammation (9). Fusobacteria are established oral pathogens well-implicated in colorectal cancer, where they upregulate the pro-inflammatory cytokines tumor necrosis factor alpha, interleukin-6, and interleukin-8 as well as cyclooxygenase-2 enzyme (50). As gram-negative microorganisms, Fusobacteria also contribute to inflammation via the LPS component of their cell wall (51). In addition to increased LPS release, elevated cytotoxic reactive oxygen species (ROS) levels, reduced bioavailability of nitric oxide (a central regulator of energy metabolism and body composition), and decreased SCFA production occur with obesity (26, 27, 43). These events create conditions that promote inflammation, induce endothelial dysfunction, and reduce insulin sensitivity, which leads to further inflammation, dyslipidemia, hyperglycemia, and other cardiometabolic dysfunction.

Diet- and oral PPI-induced dysbiosis

Diet composition and oral ingestion of medications substantially influence microbiota diversity (23, 26, 27, 52). Diets enriched in saturated fat, protein, and complex carbohydrates decrease gut microbiota biodiversity through the production of toxic metabolites or by overfeeding certain families of potentially pathogenic organisms (23). These diets increase gram-negative bacteria like *Escherichia coli* that harbor LPS and decrease the prevalence of favorable gram-positive bacteria that help maintain the gut mucosal barrier to protect against endotoxins (53, 54). Metabolic endotoxemia, approximately a two-fold increase in circulating LPS levels from baseline, is one mechanism by which dysbiosis and leaky gut elicit the systemic inflammation and insulin resistance that characterize MUO (55, 56). Systemic administration of LPS to lean mice increases fat deposition, systemic and tissue-specific inflammation, and insulin resistance to a similar extent as that caused by diet-induced obesity (DIO) (55). Furthermore, serum LPS levels are 1.5-fold greater in obese mice fed a normal chow diet than in lean mice fed a HFD (55). LPS binds with LPS-binding protein (LBP) to trigger the toll-like receptor 4 signaling cascade, which activates the inflammatory immune response (56). Both LPS and LBP are elevated in individuals with obesity or T2DM compared to healthy controls (56). Poor diet is a major culprit in the etiology and pathogenesis of obesity partly through an LPS-mediated mechanism and is linked to GERD, driving PPI use. Obesogenic diets, particularly those high in fat, increase GERD risk by lowering esophageal sphincter (LES) tone, increasing transient LES relaxation, and delaying gastric emptying (57, 58). These diets also elevate intestinal amounts of LPS-releasing, gram-negative bacteria that promote the pro-inflammatory state implicated in abnormal LES relaxation (59, 60). Esophageal microbiome analyses reveal a skewing towards gram-negative populations in esophagitis and Barrett's esophagus. This profile is strongly linked to GERD-related pathology through LPS-mediated induction of NO, promoting LES relaxtion (61, 62). Several oral medication classes alter the microbiome. With only short-term use, repeated exposure to antibiotics negatively alters microbiome composition, possibly long-term (23). A positive association between antibiotic exposure and weight gain in children has been reported (63). Compared to other commonly used medications such as statins, antibiotics, antidepressants, and metformin, PPIs impart the greatest and most consistent inter-individual variability in gut microbiota (64–68). PPI use is linked to increased risk of CDI by altering CDI-associated taxa, increasing gastric pH, and delaying gastric emptying (69–72). Comprehensive meta-analyses determined that PPI use increases the risk of developing initial and recurrent CDI by two- and 1.5-fold, respectively (73, 74). Strong evidence for PPI-induced risk prompted an FDA-issued drug safety warning (75). Daily PPI use is recognized as a sole, avoidable, independent risk factor for CDI-associated mortality in a dose-dependent fashion (76).

Although PPIs are not pro-inflammatory *per se*, they induce changes in the gut microbiota that cause inflammation. Intestinal amounts of *Enterococcus*, *Clostridium*, and *Lactobacillus* increase with PPI use, whereas those of *Bacteroides* and *Bifidobacteria* decrease, elevating the F/B ratio (77–80). Compared to pre-treatment values, human participants undergoing 8-week treatment with the PPIs esomeprazole, rabeprazole, or lansoprazole had increased fecal amounts of Firmicutes due to bacterial translocation from the oral, nasal, and throat cavities to the intestine (81). Confoundingly, this study did not control for any change in diet post-GERD relief (81). Once daily administration of esomeprazole for 4 weeks increases the fecal abundance of *Streptococcus* (normally found in the upper GI tract), with trends for increased amounts in the saliva and periodontal pocket also observed (81). *Streptococcus* increases oxidative stress in the GI tract via ROS production (80). Increased *Streptococcus* is also associated with duodenal eosinophil infiltration both after short- and long-term PPI therapy (79). The resultant intestinal inflammation is a key factor in the development of systemic, low-grade inflammation. Omeprazole use also increases the abundance of Fusobacteria and Firmicutes in the gastric mucosa of healthy dogs (82). In rats, long-term administration of lansoprazole reduces microbiota diversity and richness, with reduced abundance of *Clostridium* and members of Actinobacteria and Bacteroidetes (28).

Obesity likely increases the risk of stress, anxiety, and depression, especially when metabolic disturbances are present (83). In line with these findings, increased intestinal permeability stemming from PPI use and dysbiosis of gut microbiota is enhanced during psychological stress (78). In mice subjected to water avoidance stress (WAS), once daily administration of the PPIs rabeprazole, omeprazole, or lansoprazole post-stress session exacerbated WAS-induced increases in intestinal permeability and duodenal mast cell infiltration both *in vivo* and *ex vivo*; these phenomena are transferrable via gut microbiome transplantation (78). Expression of multiple duodenal tight junction adhesion molecules (at both the gene and protein levels) is also decreased with PPI treatment (78). Strengthening the notion that stress plays a causal role in the pathogenesis of obesity, PPIs do not increase intestinal permeability in the absence of stress (78).

Obesity-related and PPI-induced aberrations in short-chain fatty acid (SCFA) production

The microbiome-gut-brain axis is a bidirectional communication network amongst the central nervous system (CNS), autonomic nervous system (ANS), enteric nervous system (ENS), and hypothalamic pituitary adrenal (HPA) axis that maintains GI and neuronal homeostasis (84). Hypothalamic neurons sense microbiota cell wall components to regulate food intake and EE (85). SCFAs are involved in microbiota-gut-brain interactions as substrates of G protein-coupled receptors (GPCRs) to positively influence host functions such as appetite, glucose homeostasis, EE, immunomodulation, and functional integrity of the GI tract (28, 52, 86, 87).

The most common SCFAs produced by the microbiome are butyrate, propionate, and acetate. Butyrate's protective effects against obesity are pleiotropic (88). Butyrate regulates body weight by promoting EE and reducing energy intake. It induces mitochondrial function in association with up-regulated expression of genes involved in lipolysis and fatty acid β-oxidation. In brown adipose tissue, it promotes thermogenesis via activation of lysine-specific demethylase and β_3-adrenergic receptors. Along the gut-brain axis, it inhibits weight gain by promoting satiety and reducing food intake by suppressing the activity of hypothalamic orexigenic neurons. Butyrate's hypophagic and anorectic effects are mediated by increased levels of glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, and gut hormone peptide YY, as well as up-regulation of the mu-opioid receptor. In the liver, butyrate upregulates antioxidant systems by promoting β-oxidation and stimulating fibroblast growth factor 21 through activation of peroxisome proliferator-activated receptor α. These hepatic events are accompanied by reduced inflammation, lipid deposition, and cholesterol synthesis. In adipose tissue, it induces leptin production and secretion, promotes β-oxidation, and inhibits inflammation. In the pancreas, it promotes insulin secretion and inhibits glucagon secretion. In the gut, it influences the expression of colonic tight junction proteins to control gut permeability (88).

Decreased SCFA production, particularly butyrate-producing microbes, as a consequence of consuming a Western-style diet is implicated in obesity and other metabolic diseases (88, 89). Conversely, dietary supplementation with acetate, propionate, butyrate, or their admixture inhibits HFD-induced weight gain in mice (36). GPR41 and GPR43 are mammalian GPCRs located in adipose tissue, GI epithelium, and lymphatic tissue that are upregulated by circulating LPS and systemic inflammation (90). HFD intake lowers gene transcript levels of GPR41 and GPR43 in adipose tissue and elevates levels in colon vs. lean mice; SCFA supplementation reverses these effects (36). Long-term administration of lansoprazole to rats reduces intestinal and colonic butyrate concentrations, especially in old age (91). Moreover, the abundance of *Lactobacillus* in the ileum is significantly and positively correlated with butyrate concentration in the duodenum and ascending colon and positively correlated with butyrate levels in the jejunum (91). Of note, SCFAs do not always impart beneficial effects on metabolic health. Some preclinical data indicate that signaling at GPR41 and GPR43 is associated with DIO and inflammatory disease (90). These observations reflect the complex manner through which the microbiome regulates inflammation and metabolism.

Discussion

Obesity is a multifactorial condition associated with multiple concomitant diseases through a myriad of complex mechanisms. Obesity resides on a spectrum ranging from healthy to unhealthy, whereby adipogenesis and inflammation mediate its comorbidities including dyslipidemia, cardiovascular dysfunction, and insulin resistance. FMT data indicate that MUO may stem from unfavorable alterations in gut microbiota (39–42). This dysbiosis

simultaneously inhibits the production of beneficial, health-promoting metabolites (*i.e.*, SCFAs) and promotes the production of pro-inflammatory, harmful ones (*i.e.*, LPS).

Genetic and environmental factors influence the microbiome. Diet composition is one key environmental factor. Oral medications such as antibiotics also negatively alter the microbiome, potentially compromising its natural diversity years after initial exposure. Emerging evidence identifies PPIs as another culprit medication class associated with dysbiosis. In most cases, the intended duration of PPI use is only up to 8 weeks. Alarmingly, long-term PPI use is increasingly common in obese and pediatric populations (92, 93). This could permanently alter microbiome composition, and many associative findings and emerging causal evidence indicate that it deleteriously affects metabolic health long-term. Yet the full impact of short- and long-term PPI use on altering gut microbiome composition and the extent to which dysbiosis contributes to MUO in humans remains largely unknown, as no clinical trials have examined these questions to date.

Attempts to prevent/attenuate negative impacts on metabolic health related to PPI-associated dysbiosis might involve curtailing the following: physician overprescribing, direct-to-consumer advertising, misdiagnosis, self-diagnosis, and treating symptoms rather than the cause(s) of acid reflux. Although data are limited, taking probiotics and eating prebiotic foods rich in antioxidants and dietary fiber appear to be beneficial (92, 94, 95). High fiber diet improves metabolic health and mood in T2DM patients (96). In children, once daily co-administration of probiotics substantially reduced dysbiosis occurrence in response to 12-week, once daily esomeprazole vs. esomeprazole treatment alone from 56.2% to 6.2%, respectively (92). Other studies report mixed findings regarding the beneficial effects of supplementation with *Streptococcus*, *Lactobacillus* and/or *Bifidobacterium* on body weight, BMI, waist circumference, and fat mass (97). A clinical trial analyzing the effects of probiotics to reduce dysbiosis and GI discomfort in adult GERD patients using PPIs long-term is currently underway (98). The benefits of probiotic use outweigh any potential risks. Namely, probiotics prevent and treat antibiotic-associated dysbiosis and diarrhea (99). Probiotic use would likely be equally beneficial for PPI-induced dysbiosis and associated metabolic dysfunction.

Author contributions

MB, TS, TF, and AW conceptualized and drafted the manuscript. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

References

1. Bray GA, Kim KK, Wilding JPH. Obesity: a chronic relapsing progressive disease process. a position statement of the world obesity federation. *Obes Rev* (2017) 18 (7):715–23. doi: 10.1111/OBR.12551

2. Warren M, Beck S, West M. *The state of obesity 2022. trust for america's health*. Available at: https://www.tfah.org/wp-content/uploads/2022/09/2022ObesityReport_FINAL3923.pdf (Accessed January 17, 2023).

3. Ward ZJ, Bleich SN, Long MW, Gortmaker SL. Association of body mass index with health care expenditures in the united states by age and sex. *PloS One* (2021) 16(3). doi: 10.1371/JOURNAL.PONE.0247307

4. Blüher M. Metabolically healthy obesity. *Endocr Rev* (2020) 41(3):405–20. doi: 10.1210/ENDREV/BNAA004

5. Iacobini C, Pugliese G, Blasetti Fantauzzi C, Federici M, Menini S. Metabolically healthy versus metabolically unhealthy obesity. *Metabolism* (2019) 92:51–60. doi: 10.1016/j.metabol.2018.11.009

6. Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Carlos A. Camargo jr. association between body mass index and gastroesophageal reflux symptoms in both normal weight and overweight women. *N Engl J Med* (2006) 354(22):2340. doi: 10.1056/NEJMOA054391

7. Wu J, Wang K, Wang X, Pang Y, Jiang C. The role of the gut microbiome and its metabolites in metabolic diseases. *Protein Cell* (2021) 12(5):360. doi: 10.1007/S13238-020-00814-7

8. El-Serag HB, Graham DY, Satia JA, Rabeneck L. Obesity is an independent risk factor for GERD symptoms and erosive esophagitis. *Am J Gastroenterol* (2005) 100 (6):1243–50. doi: 10.1111/j.1572-0241.2005.41703.x

9. Kim MH, Yun KE, Kim J, Park E, Chang Y, Ryu S, et al. Gut microbiota and metabolic health among overweight and obese individuals. *Sci Rep* (2020) 10:1. doi: 10.1038/s41598-020-76474-8

10. Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. *J Clin Invest* (2019) 129(10):3978. doi: 10.1172/JCI129186

11. Saqui-Salces M, Tsao AC, Gillilland MG, Merchant JL. Weight gain in mice on a high caloric diet and chronically treated with omeprazole depends on sex and genetic background. *Am J Physiol Gastrointest Liver Physiol* (2017) 312(1):G15–23. doi: 10.1152/ajpgi.00211.2016

12. Kim TJ, Lee H, Baek SY, Kim K, Min YW, Min BH, et al. Metabolically healthy obesity and the risk of erosive esophagitis: a cohort study. *Clin Transl Gastroenterol* (2019) 10(9). doi: 10.14309/ctg.0000000000000077

13. Shin JM, Sachs G. Pharmacology of proton pump inhibitors. *Curr Gastroenterol Rep* (2008) 10(6):528. doi: 10.1007/S11894-008-0098-4

14. Strand DS, Kim D, Peura DA. 25 years of proton pump inhibitors: a comprehensive review. *Gut Liver* (2017) 11(1):27. doi: 10.5009/GNL15502

15. *Prescription to over-the-Counter (OTC) switch list*. FDA. Available at: https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/prescription-over-counter-otc-switch-list (Accessed January 18, 2023).

16. Benmassaoud A, McDonald EG, Lee TC. Potential harms of proton pump inhibitor therapy: rare adverse effects of commonly used drugs. *CMAJ* (2016) 188 (9):657–62. doi: 10.1503/cmaj.150570

17. Rotman SR, Bishop TF. Proton pump inhibitor use in the U.S. ambulatory setting, 2002–2009. *PloS One* (2013) 8(2):e56060. doi: 10.1371/JOURNAL.PONE.0056060

18. Haastrup PF, Jarbøl DE, Thompson W, Hansen JM, Søndergaard J, Rasmussen S. When does proton pump inhibitor treatment become long term? a scoping review. *BMJ Open Gastroenterol* (2021) 8(1):563. doi: 10.1136/bmjgast-2020-000563

19. Reimer C. Safety of long-term PPI therapy. *Best Pract Res Clin Gastroenterol* (2013) 27(3):443–54. doi: 10.1016/j.bpg.2013.06.001

20. Scarpignato C, Tolone S. Addressing long-term PPI safety. *Digestive Liver Dis* (2020) 52(8):853–6. doi: 10.1016/j.dld.2020.05.025

21. Ambizas EM, Etzel JV. Proton pump inhibitors: considerations with long-term use. *US Pharmacist* (2017) 42(7):4–7.

22. Ciardullo S, Rea F, Savaré L, Morabito G, Perseghin G, Corrao G. Prolonged use of proton pump inhibitors and risk of type 2 diabetes: results from a large population-based nested case-control study. *J Clin Endocrinol Metab* (2022) 107(7):e2671–9. doi: 10.1210/clinem/dgac231

23. Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. *Cell Mol Life Sci* (2017) 74:16. doi: 10.1007/S00018-017-2509-X

24. Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* (2017) 15 (1):73. doi: 10.1186/s12967-017-1175-y

25. Bolte LA, Vich Vila A, Imhann F, Collij V, Gacesa R, Peters V, et al. Long-term dietary patterns are associated with pro-inflammatory and anti-inflammatory features of the gut microbiome. *Gut* (2021) 70(7):1287–98. doi: 10.1136/gutjnl-2020-322670

26. Tidjani Alou M, Lagier JC, Raoult D. Diet influence on the gut microbiota and dysbiosis related to nutritional disorders. *Hum Microb J* (2016) 1:3–11. doi: 10.1016/J.HUMIC.2016.09.001

27. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? a changing ecosystem across age, environment, diet, and diseases. *Microorganisms* (2019) 7(1):14. doi: 10.3390/MICROORGANISMS7010014

28. Blaak EE, Canfora EE, Theis S, Frost G, Groen AK, Mithieux G, et al. Short chain fatty acids in human gut and metabolic health. *Benef Microbes* (2020) 11(5):411–55. doi: 10.3920/BM2020.0057

29. Xu Q, Ni JJ, Han BX, Yan SS, Wei XT, Feng GJ, et al. Causal relationship between gut microbiota and autoimmune diseases: a two-sample mendelian randomization study. *Front Immunol* (2021) 12:746998. doi: 10.3389/fimmu.2021.746998

30. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Burmann J, et al. Short chain fatty acids and gut microbiota differ between patients with parkinson's disease and age-matched controls. *Parkinsonism Relat Disord* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019

31. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol Aging* (2017) 49:60–8. doi: 10.1016/j.neurobiolaging.2016.08.019

32. Wang Y, Li L, Zhao X, Sui S, Wang Q, Shi G, et al. Intestinal microflora changes in patients with mild alzheimer's disease in a Chinese cohort. *J Alzheimers Dis* (2022) 88 (2):563–75. doi: 10.3233/JAD-220076

33. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* (2013) 500 (7464):541–6. doi: 10.1038/nature12506

34. Cui J, Ramesh G, Wu M, Jensen ET, Crago O, Bertoni AG, et al. Butyrate-producing bacteria and insulin homeostasis: the microbiome and insulin longitudinal evaluation study (MILES). *Diabetes* (2022) 71(11):2438–46. doi: 10.2337/db22-0168

35. Mousa WK, Chehadeh F, Husband S. Microbial dysbiosis in the gut drives systemic autoimmune diseases. *Front Immunol* (2022) 13:906258. doi: 10.3389/fimmu.2022.906258

36. Lu Y, Fan C, Li P, Lu Y, Chang X, Qi K. Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating g protein-coupled receptors and gut microbiota. *Sci Rep* (2016) 6(1):1–13. doi: 10.1038/srep37589

37. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Med* (2016) 8 (1):42. doi: 10.1186/s13073-016-0303-2

38. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, et al. The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? *Nutrients* (2020) 12(5). doi: 10.3390/nu12051474

39. de Groot P, Scheithauer T, Bakker GJ, Prodan A, Levin E, Khan MT, et al. Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time. *Gut* (2020) 69 (3):502–12. doi: 10.1136/gutjnl-2019-318320

40. Lai ZL, Tseng CH, Ho HJ, Cheung CKY, Lin JY, Chen YJ, et al. Fecal microbiota transplantation confers beneficial metabolic effects of diet and exercise on diet-induced obese mice. *Sci Rep* (2018) 8(1):15625. doi: 10.1038/s41598-018-33893-y

41. Yu EW, Gao L, Stastka P, Cheney MC, Mahabamunuge J, Torres Soto M, et al. Fecal microbiota transplantation for the improvement of metabolism in obesity: the FMT-TRIM double-blind placebo-controlled pilot trial. *PloS Med* (2020) 17(3): e1003051. doi: 10.1371/journal.pmed.1003051

42. Koopen AM, Almeida EL, Attaye I, Witjes JJ, Rampanelli E, Majait S, et al. Effect of fecal microbiota transplantation combined with Mediterranean diet on insulin sensitivity in subjects with metabolic syndrome. *Front Microbiol* (2021) 12:662159. doi: 10.3389/fmicb.2021.662159

43. Tseng CH, Wu CY. The gut microbiome in obesity. *J Formosan Med Assoc* (2019) 118:S3–9. doi: 10.1016/J.JFMA.2018.07.009

44. Mathur R, Barlow GM. Obesity and the microbiome. *Expert Rev Gastroenterol Hepatol* (2015) 9(8):1087–99. doi: 10.1586/17474124.2015.1051029

45. Olivares PDSG, Pacheco ABF, Aranha LN, Oliveira BS, Santos AA, Neto JFN, et al. Gut microbiota of adults with different metabolic phenotypes. *Nutrition* (2021) 90:111293. doi: 10.1016/j.nut.2021.111293

46. Zeng Q, Yang Z, Wang F, Li D, Liu Y, Wang D, et al. Association between metabolic status and gut microbiome in obese populations. *Microb Genom* (2021) 7(8). doi: 10.1099/mgen.0.000639

47. Alcazar M, Escribano J, Ferré N, Closa-Monasterolo R, Selma-Royo M, Feliu A, et al. Gut microbiota is associated with metabolic health in children with obesity. *Clin Nutr* (2022) 41(8):1680–8. doi: 10.1016/j.clnu.2022.06.007

48. Proffitt C, Bidkhori G, Moyes D, Shoaie S. Disease, drugs and dysbiosis: understanding microbial signatures in metabolic disease and medical interventions. *Microorganisms* (2020) 8(9):1–16. doi: 10.3390/microorganisms8091381

49. Sedighi M, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, et al. Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. *Microb Pathog* (2017) 111:362–9. doi: 10.1016/j.micpath.2017.08.038

50. Kelly D, Yang L, Pei Z. Gut microbiota, fusobacteria, and colorectal cancer. *Diseases* (2018) 6(4):109. doi: 10.3390/DISEASES6040109

51. Su W, Chen Y, Cao P, Chen Y, Guo Y, Wang S, et al. Fusobacterium nucleatum promotes the development of ulcerative colitis by inducing the autophagic cell death of intestinal epithelial. *Front Cell Infect Microbiol* (2020) 10:594806. doi: 10.3389/fcimb.2020.594806

52. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. *World J Gastroenterol* (2015) 21(29):8787. doi: 10.3748/WJG.V21.I29.8787

53. Amabebe E, Robert FO, Agbalalah T, Orubu ESF. Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. *Br J Nutr* (2020) 123(10):1127–37. doi: 10.1017/S0007114520000380

54. Akagbosu CO, Nadler EP, Levy S, Hourigan SK. The role of the gut microbiome in pediatric obesity and bariatric surgery. *Int J Mol Sci* (2022) 23(23). doi: 10.3390/IJMS232315421

55. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: is it real and is it relevant? *Biochimie* (2016) 124:11. doi: 10.1016/J.BIOCHI.2015.06.020

56. Mohammad S, Thiemermann C. Role of metabolic endotoxemia in systemic inflammation and potential interventions. *Front Immunol* (2020) 11:594150. doi: 10.3389/FIMMU.2020.594150

57. Nebel OT, Castell DO. Lower esophageal sphincter pressure changes after food ingestion. *Gastroenterology* (1972) 63(5):778–83. doi: 10.1016/S0016-5085(19)33219-6

58. Nebel OT, Castell DO. Inhibition of the lower oesophageal sphincter by fat–a mechanism for fatty food intolerance. *Gut* (1973) 14(4):270–4. doi: 10.1136/gut.14.4.270

59. Yang L, Francois F, Pei Z. Molecular pathways: pathogenesis and clinical implications of microbiome alteration in esophagitis and Barrett esophagus. *Clin Cancer Res* (2012) 18(8):2138–44. doi: 10.1158/1078-0432.CCR-11-0934

60. D'Souza SM, Houston K, Keenan L, Yoo BS, Parekh PJ, Johnson DA. Role of microbial dysbiosis in the pathogenesis of esophageal mucosal disease: a paradigm shift from acid to bacteria? *World J Gastroenterol* (2021) 27(18):2054–72. doi: 10.3748/wjg.v27.i18.2054

61. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology* (2009) 137(2):588–97. doi: 10.1053/j.gastro.2009.04.046

62. Fan YP, Chakder S, Gao F, Rattan S. Inducible and neuronal nitric oxide synthase involvement in lipopolysaccharide-induced sphincteric dysfunction. *Am J Physiol Gastrointest Liver Physiol* (2001) 280(1):G32–42. doi: 10.1152/ajpgi.2001.280.1.G32

63. Duong QA, Pittet LF, Curtis N, Zimmermann P. Antibiotic exposure and adverse long-term health outcomes in children: a systematic review and meta-analysis. *J Infection* (2022) 85(3):213–300. doi: 10.1016/j.jinf.2022.01.005

64. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* (2016) 352(6285):565–9. doi: 10.1126/science.aad3369

65. Imhann F, Vich Vila A, Bonder MJ, Lopez Manosalva AG, Koonen DPY, Fu J, et al. The influence of proton pump inhibitors and other commonly used medication on the gut microbiota. *Gut Microbes* (2017) 8(4):351–8. doi: 10.1080/19490976.2017.1284732

66. Vich Vila A, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat Commun* (2020) 11(1):362. doi: 10.1038/s41467-019-14177-z

67. Weersma RK, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. *Gut* (2020) 69(8):1510–9. doi: 10.1136/gutjnl-2019-320204

68. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* (2018) 555 (7698):623–8. doi: 10.1038/nature25979

69. Bavishi C, DuPont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther* (2011) 34(11-12):1269–81. doi: 10.1111/j.1365-2036.2011.04874.x

70. Imhann F, Bonder MJ, Vila AV, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. *Gut* (2016) 65(5):740. doi: 10.1136/GUTJNL-2015-310376

71. Freedberg DE, Toussaint NC, Chen SP, Ratner AJ, Whittier S, Wang TC, et al. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. *Gastroenterology* (2015) 149(4):883–5.e9. doi: 10.1053/j.gastro.2015.06.043

72. Sanaka M, Yamamoto T, Kuyama Y. Effects of proton pump inhibitors on gastric emptying: a systematic review. *Dig Dis Sci* (2010) 55(9):2431–40. doi: 10.1007/s10620-009-1076-x

73. Reveles KR, Ryan CN, Chan L, Cosimi RA, Haynes WL. Proton pump inhibitor use associated with changes in gut microbiota composition. *Gut* (2018) 67(7):1369–70. doi: 10.1136/gutjnl-2017-315306

74. Kwok CS, Arthur AK, Anibueze CI, Singh S, Cavallazzi R, Loke YK. Risk of clostridium difficile infection with acid suppressing drugs and antibiotics: meta-analysis. *Am J Gastroenterol* (2012) 107(7):1011–9. doi: 10.1038/ajg.2012.108

75. Maes ML, Fixen DR, Linnebur SA. Adverse effects of proton-pump inhibitor use in older adults: a review of the evidence. *Ther Adv Drug Saf* (2017) 8(9):273–97. doi: 10.1177/2042098617715381

76. Lin CY, Cheng HT, Kuo CJ, Lee YS, Sung CM, Keidan M, et al. Proton pump inhibitor-induced gut dysbiosis increases mortality rates for patients with clostridioides difficile infection. *Microbiol Spectr* (2022) 10(4). doi: 10.1128/spectrum.00486-22

77. Bruno G, Zaccari P, Rocco G, Scalese G, Panetta C, Porowska B, et al. Proton pump inhibitors and dysbiosis: current knowledge and aspects to be clarified. *World J Gastroenterol* (2019) 25(22):2706–19. doi: 10.3748/wjg.v25.i22.2706

78. Takashima S, Tanaka F, Kawaguchi Y, Usui Y, Fujimoto K, Nadatani Y, et al. Proton pump inhibitors enhance intestinal permeability via dysbiosis of gut microbiota under stressed conditions in mice. *Neurogastroenterol Motil* (2020) 32(7). doi: 10.1111/nmo.13841

79. Wauters L, Tito RY, Ceulemans M, Lambaerts M, Accarie A, Rymenans L, et al. Duodenal dysbiosis and relation to the efficacy of proton pump inhibitors in functional dyspepsia. *Int J Mol Sci* (2021) 22(24). doi: 10.3390/ijms222413609

80. Shi YC, Cai ST, Tian YP, Zhao HJ, Zhang YB, Chen J, et al. Effects of proton pump inhibitors on the gastrointestinal microbiota in gastroesophageal reflux disease. *Genomics Proteomics Bioinf* (2019) 17(1):52–63. doi: 10.1016/J.GPB.2018.12.004

81. Hojo M, Asahara T, Nagahara A, Takeda T, Matsumoto K, Ueyama H, et al. Gut microbiota composition before and after use of proton pump inhibitors. *Dig Dis Sci* (2018) 63(11):2940–9. doi: 10.1007/s10620-018-5122-4

82. Garcia-Mazcorro JF, Suchodolski JS, Jones KR, Clark-Price SC, Dowd SE, Minamoto Y, et al. Effect of the proton pump inhibitor omeprazole on the gastrointestinal bacterial microbiota of healthy dogs. *FEMS Microbiol Ecol* (2012) 80 (3):624–36. doi: 10.1111/j.1574-6941.2012.01331.x

83. Abiri B, Hosseinpanah F, Banihashem S, Madinehzad SA, Valizadeh M. Mental health and quality of life in different obesity phenotypes: a systematic review. *Health Qual Life Outcomes* (2022) 20(1). doi: 10.1186/s12955-022-01974-2

84. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* (2015) 28(2):203–9.

85. Gabanyi I, Lepousez G, Wheeler R, Vieites-Prado A, Nissant A, Wagner S, et al. Bacterial sensing via neuronal Nod2 regulates appetite and body temperature. *Sci (1979)* (2022) 376(6590). doi: 10.1126/science.abj3986

86. Jung TH, Han KS, Park JH, Hwang HJ. Butyrate modulates mucin secretion and bacterial adherence in LoVo cells via MAPK signaling. *PloS One* (2022) 17. doi: 10.1371/journal.pone.0269872

87. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol (Lausanne)* (2020) 11:25. doi: 10.3389/fendo.2020.00025

88. Coppola S, Avagliano C, Calignano A, Berni Canani R. The protective role of butyrate against obesity and obesity-related diseases. *Molecules* (2021) 26(3). doi: 10.3390/molecules26030682

89. Gentile CL, Weir TL. The gut microbiota at the intersection of diet and human health. *Sci (1979)* (2018) 362(6416):776–80. doi: 10.1126/science.aau5812

90. Ang Z, Ding JL. GPR41 and GPR43 in obesity and inflammation – protective or causative? *Front Immunol* (2016) 7:28. doi: 10.3389/FIMMU.2016.00028

91. Lee SM, Kim N, Nam RH, Park JH, Choi SI, Park YT, et al. Gut microbiota and butyrate level changes associated with the long-term administration of proton pump inhibitors to old rats. *Sci Rep* (2019) 9(1). doi: 10.1038/s41598-019-43112-x

92. Belei O, Olariu L, Dobrescu A, Marcovici T, Marginean O. Is it useful to administer probiotics together with proton pump inhibitors in children with gastroesophageal reflux? *J Neurogastroenterol Motil* (2018) 24(1):51–7. doi: 10.5056/jnm17059

93. Levy EI, Hoang DM, Vandenplas Y. The effects of proton pump inhibitors on the microbiome in young children. *Acta Paediatr* (2020) 109(8):1531–8. doi: 10.1111/apa.15213

94. Deledda A, Annunziata G, Tenore GC, Palmas V, Manzin A, Velluzzi F. Diet-derived antioxidants and their role in inflammation, obesity and gut microbiota modulation. *Antioxidants* (2021) 10(5). doi: 10.3390/antiox10050708

95. Djuric Z. Dietary approaches for normalizing dysbiosis induced by high-fat, obesogenic diets. *Curr Opin Clin Nutr Metab Care* (2023) 26(3):293–301. doi: 10.1097/MCO.0000000000000917

96. Chen L, Liu B, Ren L, Du H, Fei C, Qian C, et al. High-fiber diet ameliorates gut microbiota, serum metabolism and emotional mood in type 2 diabetes patients. *Front Cell Infect Microbiol* (2023) 13:1069954. doi: 10.3389/fcimb.2023.1069954

97. Abenavoli L, Scarpellini E, Colica C, Boccuto L, Salehi B, Sharifi-Rad J, et al. Gut microbiota and obesity: a role for probiotics. *Nutrients* (2019) 11(11). doi: 10.3390/nu11112690

98. Liu W, Xie Y, Li Y, Zheng L, Xiao Q, Zhou X, et al. Protocol of a randomized, double-blind, placebo-controlled study of the effect of probiotics on the gut microbiome of patients with gastro-oesophageal reflux disease treated with rabeprazole. *BMC Gastroenterol* (2022) 22(1). doi: 10.1186/s12876-022-02320-y

99. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JNV, Shanman R, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* (2012) 307(18):1959–69. doi: 10.1001/jama.2012.3507

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The gut-retina axis: a new perspective in the prevention and treatment of diabetic retinopathy

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Diabetic retinopathy (DR) is a microvascular lesion that occurs as a complication of diabetes mellitus. Many studies reveal that retinal neurodegeneration occurs early in its pathogenesis, and abnormal retinal function can occur in patients without any signs of microvascular abnormalities. The gut microbiota is a large, diverse colony of microorganisms that colonize the human intestine. Studies indicated that the gut microbiota is involved in the pathophysiological processes of DR and plays an important role in its development. On the one hand, numerous studies demonstrated the involvement of gut microbiota in retinal neurodegeneration. On the other hand, alterations in gut bacteria in RD patients can cause or exacerbate DR. The present review aims to underline the critical relationship between gut microbiota and DR. After a brief overview of the composition, function, and essential role of the gut microbiota in ocular health, and the review explores the concept of the gut-retina axis and the conditions of the gut-retina axis crosstalk. Because gut dysbiosis has been associated with DR, the review intends to determine changes in the gut microbiome in DR, the hypothesized mechanisms linking to the gut-retina axis, and its predictive potential.

KEYWORDS

diabetes mellitus (DM), diabetic retinopathy (DR), gut-retina axis, gut microbiota, retina, mechanics, treatment

1 Introduction

Diabetic retinopathy (DR) has emerged as a leading cause of visual impairment in working-age people in various regions (1–3). According to the International Diabetes Federation (IDF), more than 500 million people worldwide will have diabetes mellitus (DM) by 2021 (4). The presence and progression of DR are associated with a significant increase in healthcare costs. Diabetes-related direct health expenditures were USD 760 billion in 2019 and are expected to rise to USD 825 billion by 2045 (5). Numerous studies demonstrated that approximately one in every three diabetic patients has DR (6). Given the

high incidence of DR and the Global Burden of Disease estimate, it is critical to investigate the predictive potential for DR progression and potential therapeutic targets.

DR has been considered a microvascular complication, a known complication of DM (7–9). Studies suggest that neurodegeneration is an early event in its pathogenesis, and abnormalities in retinal function can occur in patients with no evidence of microvascular abnormalities (10, 11). The American Diabetes Association (ADA) recently defined DR as a precise neurovascular complication (12). Although studies revealed that DR is caused by chronic hyperglycemia, with retinal neovascularization, chronic inflammation, disorders of glucolipid metabolism, and immune response as its hallmarks (13–16), the exact pathogenesis remains unknown. The intestinal microbiota is a large, diverse colony of microorganisms colonizing the human intestine. The intestinal microbiota evolved symbiotically with its host and plays an important role in regulating nutrient absorption (17) and metabolism (18–20), maintaining the intestinal mucosal barrier (21), intestinal immunity and pathogen defense (22, 23). Studies indicated that the intestinal microbiota is involved in the pathophysiological processes of DR and plays an important role in DR development (24, 25).

The gut microbiota is primarily made up of bacteria. However, it also contains other commensals such as archaea, viruses, fungi, and protists (The term "microbiota" refers to consortia of microorganisms living in a specific environment, whereas "commensals" refers to microorganisms that colonize hosts without causing disease) (26). Following their functions, the intestinal microbiota can be classified as commensal, probiotic, or pernicious bacteria. The primary role of probiotics is to improve nutrient digestion and absorption, regulate lipid metabolism, and reduce the inflammatory response (27–29). Simultaneously, pernicious bacteria can activate the inflammatory response *in vivo*, disrupt the function of the intestinal epithelial barrier, and cause metabolic disorders (30, 31). Dysbiosis of the gut microbiota, also known as gut dysbiosis, is primarily characterized by a reduction in the diversity and abundance of bacteria and fungi, particularly those associated with dysfunction and various pathologies (32).

Moreover, dysbiosis of the gut microbiota can result in various gastrointestinal diseases and processes in distal tissues other than the intestine, such as joints, mucous membranes, and the eyes, which are common sites of invasion (33–35).In addition, new molecular biology-based techniques enable the identification and quantification of microbiota by analyzing DNA and RNA extracted from fecal samples. The studies described in the preceding sections support the notion that gut microbiota has become a hotspot for disease research.

Since Rowan (36) et al. introduced the concept of the "gut-retina axis" and demonstrated the existence of gut-retina crosstalk, the significance of gut microbes as important modulators of ocular disease have grown (37). Scientists identified that diet, probiotics, and antibiotics could influence the gut microbiota and, thus, the development of retinal disease (38). Increased intraocular pressure, glucose accumulation in vessels, and neovascularization can affect the health of the eye in poorly controlled diabetes (39). These processes are associated with microvascular complications in the eye, such as cataracts, glaucoma, and DR (40). DR, a complication of poorly controlled diabetes, can eventually lead to blindness (41). Dysbiosis of the gut microbiota is closely linked to the occurrence, development, and prognosis of DR.

On the one hand, an increasing number of studies have demonstrated that gut microbiota plays a role in retinal neurodegeneration (42, 43), in retinal inflammatory processes (44), and affect glucose metabolism, insulin resistance, and entero-insulin secretion (45). Conversely, alterations in the gut bacterial microbiome in people with RD, and thus dysbiosis of gut microbiota, can also cause or aggravate DR (46). For example, carnosine was depleted in DR patients compared to healthy controls. Carnosine is an endogenous dipeptide composed of β-alanine and L-histidine with significant antioxidant and anti-inflammatory properties (47). These findings suggest that gut microbiota mediates gut-retinal communication, which is important in DR.

The present review aims to highlight the importance of gut microbiota in DR. The critical role of gut dysbiosis in the development and progression of DR is discussed briefly. Subsequently, the concept of the gut-retina axis and the mediators and conditions that allow gut-retina crosstalk will be investigated, focusing on the mechanisms involved in regulating DR by the intestinal microbiota. Finally, diet and antibiotics strategies for treating DR via the regulated intestinal microbiota and, thus, the treatment of DR will be described.

2 Microbiota and ocular diseases

2.1 Gut microbiota and ocular diseases

Recent studies have confirmed the presence of many microorganisms, such as bacteria, viruses, and fungi, on the human body surface and within the body (48). These microorganisms are ten times higher than the body's cells and have 100 times more genes than the body's genome, with 1000 to 1150 bacteria colonizing the intestine (49, 50). Although many microorganisms exist in the human gut, only about 160 species belong to the advantage bacterium group (51). The human gut microbiota is primarily composed of two dominant bacterial phyla (human microorganisms are classified by phylum, order, family, genus, and species), *Firmicutes* and *Bacteroidetes*, which account for more than 90% of the entire community, and other subdominant phyla such as *Proteobacteria*, *Aspergillus*, *Actinomycetes*, and *Clostridium* (52). Different intestinal flora interacts in the intestinal micro-ecosystem, and the intestinal flora and their host have a mutually beneficial commensal relationship (53, 54). It maintains a complex dynamic balance in healthy populations that can help the body with various physiological functions, mainly limiting the colonization of pathogenic intestinal bacteria and maintaining the integrity of the intestinal epithelial barrier and immune homeostasis (55, 56). In addition, the intestinal flora decomposes and utilizes food residues to provide humans with

essential vitamins, amino acids, and other nutrients through the mediation of a series of digestive enzymes. It can also metabolize harmful substances like nitrosamines and lactic acid. Therefore, intestinal microorganisms play an important role in the human micro-ecosystem. When there is gut dysbiosis, the intestinal micro-ecosystem is disrupted, resulting in chronic inflammatory responses and immune diseases in the eye (Table 1), such as fungal keratitis (61), DR (62), age-related macular degeneration (AMD) (63), and uveitis (UVT) (64). In addition, there is a link between inflammatory bowel diseases and ocular diseases; 10% of subjects with inflammatory bowel disease have ocular diseases (such as episcleritis, uveitis, and conjunctivitis) (65). In humans, patients with DR have a significantly lower proportion of *Bacteroidetes* and *Actinobacteria* than healthy individuals (45, 46).

2.2 Ocular surface microbiota in patients with DR

Several studies have used traditional microbial cultures and 16S rRNA gene sequencing to describe the commensal microbiota on the ocular surface. Under normal physiological conditions, the microbiota is relatively stable, with low diversity and abundance, while still playing an important role in maintaining ocular surface homeostasis (66, 67). However, Suwajanakorn et al. (68) used next-generation sequencing analysis to demonstrate the importance of DR and glycemic control status in influencing changes in the ocular surface microbiome. Subsequent studies identified that microbes could be transferred to the retina of type 1 diabetic mice with retinopathy through gut and plasma microbiota (69). Furthermore, the microbiota composition

TABLE 1 Alterations of bacteria in the fecal microbiota of patients with ocular diseases.

Study (Author, (Publication Year)	Ocular Diseases	Study design	Major Results	Conclusion
Healthy controls (HC) and Subjects with uveitis (UVT) were compared.			1. It revealed reduced diversity of several anti-inflammatory organisms, including *Faecalibacterium, Bacteroides*, *Lachnospira*, *Ruminococcus*, and members of *Lachnospiraceae* and *Ruminococcaceae* families, while enrichment of *Prevotella* (pro-inflammatory) and *Streptococcus* (pathogenic) OTUs in UVT microbiomes compared to HC. 2. Decrease in probiotic and antibacterial organisms was observed in UVT compared to HC microbiomes.	The study demonstrates dysbiosis in the gut bacterial communities of UVT patients in an Indian cohort.
Kalyana et al. (57) (2018),	UVT	Randomized (HC, n = 13; UVT, n = 13), Indian cohort		
Cases with Dry Eye and controls were compared.			Among cases, 27 were relatively more abundant, including ten *Lactobacillus* and four *Bifidobacterium* species. A relative depletion of five species was identified in patients compared with controls, notably *Fusobacterium* varium and *Prevotella stercorea*.	Differences in gut microbiome composition were found in individuals with Dry Eye compared with controls.
Goodman et al. (58) (2022),	Dry Eye	Case-Control Study (Cases, n = 13; Controls, n = 13)		
Patients with AMD and controls were compared.			1. The genera *Anaerotruncus*, Oscillibacter, Ruminococcus torques, and *Eubacterium ventriosum* were relatively enriched in patients with AMD, whereas *Bacteroides eggerthii* was enriched in controls. 2. Patient's intestinal microbiomes were enriched in genes of L-alanine fermentation, glutamate degradation, and arginine biosynthesis pathways and decreased in fatty acid elongation pathway genes.	Modifications in the intestinal microbiome are associated with AMD,
Zinkernagel et al. (59) (2017),	AMD	Randomized (Patients with AMD, n = 12; controls, n = 11), Participants were recruited from the Department of Ophthalmology, University Hospital Bern (Inselspital), Switzerland.		
The healthy controls (HC) and fungal keratitis (FK) patients were compared.			*Faecalibacterium prausnitzii*, an anti-inflammatory bacterium, and *Megasphaera*, *Mitsuokella multacida*, and *Lachnospira* are butyrate producers enriched in HC. In contrast, *Treponema* and *Bacteroides fragilis*, which are pathogenic, were abundant in FK patients.	1. The distinct patterns of gut bacterial composition in FK and HC samples. 2. Dysbiosis in the gut bacterial microbiomes of FK patients compared to HC
Kalyana et al. (60) (2018),	fungal Keratitis	Randomized (HC, n = 31; FK, n = 32, The participants were recruited from the southern part of India.		
The healthy controls (HC, n = 30), people with T2DM without DR (n = 25), and people with T2DM and DR (n = 28) were compared			The microbiomes of people with T2DM and DR were significantly different. Both DM and DR microbiomes revealed a decrease in anti-inflammatory, probiotics, and other bacteria that could be pathogenic compared to HC, and the observed change was more pronounced in people with DR.	Dysbiosis in the gut microbiomes, at phyla and genera levels, was observed in people with T2DM and DR compared to HC. People with DR exhibited more significant discrimination from HC.
Das et al. (46) (2021),	DR	Randomized, subjects were recruited from South India		

UVT, Uveitis; AMD, Age-related macular degeneration; T2DM, Type 2 diabetes; DR, Diabetic retinopathy.

varies throughout the body, including the eye. Although the overall gut microbiota comprises *Firmicutes* and *Bacteroidetes* (70), the ocular surface microbiota primarily comprises *Proteobacteria* and *Actinobacteria* (71, 72). *Proteobacteria*, *Actinobacteria*, and *Firmicutes* account for over 87% of all microorganisms present in the eye (73). With further investigation, the doctrine that active microbiota is present in the eye has been broken. For example, the internal eye compartment is sterile, Whereas the external compartment is exposed to environmental microorganisms (74).

2.3 Prevalence of gut dysbiosis in DR patients

As a metabolic disease caused by multiple factors, the gut microbiota composition differs between T2DM patients and healthy individuals. For example, significant reductions in the proportion of *Firmicutes* and *Clostridium* appear in the microbiota of diabetic patients compared to healthy controls (75). Subsequent studies have confirmed the role of gut flora in systemic metabolism and T2DM. Qin et al. (76) and Karlsson et al. (77) performed metagenomic sequencing in Chinese and Swedish diabetic patients, respectively, demonstrating that T2DM was characterized by gut dysbiosis. Further research has linked dysbiosis of the gut flora to insulin resistance (IR) and abnormal lipid metabolism, which are important factors in the pathogenesis of T2DM (78). In addition, *Bacteroidetes* to *Firmicutes* ratio (B/F ratio) is a potential diagnostic biomarker for DM (79, 80).

Previous studies have demonstrated that the pathogenesis of T2DM is commonly associated with altered gut microbiota. However, it is unclear whether diabetic patients with or without retinopathy have different gut microbial dysbiosis. Scientists investigated this and identified that DR patients have intestinal dysbiosis similar to T2DM patients, with the main differences being a decrease in microbial diversity (81), changes in microbial composition and structure (37, 46), low levels of beneficial microflora and higher levels of pathogenic bacteria (46, 82). Huang et al. (83) found increased *Bifidobacterium* and *Lactobacillus* levels and decreased *Escherichia-Shigella*, *Faecalibacterium*, *Eubacterium_hallii*_group, and *Clostridium* genera in DM and DR patients compared to the healthy population. Furthermore, patients with DR have a different gut microbiota than those with diabetes, but little variability exists among them. Moreover, Prasad et al. examined the retinas of diabetic mice and determined that gut microbial dysbiosis aggravated retinal impairment and inflammation (69). All these studies confirm that dysbiotic gut microbiota characterized DM and DR.

3 Overview of gut-retina axis

For decades, scientists have studied the relationship between the gastrointestinal (GI) tract and the brain, and numerous studies have confirmed the existence of the brain-gut axis. The "gut-brain axis" refers to the specific linkage between the GI tract and the central nervous system (CNS), which consists of a bidirectional exchange between the two (84). The presence of the brain-gut axis suggests that CNS regulates and governed gut metabolic activity, and there is growing evidence that not only the brain (CNS) can influence GI tract function, but the gut flora can also influence the development of CNS diseases (85, 86). For example, several studies demonstrated that amyloid deposits and neuronal fiber tangle deposits in the enteric nervous system (ENS) of patients with Alzheimer's disease are similar to those found in the brain parenchyma (87, 88). Lewy vesicles, which appear in the brain of patients with Parkinson's disease, have also been identified in enteric neurons (89).

The possibility of an interconnection between the eye and CNS has long been debated because the retina is an extension of the brain (90). The retina is the light-sensitive neural tissue that lines the back of the eye. In anatomical and developmental terms, the retina is a brain extension known as the 'peripheral brain.' Both organs consist of neurons derived from a neural tube with a multilayered cellular structure and synaptic connections. Moreover, the retina transmits information to the brain's visual cortex via the optic nerve, which converts optical signals into nerve impulses. In addition, the retina has the advantages of clear structural stratification, visualization, ease of observation, and relative ease of functional testing compared to the brain, making it an ideal model for observing and studying neurological diseases (91).

Many features of neurodegenerative processes in the CNS are similar to those observed in the retina, and some CNS neurodegenerative diseases can affect the retina and vice versa (92). Retinal lesions, such as ganglion cell layer thinning, can occur early in Alzheimer's disease (93). Retinal chronic progressive neurodegeneration, which can happen in the elderly, can result in eye disorders like glaucoma, AMD, and DR (94). Therefore, scholars have questioned whether the concept of a brain-gut axis applies to the retina independently of the brain, that is, whether the gut-retina axis can be distinguished from the gut-brain axis (64).

Subsequent studies have confirmed the existence of the gut-retina axis and demonstrated that dysregulation of the gut microbiota contributes to the development of ocular diseases (95–97). Moreover, the concept of "gut-retina axis" was formally proposed (98, 99), demonstrating that the gut-retina axis is closely related to ocular immune system homeostasis and plays an important role in various ocular diseases, such as AMD (36, 63), UVT (100), and glaucoma (101). The intestinal-ocular axis has emerged as a new area of basic and clinical research in ophthalmology. However, more in-depth research is needed to confirm and support the existence of the gut-retinal axis.

4 How the intestinal microbiota achieves mutual communication between the gut-retina

The gut-retina axis is an emerging concept that describes a strong interaction between the gut host-microbiota interface and the retina. Because the retina is immune-privileged, a critical question is how this gut-retina crosstalk can be validated. The retina is a ten-layer complex composed of numerous cells, including glial cells (Müller cells, astrocytes, and microglia), retinal microvascular endothelial cells (RMECs), retinal pigment epithelium (RPE), and all types of neurons (102, 103). RPEs, RMECs, and tight junctures form the outer and inner

blood-retinal barrier (BRB). The integrity of the BRB is crucial for the function of various cells within the retina because it prevents the entry of peripheral pathogens, pathogen-associated molecular patterns (PAMPs), and leukocytes, rendering the retina an immune-privileged tissue (104). Therefore, scientists have conducted numerous studies that have revealed that the crosstalk between the gut and retina is primarily achieved through the following pathways.

The interaction between microbes, gut-derived products, and the retina can be explained by the disruption of the BRB, which is common in retinal diseases (105, 106). The GI epithelium serves as a broad interface with the external environment. Single epithelial cells, also called intestinal cells, are tightly connected and cover the inner surface of our intestinal mucosa. These cells provide a barrier by using transcellular and paracellular transport mechanisms to selectively regulate the exchanges of luminal toxins, antigens, nutrients, and water absorption between the inner and outer environments (107). Conversely, the GI epithelial barrier must maintain rapid cell renewal and barrier integrity while being exposed to continuous environmental assaults. Dysbiosis of the intestinal microbiota and inflammatory response in the presence of specific eye diseases (such as DR and UVT) can lead to intestinal barrier impairment, which increases permeability (43). Consequently, impaired gut barrier function leads to the excessive translocation of gut-derived products (such as LTA, PGN, and LPS) and even live gut bacteria into the bloodstream (108). A recent study found microbiota in the intraocular environment of healthy populations and patients with ocular diseases, breaching the dogma that the intraocular environment is sterile (109).

Short-chain fatty acids (SCFAs) are beneficial microbial metabolites produced only in the gut. Chen et al. (110) demonstrate that SCFAs can cross the BRB via systemic circulation and reach the retina, triggering an innate immune response. Consistent with this, data indicate that increased intestinal permeability caused by altered gut microbiota may allow for more significant translocation of gut metabolites and products, which may modulate retina-specific immune cells (111). In addition, studies have confirmed that SCFAs entering the systemic circulation are transported via the monocarboxylate transporter (MCT-1) across the blood-brain barrier and function in the CNS (112). SCFAs may be able to enter the retina and exert regulatory effects because MCT-1 is also present in BRB (113). All these studies suggest the presence of intraocular crosstalk.

5 Mechanism of gut-retina axis regulation in DR

The studies described in the preceding sections support a causative role of microbiota in triggering DR, but the specific mechanisms involved remain elusive. Gut dysbiosis has been associated with DM and DR. The gut-retina axis could be a potential target for preventing DR, a well-known complication of DM. A critical question now is how the gut microbiota influences the development and treatment of DR through the gut-retina axis. We reviewed the literature and identified that the hypothesized mechanisms relating to the gut-retina axis include disruption of intestinal barrier function, activation of the stimulator of interferon genes (STING) signaling pathway, production of lipopolysaccharide (LPS), angiotensin-converting enzyme 2 (ACE2) deficiency, and affecting gut microbiota metabolites (Figure 1).

5.1 Affecting intestinal barrier system function

The intestinal mechanical and biological barriers are formed by the hierarchical and regular distribution of intrinsic intestinal bacteria in the intestinal epithelial cells, mucus on the mucosal surface, and the tight connection between cells. Both contribute to the human intestinal barrier system, which protects the organism from harmful or foreign pathogenic bacteria. The intestinal biological barrier primarily consists of *Bifidobacterium* and other bacteria found in the deep layer and *Peptostreptococcus* in the intermediate layer, which accounts for more than 99% of intestinal bacteria. These intrinsic intestinal bacteria act as a biological barrier by pre-empting colonization sites, competing for nutrients, producing organic acids and SCFAs to lower intestinal pH, producing bacterins, and inducing a moderate inflammatory response (114).

Gut microbiota is being extensively investigated for its role in DM and its complications. Changes in the gut-microbiome cause pathological inflammation and accelerate DR progression. Consequently, it influences the immune system and homeostasis locally (within the gut) and systemically (115). In this context, increased intestinal permeability and associated microbial translocation are important in the pathogenesis of DR (116). Furthermore, this contributes to the chronic systemic inflammatory process and further disrupts the intestinal barrier system. However, it has been demonstrated that even in the absence of ocular infection, the eye is susceptible to inflammatory disease, which is influenced by intraocular microbiota dysbiosis (103). In contrast, the relationship between the initiating factors of intraocular microbiota dysbiosis and DM leading to inflammation requires further investigation.

5.2 Stimulator of interferon genes signaling pathway-mediated inflammation

In various inflammatory diseases, aberrant regulation of the STING pathway has emerged as a critical pathogenic mechanism (117). STING is an endoplasmic reticulum (ER) adaptor protein commonly expressed in the ER. STING activation by the cytoplasmic DNA sensor cycle GMP-AMP synthase (cGAS) causes the activation of the nuclear factor-κB (NF-κB) and the transcription factor interferon regulatory factor 3 (IRF3) (118). A positive feedback loop between dysbiosis and abnormal activation of the STING pathway in the intestine is associated with increased intestinal permeability (119). There is a possibility that dysbiosis in DR patients disrupts intestinal homeostasis and aggravates barrier dysfunction through the erroneous accumulation of STING in the gut. Subsequent translocation of microbial products into the blood allows access to the retina via the impaired BRB, resulting in chronic activation of the STING pathway in the retina, contributing to disease progression (119). In addition, the STING pathway has been linked to changes in the retina and retinal cells of patients with ocular diseases (120).

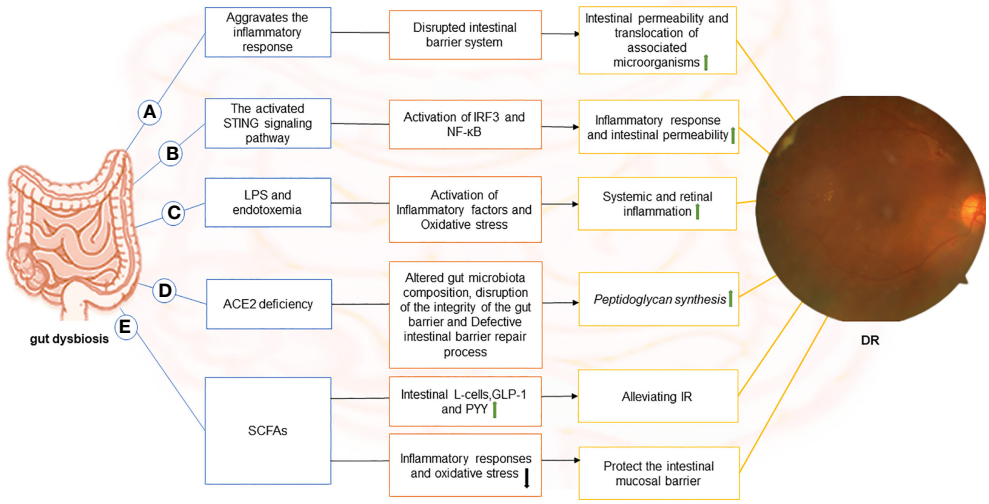

FIGURE 1
The image depicts the various possible mechanisms that connect the gut to the retina in DR and the components of each hypothetical gut-retina axis. **(A)** dysbiosis of the gut microbiota in diabetic patients causes local (including gut) and systemic inflammation. Subsequently, with inflammation, the intestinal barrier system is compromised. In this context, intestinal permeability increases, and associated microorganisms translocate. **(B)** stimulator of interferon genes (STING) pathway-mediated inflammatory signaling is activated, leading to activation of interferon regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB), which exacerbates intestinal barrier disruption. In this case, gut microbial products translocate into the blood and reach the retina through the damaged blood-retinal barrier, causing chronic activation of the STING pathway in the retina and contributing to the progression of DR disease. **(C)** endotoxemia can result from the release of lipopolysaccharide (LPS) in the gut and its entry into the blood. LPS can diffuse directly into the circulatory system and promote the release of inflammatory factors through receptor-ligand binding (primarily to CD14 and TLR4) in DM due to increased intestinal permeability or absorption via enterocytes. Moreover, binding to TLR4 increases oxidative stress, leading to systemic and retinal inflammation.**(D)** Angiotensin-converting enzyme 2 (ACE2) deficiency alters the gut microbiome composition in diabetic mice, disrupts intestinal barrier integrity, and results in intestinal barrier repair defects. The disruption of the intestinal vascular barrier causes peptidoglycan synthesis in mice, which enters the plasma and promotes DR. **(E)**Short-chain fatty acids (SCFAs) inhibit inflammatory responses and oxidative stress, suppress endotoxin-induced inflammation, and protect the intestinal mucosal barrier. It also stimulates intestinal L-cells and promotes the secretion of glucagon-like peptide-1 (GLP-1) and endocrine-regulating peptide (PYY) to alleviate IR. .

5.3 LPS

LPS, a gut microbial-derived product composed of lipid A-based glycolipid found on the outer membrane of gram-negative bacteria, is thought to be a pro-inflammatory mediator of insulin resistance. It is challenging to shed from the outer membrane of gram-negative bacteria in healthy populations, but it becomes detached and toxic when bacteria are lysed or damaged (121). LPS is released into the gut, and when it enters the blood, it causes LPS-related toxicity, known as endotoxemia (122). LPS can enter the circulatory system by direct diffusion due to increased intestinal permeability in DR or by absorption through enterocytes. LPS is transported in the blood by lipopolysaccharide-binding proteins, binding CD14 and Toll-Like Receptor 4(TLR4) in peripheral tissues such as skeletal muscle and adipose, causing macrophage aggregation in adipose tissue, promoting the release of inflammatory factors, inducing abnormal phosphorylation of IRS-1, and leading to IR (123). When it binds to TLR4, it activates NF-κB and increases oxidative stress, leading to systemic and retinal inflammation (123).

LPS causes endotoxemia and promotes inflammation, whereas other microorganisms produce protective effects. For example, *Lactobacillus*, *Bacteroides*, *Faecalibacterium*, *Akkermansia muciniphila* (*A. muciniphila*), and *Roseburia* are known to down-regulate the pro-inflammatory cytokines in the intestine. *Bacteroides* and *A. muciniphila* improve intestinal barrier function. *Bacteroides* reduce intestinal permeability, decrease LPS production, and improve endotoxemia by up-regulating the colonic tight junction gene expression (122). *A. muciniphila* reduces intestinal permeability by regulating extracellular vesicles, which improves intestinal tight junctions by activating AMP-activated protein kinase in intestinal epithelial cells, thereby enhancing intestinal defence (124). In addition, the outer membrane protein of *A. muciniphila* up-regulates tight junction protein expression and inhibits CB-1, improving intestinal integrity and reducing LPS levels (125).

5.4 The gut-retina axis regulates DR via angiotensin-converting enzyme 2 and peptidoglycan

Takkar et al. (126) reported that ACE2 and peptidogly can play an important role in regulating the pathogenesis of DR. In type 1 diabetic mice, ACE2 deficiency alters gut microbiome composition and gut integrity, as well as defects in the gut barrier repair process (127). Disruption of the intestinal vascular barrier and increased growth of *Bifidobacterium animalis* contributes to peptidoglycan synthesis. Consequently, bacterial peptidoglycan enters the bloodstream and promotes DR (128). ACE2 regulates bone marrow-derived myeloid angiogenesis, restoring intestinal

epithelial and endothelial functions disrupted in DM (129, 130), and alters peptidoglycan biosynthesis by reducing microbiome-associated genes (128).

5.5 Affecting metabolites of gut microbiota

SCFAs, composed of acetate, propionate, and butyrate, are small organic metabolites produced by the fermentation of dietary fibers and resistant starch. They have numerous benefits in energy metabolism, intestinal homeostasis, and immune response regulation. It can inhibit the inflammatory response and oxidative stress and affect glycolipid metabolism as a signaling molecule between intestinal flora and the host (110). Glucolipid metabolism disorders and insulin resistance are characteristic manifestations of DR. SCFAs primarily influence glucose and lipid metabolism by regulating the endocrine system. It (especially acetate and butyrate) can specifically stimulate intestinal L-cells and promote the secretion of glucagon-like peptide-1 (GLP-1) and endocrine-regulated peptide (PYY). In obese mice, this improves insulin sensitivity and increases energy expenditure, preventing and treating diet-induced IR (131, 132). In addition, SCFAs reduce IR by inhibiting endotoxin-related inflammation and protecting the intestinal mucosal barrier (133).

6 Regulating gut microbiota as a therapeutic strategy for DR treatment

The external environment gradually shapes the diversity of the human intestinal microbiota. Before birth, the fetus is sterile in the intestine and progressively accumulates a specific intestinal flora through exposure to the surrounding environment (134). Childhood and adolescence are critical periods for forming intestinal flora, and individual-specific intestinal flora is formed in adulthood (135). Although the intestinal flora is relatively stable in adulthood, it is also modifiable, with its composition changing with age, diet, lifestyle, and environmental exposure (134). When the gut microbiota in patients with DM and DR is dysregulated, dietary modifications (e.g., probiotic/prebiotic supplementation and low-sugar diet) and fecal transplantation can maintain intestinal homeostasis and improve the condition (136). Moreover, studies on new technologies, such as gut flora editing and synthesis of the gut microbiome to regulate and synthesize gut flora, have been reported (137), providing ideas for using gut flora in treating DR.

Genetic factors have a limited impact on the composition of the host gut microbiota. For example, diets can influence gut microbes in healthy individuals. A high-fat diet is associated with an increased abundance of *Bacteroides*, whereas a high fiber intake is associated with an increased abundance of *Prevotella* (138). The diet also influences the production of intestinal flora metabolites, such as SCFAs, LPS, bile acids, and branched-chain amino acids (BCAA; valine, leucine, and isoleucine) (122). Beli et al. (42) reported that intermittent fasting (IF) can reduce retinal complications (DR) in diabetic mice. In particular, IF can reduce intestinal permeability and promote the production of *tauroursodeoxycholic acid* (TUDCA), a potent activator of Takeda-G-protein-receptor-5 (TGR5) in the retinal ganglion cell layer and can act as a neuroprotective agent. IF also improves intestinal vascular barrier function and lowers plasma peptidoglycan levels. Peptidogly can activates TLR2-mediated signaling cascades and exacerbates DR by interfering with the integrity of retinal endothelial cell junctions (42). In conclusion, these findings reveal that remodeling the intestinal microbiome has a protective effect on the retina, preventing the development of DR. In addition, it has been suggested that the potential mechanism by which DR does not occur in diabetic patients is closely related to intestinal microbiota imbalance, which varies between individuals (139).

7 Conclusion and future perspectives

The gut-retina axis concept was developed in response to the dysregulation of gut microbiota observed in patients with retinal diseases such as AMD, DR, and glaucoma. Researchers used antibiotics, probiotics, and diet to reshape the gut microbiota, and the results improved eye disease, providing the link between the gut microbiota and the retina. Subsequent studies revealed further crosstalk between the eye and the gut.

The specificity of the abundance and function of microorganisms and their metabolites in retinal diseases is slowly being elucidated. Scientists have made several advances in the enumeration, characterization, and classification of the human microbiota since the advent of high-throughput sequencing and culture group technologies (134). The most commonly used method for determining microbiome composition was 16S rRNA gene sequencing, which had many limitations for strain-level identification and classification of microorganisms (140). The integrated application of multi-omics, such as macro-genomics, macro-proteomics, and macro-metabolomics, can provide a more accurate and direct interpretation of the functional properties of the intestinal flora for a more accurate understanding of the human micro-ecosystem (141). However, due to individual heterogeneity and the limitations of current diagnostic techniques, interventions on the gut microbiota for disease treatment still be carefully considered. Meanwhile, studies on gut microecology and DR have been reported infrequently compared to other disciplines, and more high-quality studies are required to support this in the future.

The link between microbiota and DR is now well established, and identifying pathogenic or protective microbes is an important step to follow in future. In conclusion, the concept of a gut-retina axis driven by various pathways is being actively investigated, and available data in animals and humans suggest possible therapeutic applications for disease through targeted manipulation of the microbiome.

Author contributions

HZ reviewed the literature and drafted this review. YM reviewed the literature, gave critical comments, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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References

1. Ipp E, Liljenquist D, Bode B, Shah VN, Silverstein S, Regillo CD, et al. Pivotal evaluation of an artificial intelligence system for autonomous detection of referrable and vision-threatening diabetic retinopathy. *JAMA Netw Open* (2021) 4:e2134254. doi: 10.1001/jamanetworkopen.2021.34254

2. Frudd K, Sivaprasad S, Raman R, Krishnakumar S, Revathy YR, Turowski P. Diagnostic circulating biomarkers to detect vision-threatening diabetic retinopathy: potential screening tool of the future? *Acta Ophthalmol* (2022) 100:e648–68. doi: 10.1111/aos.14954

3. Wong TY, Cheung CMG, Larsen M, Sharma S, Simó R. Diabetic retinopathy. *Nat Rev Dis Primers* (2016) 2:16012. doi: 10.1038/nrdp.2016.12

4. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

5. Williams R, Karuranga S, Malanda B, Saeedi P, Basit A, Besançon S, et al. Global and regional estimates and projections of diabetes-related health expenditure: results from the international diabetes federation diabetes atlas, 9th edition. *Diabetes Res Clin Pract* (2020) 162:108072. doi: 10.1016/j.diabres.2020.108072

6. Tan GS, Cheung N, Simó R, Cheung GCM, Wong TY. Diabetic macular oedema. *Lancet Diabetes Endocrinol* (2017) 5:143–55. doi: 10.1016/S2213-8587(16)30052-3

7. Hammes HP. Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond. *Diabetologia* (2018) 61:29–38. doi: 10.1007/s00125-017-4435-8

8. Rao H, Jalali JA, Johnston TP, Koulen P. Emerging roles of dyslipidemia and hyperglycemia in diabetic retinopathy: molecular mechanisms and clinical perspectives. *Front Endocrinol (Lausanne)* (2021) 12:620045. doi: 10.3389/fendo.2021.620045

9. Feng Y, Wang Y, Yang Z, Wu L, Hoffmann S, Wieland T, et al. Chronic hyperglycemia inhibits vasoregression in a transgenic model of retinal degeneration. *Acta diabetologica* (2014) 51(2):211–8. doi: 10.1007/s00592-013-0488-4

10. Soni D, Sagar P, Takkar B. Diabetic retinal neurodegeneration as a form of diabetic retinopathy. *Int Ophthalmol* (2021) 41:3223–48. doi: 10.1007/s10792-021-01864-4

11. Sachdeva MM. Retinal neurodegeneration in diabetes: an emerging concept in diabetic retinopathy. *Curr Diabetes Rep* (2021) 21:65. doi: 10.1007/s11892-021-01428-x

12. Simó-Servat O, Hernández C, Simó R. Diabetic retinopathy in the context of patients with diabetes. *ORE* (2019) 62:211–7. doi: 10.1159/000499541

13. Youngblood H, Robinson R, Sharma A, Sharma S. Proteomic biomarkers of retinal inflammation in diabetic retinopathy. *Int J Mol Sci* (2019) 20:4755. doi: 10.3390/ijms20194755

14. Tang L, Xu GT, Zhang JF. Inflammation in diabetic retinopathy: possible roles in pathogenesis and potential implications for therapy. *Neural Regener Res* (2023) 18:976–82. doi: 10.4103/1673-5374.355743

15. Pelikánová T. Diabetic retinopathy: pathogenesis and therapeutic implications. *Vnitr Lek* (2016) 62:620–8.

16. Kowluru RA, Mishra M. Regulation of matrix metalloproteinase in the pathogenesis of diabetic retinopathy. *Prog Mol Biol Transl Sci* (2017) 148:67–85. doi: 10.1016/bs.pmbts.2017.02.004

17. Domingo JJS, Sánchez SC. From the intestinal flora to the microbiome. *Rev Esp Enferm Dig* (2018) 110:51–6. doi: 10.17235/reed.2017.4947/2017

18. Wang Q, Hao C, Yao W, Zhu D, Lu H, Li L, et al. Intestinal flora imbalance affects bile acid metabolism and is associated with gallstone formation. *BMC Gastroenterol* (2020) 20:59. doi: 10.1186/s12876-020-01195-1

19. Ramírez-Pérez O, Cruz-Ramón V, Chinchilla-López P, Méndez-Sánchez N. The role of the gut microbiota in bile acid metabolism. *Ann Hepatol* (2017) 16:s15–20. doi: 10.5604/01.3001.0010.5494

20. Węgielska I, Suliburska J. The role of intestinal microbiota in the pathogenesis of metabolic diseases. *Acta Sci Pol Technol Aliment* (2016) 15:201–11. doi: 10.17306/J.AFS.2016.2.20

21. Li XY, He C, Zhu Y, Lu NH. Role of gut microbiota on intestinal barrier function in acute pancreatitis. *World J Gastroenterol* (2020) 26:2187–93. doi: 10.3748/wjg.v26.i18.2187

22. Zhou B, Yuan Y, Zhang S, Guo C, Li X, Li G, et al. Intestinal flora and disease mutually shape the regional immune system in the intestinal tract. *Front Immunol* (2020) 11:575. doi: 10.3389/fimmu.2020.00575

23. Wang C, Li Q, Ren J. Microbiota-immune interaction in the pathogenesis of gut-derived infection. *Front Immunol* (2019) 10:1873. doi: 10.3389/fimmu.2019.01873

24. Ding Y, Zhao J, Liu G, Li Y, Jiang J, Meng Y, et al. Total bilirubin predicts severe progression of diabetic retinopathy and the possible causal mechanism. *J Diabetes Res* (2020) 2020:7219852. doi: 10.1155/2020/7219852

25. Xue L, Huang L, Tian Y, Cao X, Song Y. Trimethylamine-N-Oxide promotes high-Glucose-Induced dysfunction and NLRP3 inflammasome activation in retinal microvascular endothelial cells. *J Ophthalmol* (2023) 2023:8224752. doi: 10.1155/2023/8224752

26. Matijašić M, Meštrović T, Paljetak HČ, Perić M, Barešić A, Verbanac D. Gut microbiota beyond bacteria-mycobiome, virome, archaeome, and eukaryotic parasites in IBD. *Int J Mol Sci* (2020) 21:2668. doi: 10.3390/ijms21082668

27. Baroja LM, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. *Clin Exp Immunol* (2007) 149:470–9. doi: 10.1111/j.1365-2249.2007.03434.x

28. Keshteli AH, Valcheva R, Nickurak C, Park H, Mandal R, van Diepen K, et al. Anti-inflammatory diet prevents subclinical colonic inflammation and alters metabolomic profile of ulcerative colitis patients in clinical remission. *Nutrients* (2022) 14:3294. doi: 10.3390/nu14163294

29. Li KL, Wang BZ, Li ZP, Li YL, Liang JJ. Alterations of intestinal flora and the effects of probiotics in children with recurrent respiratory tract infection. *World J Pediatr* (2019) 15:255–61. doi: 10.1007/s12519-019-00248-0

30. Quaglio AEV, Grillo TG, De-Oliveira ECS, Di-Stasi LC, Sassaki LY. Gut microbiota, inflammatory bowel disease and colorectal cancer. *World J Gastroenterol* (2022) 28:4053–60. doi: 10.3748/wjg.v28.i30.4053

31. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol* (2018) 11:1–10. doi: 10.1007/s12328-017-0813-5

32. Verhaar BJH, Prodan A, Nieuwdorp M, Muller M. Gut microbiota in hypertension and atherosclerosis: a review. *Nutrients* (2020) 12:2982. doi: 10.3390/nu12102982

33. Boyajian JL, Ghebretatios M, Schaly S, Islam P, Prakash S. Microbiome and human aging: probiotic and prebiotic potentials in longevity, skin health and cellular senescence. *Nutrients* (2021) 13:4550. doi: 10.3390/nu13124550

34. Myers B, Brownstone N, Reddy V, Chan S, Thibodeaux Q, Truong A, et al. The gut microbiome in psoriasis and psoriatic arthritis. *Best Pract Res Clin Rheumatol* (2019) 33:101494. doi: 10.1016/j.berh.2020.101494

35. He J, Chu Y, Li J, Meng Q, Liu Y, Jin J, et al. Intestinal butyrate-metabolizing species contribute to autoantibody production and bone erosion in rheumatoid arthritis. *Sci Adv* (2022) 8:eabm1511. doi: 10.1126/sciadv.abm1511

36. Rowan S, Jiang S, Korem T, Szymanski J, Chang ML, Szelog J, et al. Involvement of a gut-retina axis in protection against dietary glycemia-induced age-related macular degeneration. *Proc Natl Acad Sci U.S.A.* (2017) 114:E4472–81. doi: 10.1073/pnas.1702302114

37. Jayasudha R, Das T, Kalyana CS, Sai PG, Bhargava A, Tyagi M, et al. Gut mycobiomes are altered in people with type 2 diabetes mellitus and diabetic retinopathy. *PloS One* (2020) 15:e0243077. doi: 10.1371/journal.pone.0243077

38. Bringer MA, Gabrielle PH, Bron AM, Creuzot-Garcher C, Acar N. The gut microbiota in retinal diseases. *Exp Eye Res* (2022) 214:108867. doi: 10.1016/j.exer.2021.108867

39. Gourgari E, Dabelea D, Rother K. Modifiable risk factors for cardiovascular disease in children with type 1 diabetes: can early intervention prevent future cardiovascular events? *Curr Diabetes Rep* (2017) 17:134. doi: 10.1007/s11892-017-0968-y

40. Papatheodorou K, Papanas N, Banach M, Papazoglou D, Edmonds M. Complications of diabetes 2016. *J Diabetes Res* (2016) 2016:6989453. doi: 10.1155/2016/6989453

41. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* (2010) 376:124–36. doi: 10.1016/S0140-6736(09)62124-3

42. Dong L, Zhang Z, Liu X, Wang Q, Hong Y, Li X, et al. RNA Sequencing reveals BMP4 as a basis for the dual-target treatment of diabetic retinopathy. *J Mol Med (Berl)* (2021) 99:225–40. doi: 10.1007/s00109-020-01995-8

43. Beli E, Yan Y, Moldovan L, Vieira CP, Gao R, Duan Y, et al. Restructuring of the gut microbiome by intermittent fasting prevents retinopathy and prolongs survival in db/db mice. *Diabetes* (2018) 67:1867–79. doi: 10.2337/db18-0158

44. Yang G, Wei J, Liu P, Zhang Q, Tian Y, Hou G, et al. Role of the gut microbiota in type 2 diabetes and related diseases. *Metabolism* (2021) 117:154712. doi: 10.1016/j.metabol.2021.154712

45. Iatcu CO, Steen A, Covasa M. Gut microbiota and complications of type-2 diabetes. *Nutrients* (2021) 14:166. doi: 10.3390/nu14010166

46. Das T, Jayasudha R, Chakravarthy S, Prashanthi GS, Bhargava A, Tyagi M, et al. Alterations in the gut bacterial microbiome in people with type 2 diabetes mellitus and diabetic retinopathy. *Sci Rep* (2021) 11:2738. doi: 10.1038/s41598-021-82538-0

47. Caruso G, Fresta CG, Fidilio A, O'Donnell F, Musso N, Lazzarino G, et al. Carnosine decreases PMA-induced oxidative stress and inflammation in murine macrophages. *Antioxidants (Basel)* (2019) 8:281. doi: 10.3390/antiox8080281

48. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* (2010) 464:59–65. doi: 10.1038/nature08821

49. de-Oliveira GLV, Leite AZ, Higuchi BS, Gonzaga MI, Mariano VS. Intestinal dysbiosis and probiotic applications in autoimmune diseases. *Immunology* (2017) 152:1–12. doi: 10.1111/imm.12765

50. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, et al. An integrated catalog of reference genes in the human gut microbiome. *Nat Biotechnol* (2014) 32:834–41. doi: 10.1038/nbt.2942

51. Zhu J, Ren H, Zhong H, Li X, Zou Y, Han M, et al. An expanded gene catalog of mouse gut metagenomes. *mSphere* (2021) 6:e01119–20. doi: 10.1128/mSphere.01119-20

52. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, et al. The Firmicutes/Bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? *Nutrients* (2020) 12:1474. doi: 10.3390/nu12051474

53. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell* (2012) 148:1258–70. doi: 10.1016/j.cell.2012.01.035

54. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* (2006) 124:837–48. doi: 10.1016/j.cell.2006.02.017

55. De-Luca F, Shoenfeld Y. The microbiome in autoimmune diseases. *Clin Exp Immunol* (2019) 195:74–85. doi: 10.1111/cei.13158

56. Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res* (2017) 4:14. doi: 10.1186/s40779-017-0122-9

57. Kalyana CS, Jayasudha R, Sai PG, Ali MH, Sharma S, Tyagi M, et al. Dysbiosis in the gut bacterial microbiome of patients with uveitis, an inflammatory disease of the eye. *Indian J Microbiol* (2018) 58:457–69. doi: 10.1007/s12088-018-0746-9

58. Goodman CF, Doan T, Mehra D, Betz J, Locatelli E, Mangwani-Mordani S, et al. Case-control study examining the composition of the gut microbiome in individuals with and without immune-mediated dry eye. *Cornea* (2022) 13:10. doi: 10.1097/ICO.0000000000003195

59. Zinkernagel MS, Zysset-Burri DC, Keller I, Berger LE, Leichtle AB, Largiadèr CR, et al. Association of the intestinal microbiome with the development of neovascular age-related macular degeneration. *Sci Rep* (2017) 7:40826. doi: 10.1038/srep40826

60. Kalyana CS, Jayasudha R, Ranjith K, Dutta A, Pinna NK, Mande SS, et al. Alterations in the gut bacterial microbiome in fungal keratitis patients. *PloS One* (2018) 13:e0199640. doi: 10.1371/journal.pone.0199640

61. Jayasudha R, Chakravarthy SK, Prashanthi GS, Sharma S, Garg P, Murthy SI, et al. Alterations in gut bacterial and fungal microbiomes are associated with bacterial keratitis, an inflammatory disease of the human eye. *J Biosci* (2018) 43:835–56. doi: 10.1007/s12038-018-9798-6

62. Jabbehdari S, Sallam AB. Gut microbiome and diabetic retinopathy. *Eur J Ophthalmol* (2022) 32:2494–7. doi: 10.1177/11206721221083068

63. Rinninella E, Mele MC, Merendino N, Cintoni M, Anselmi G, Caporossi A, et al. The role of diet, micronutrients and the gut microbiota in age-related macular degeneration: new perspectives from the gut¯Retina axis. *Nutrients* (2018) 10:1677. doi: 10.3390/nu10111677

64. Horai R, Caspi RR. Microbiome and autoimmune uveitis. *Front Immunol* (2019) 10:232. doi: 10.3389/fimmu.2019.00232

65. Rogler G, Singh A, Kavanaugh A, Rubin DT. Extraintestinal manifestations of inflammatory bowel disease: current concepts, treatment, and implications for disease management. *Gastroenterology* (2021) 161:1118–32. doi: 10.1053/j.gastro.2021.07.042

66. Willcox MD. Characterization of the normal microbiota of the ocular surface. *Exp Eye Res* (2013) 117:99–105. doi: 10.1016/j.exer.2013.06.003

67. Deng Y, Wen X, Hu X, Zou Y, Zhao C, Chen X, et al. Geographic difference shaped human ocular surface metagenome of young han Chinese from Beijing, wenzhou, and guangzhou cities. *Invest Ophthalmol Vis Sci* (2020) 61:47. doi: 10.1167/iovs.61.2.47

68. Suwajanakorn O, Puangsricharern V, Kittipibul T, Chatsuwan T. Ocular surface microbiome in diabetes mellitus. *Sci Rep* (2022) 12:21527. doi: 10.1038/s41598-022-25722-0

69. Prasad R, Asare-Bediko B, Harbour A, Floyd JL, Chakraborty D, Duan Y, et al. Microbial signatures in the rodent eyes with retinal dysfunction and diabetic retinopathy. *Invest Ophthalmol Vis Sci* (2022) 63:5. doi: 10.1167/iovs.63.1.5

70. Damms-Machado A, Mitra S, Schollenberger AE, Kramer KM, Meile T, Königsrainer A, et al. Effects of surgical and dietary weight loss therapy for obesity on gut microbiota composition and nutrient absorption. *BioMed Res Int* (2015) 2015:806248. doi: 10.1155/2015/806248

71. Huang Y, Yang B, Li W. Defining the normal core microbiome of conjunctival microbial communities. *Clin Microbiol Infect* (2016) 22:643.e7–643.e12. doi: 10.1016/j.cmi.2016.04.008

72. Ozkan J, Willcox M, Wemheuer B, Wilcsek G, Coroneo M, Thomas T. Biogeography of the human ocular microbiota. *Ocul Surf* (2019) 17:111–8. doi: 10.1016/j.jtos.2018.11.005

73. Lu LJ, Liu J. Human microbiota and ophthalmic disease. *Yale J Biol Med* (2016) 89:325–30.

74. Caspi RR. In this issue: immunology of the eye-inside and out. *Int Rev Immunol* (2013) 32:1–3. doi: 10.3109/08830185.2012.750138

75. Hoang HT, Le DH, Le TTH, Nguyen TTN, Chu HH, Nguyen NT. Metagenomic 16S rDNA amplicon data of microbial diversity of guts in Vietnamese humans with type 2 diabetes and nondiabetic adults. *Data Brief* (2021) 34:106690. doi: 10.1016/j.dib.2020.106690

76. Qin J, Li Y, Cai Z, Li S, Zhu 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

77. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* (2013) 498:99–103. doi: 10.1038/nature12198

78. Aron-Wisnewsky J, Warmbrunn MV, Nieuwdorp M, Clément K. Metabolism and metabolic disorders and the microbiome: the intestinal microbiota associated with obesity, lipid metabolism, and metabolic health-pathophysiology and therapeutic strategies. *Gastroenterology* (2021) 160:573–99. doi: 10.1053/j.gastro.2020.10.057

79. Floyd JL, Grant MB. The gut-eye axis: lessons learned from murine models. *Ophthalmol Ther* (2020) 9:499–513. doi: 10.1007/s40123-020-00278-2

80. Moubayed NM, Bhat RS, Al Farraj D, Dihani NA, El Ansary A, Fahmy RM. Screening and identification of gut anaerobes (Bacteroidetes) from human diabetic stool samples with and without retinopathy in comparison to control subjects. *Microb Pathog* (2019) 129:88–92. doi: 10.1016/j.micpath.2019.01.025

81. Bai J, Wan Z, Zhang Y, Wang T, Xue Y, Peng Q. Composition and diversity of gut microbiota in diabetic retinopathy. *Front Microbiol* (2022) 13:926926. doi: 10.3389/fmicb.2022.926926

82. Khan R, Sharma A, Ravikumar R, Parekh A, Srinivasan R, George RJ, et al. Association between gut microbial abundance and sight-threatening diabetic retinopathy. *Invest Ophthalmol Visual Sci* (2021) 62(7):19. doi: 10.1167/iovs.62.7.19

83. Huang Y, Wang Z, Ma H, Ji S, Chen Z, Cui Z, et al. Dysbiosis and implication of the gut microbiota in diabetic retinopathy. *Front Cell Infect Microbiol* (2021) 11:646348. doi: 10.3389/fcimb.2021.646348

84. Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, et al. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. *Pharmacol Res* (2021) 172:105840. doi: 10.1016/j.phrs.2021.105840

85. Hillestad EMR, van der Meeren A, Nagaraja BH, Bjørsvik BR, Haleem N, Benitez-Paez A, et al. Gut bless you: the microbiota-gut-brain axis in irritable bowel syndrome. *World J Gastroenterol* (2022) 28:412–31. doi: 10.3748/wjg.v28.i4.412

86. Ancona A, Petito C, Iavarone I, Petito V, Galasso L, Leonetti A, et al. The gut-brain axis in irritable bowel syndrome and inflammatory bowel disease. *Dig Liver Dis* (2021) 53:298–305. doi: 10.1016/j.dld.2020.11.026

87. Hill JM, Lukiw WJ. Microbial-generated amyloids and alzheimer's disease (AD). *Front Aging Neurosci* (2015) 7:9. doi: 10.3389/fnagi.2015.00009

88. Zhao Y, Lukiw WJ. Microbiome-generated amyloid and potential impact on amyloidogenesis in alzheimer's disease (AD). *J Nat Sci* (2015) 1:e138.

89. Liu M, Geddis MS, Wen Y, Setlik W, Gershon MD. Expression and function of 5-HT4 receptors in the mouse enteric nervous system. *Am J Physiol Gastrointest Liver Physiol* (2005) 289:G1148–1163. doi: 10.1152/ajpgi.00245.2005

90. Young JB, Godara P, Williams V, Summerfelt P, Connor TB, Tarima S, et al. Assessing retinal structure in patients with parkinson's disease. *J Neurol Neurophysiol* (2019) 10:485. doi: 10.4172/2155-9562.1000485

91. Kashani AH, Asanad S, Chan JW, Singer MB, Zhang J, Sharifi M, et al. Past, present and future role of retinal imaging in neurodegenerative disease. *Prog Retin Eye Res* (2021) 83:100938. doi: 10.1016/j.preteyeres.2020.100938

92. Byun MS, Park SW, Lee JH, Yi D, Jeon SY, Choi HJ, et al. Association of retinal changes with Alzheimer disease neuroimaging biomarkers in cognitively normal individuals. *JAMA Ophthalmol* (2021) 139:548–56. doi: 10.1001/jamaophthalmol.2021.0320

93. Asanad S, Felix CM, Fantini M, Harrington MG, Sadun AA, Karanjia R. Retinal ganglion cell dysfunction in preclinical alzheimer's disease: an electrophysiologic biomarker signature. *Sci Rep* (2021) 11:6344. doi: 10.1038/s41598-021-85010-1

94. Marchesi N, Fahmideh F, Boschi F, Pascale A, Barbieri A. Ocular neurodegenerative diseases: interconnection between retina and cortical areas. *Cells* (2021) 10:2394. doi: 10.3390/cells10092394

95. Moon J, Yoon CH, Choi SH, Kim MK. Can gut microbiota affect dry eye syndrome? *Int J Mol Sci* (2020) 21:8443. doi: 10.3390/ijms21228443

96. Napolitano P, Filippelli M, Davinelli S, Bartollino S, dell'Omo R, Costagliola C. Influence of gut microbiota on eye diseases: an overview. *Ann Med* (2021) 53:750–61. doi: 10.1080/07853890.2021.1925150

97. Xue W, Li JJ, Zou Y, Zou B, Wei L. Microbiota and ocular diseases. *Front Cell Infect Microbiol* (2021) 11:759333. doi: 10.3389/fcimb.2021.759333

98. Jiao J, Yu H, Yao L, Li L, Yang X, Liu L. Recent insights into the role of gut microbiota in diabetic retinopathy. *J Inflammation Res* (2021) 14:6929–38. doi: 10.2147/JIR.S336148

99. Scuderi G, Troiani E, Minnella AM. Gut microbiome in retina health: the crucial role of the gut-retina axis. *Front Microbiol* (2021) 12:726792. doi: 10.3389/fmicb.2021.726792

100. Nakamura YK, Metea C, Karstens L, Asquith M, Gruner H, Moscibrocki C, et al. Gut microbial alterations associated with protection from autoimmune uveitis. *Invest Ophthalmol Vis Sci* (2016) 57:3747–58. doi: 10.1167/iovs.16-19733

101. Tang J, Tang Y, Yi I, Chen DF. The role of commensal microflora-induced T cell responses in glaucoma neurodegeneration. *Prog Brain Res* (2020) 256:79–97. doi: 10.1016/bs.pbr.2020.06.002

102. Masland RH. The fundamental plan of the retina. *Nat Neurosci* (2001) 4:877–86. doi: 10.1038/nn0901-877

103. Hoon M, Okawa H, Della Santina L, Wong ROL. Functional architecture of the retina: development and disease. *Prog Retin Eye Res* (2014) 42:44–84. doi: 10.1016/j.preteyeres.2014.06.003

104. Chen M, Luo C, Zhao J, Devarajan G, Xu H. Immune regulation in the aging retina. *Prog Retin Eye Res* (2019) 69:159–72. doi: 10.1016/j.preteyeres.2018.10.003

105. Eshaq RS, Aldalati AMZ, Alexander JS, Harris NR. Diabetic retinopathy: breaking the barrier. *Pathophysiology* (2017) 24:229–41. doi: 10.1016/j.pathophys.2017.07.001

106. Brockhaus K, Melkonyan H, Prokosch-Willing V, Liu H, Thanos S. Alterations in tight- and adherens-junction proteins related to glaucoma mimicked in the organotypically cultivated mouse retina under elevated pressure. *Invest Ophthalmol Vis Sci* (2020) 61:46. doi: 10.1167/iovs.61.3.46

107. Kayama H, Okumura R, Takeda K. Interaction between the microbiota, epithelia, and immune cells in the intestine. *Annu Rev Immunol* (2020) 38:23–48. doi: 10.1146/annurev-immunol-070119-115104

108. Sato J, Kanazawa A, Azuma K, Ikeda F, Goto H, Komiya K, et al. Probiotic reduces bacterial translocation in type 2 diabetes mellitus: a randomized controlled study. *Sci Rep* (2017) 7:12115. doi: 10.1038/s41598-017-12535-9

109. Deng Y, Ge X, Li Y, Zou B, Wen X, Chen W, et al. Identification of an intraocular microbiota. *Cell Discovery* (2021) 7:13. doi: 10.1038/s41421-021-00245-6

110. Chen N, Wu J, Wang J, Piri N, Chen F, Xiao T, et al. Short chain fatty acids inhibit endotoxin-induced uveitis and inflammatory responses of retinal astrocytes. *Exp Eye Res* (2021) 206:108520. doi: 10.1016/j.exer.2021.108520

111. Andriessen EM, Wilson AM, Mawambo G, Dejda A, Miloudi K, Sennlaub F, et al. Gut microbiota influences pathological angiogenesis in obesity-driven choroidal neovascularization. *EMBO Mol Med* (2016) 8:1366–79. doi: 10.15252/emmm.201606531

112. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol* (2019) 16:461–78. doi: 10.1038/s41575-019-0157-3

113. Gyawali A, Kang YS. Blood-to-Retina transport of imperatorin involves the carrier-mediated transporter system at the inner blood-retinal barrier. *J Pharm Sci* (2019) 108:1619–26. doi: 10.1016/j.xphs.2018.11.040

114. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051

115. Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* (2020) 20(1):40–54. doi: 10.1038/s41577-019-0198-4

116. Rizzetto L, Fava F, Tuohy KM, Selmi C. Connecting the immune system, systemic chronic inflammation and the gut microbiome: the role of sex. *J Autoimmun* (2018) 92:12–34. doi: 10.1016/j.jaut.2018.05.008

117. Motwani M, Pesiridis S, Fitzgerald KA. DNA Sensing by the cGAS-STING pathway in health and disease. *Nat Rev Genet* (2019) 20:657–74. doi: 10.1038/s41576-019-0151-1

118. Shmuel-Galia L, Humphries F, Lei X, Ceglia S, Wilson R, Jiang Z, et al. Dysbiosis exacerbates colitis by promoting ubiquitination and accumulation of the innate immune adaptor STING in myeloid cells. *Immunity* (2021) 54:1137–1153.e8. doi: 10.1016/j.immuni.2021.05.008

119. Qin X, Zou H, Niu C. The STING pathway: an uncharacterized angle beneath the gut–retina axis. *Exp Eye Res* (2022) 217:108970. doi: 10.1016/j.exer.2022.108970

120. Zou M, Gong L, Ke Q, Qi R, Zhu X, Liu W, et al. Heterochromatin inhibits cGAS and STING during oxidative stress-induced retinal pigment epithelium and retina degeneration. *Free Radic Biol Med* (2022) 178:147–60. doi: 10.1016/j.freeradbiomed.2021.11.040

121. Wang C, Xiao Y, Yu L, Tian F, Zhao J, Zhang H, et al. Protective effects of different bacteroides vulgatus strains against lipopolysaccharide-induced acute intestinal injury, and their underlying functional genes. *J Adv Res* (2022) 36:27–37. doi: 10.1016/j.jare.2021.06.012

122. Liu J, He Z, Ma N, Chen ZY. Beneficial effects of dietary polyphenols on high-fat diet-induced obesity linking with modulation of gut microbiota. *J Agric Food Chem* (2020) 68:33–47. doi: 10.1021/acs.jafc.9b06817

123. Lee CJ, Sears CL, Maruthur N. Gut microbiome and its role in obesity and insulin resistance. *Ann N Y Acad Sci* (2020) 1461:37–52. doi: 10.1111/nyas.14107

124. Chelakkot C, Choi Y, Kim DK, Park HT, Ghim J, Kwon Y, et al. Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Exp Mol Med* (2018) 50:e450. doi: 10.1038/emm.2017.282

125. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al. A purified membrane protein from akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* (2017) 23:107–13. doi: 10.1038/nm.4236

126. Takkar B, Sheemar A, Jayasudha R, Soni D, Narayanan R, Venkatesh P, et al. Unconventional avenues to decelerate diabetic retinopathy. *Surv Ophthalmol* (2022) 67:1574–92. doi: 10.1016/j.survophthal.2022.06.004

127. Dean RG, Burrell LM. ACE2 and diabetic complications. *Curr Pharm Des* (2007) 13:2730–5. doi: 10.2174/138161207781662876

128. Duan Y, Prasad R, Feng D, Beli E, Li Calzi S, Longhini ALF, et al. Bone marrow-derived cells restore functional integrity of the gut epithelial and vascular barriers in a model of diabetes and ACE2 deficiency. *Circ Res* (2019) 125:969–88. doi: 10.1161/CIRCRESAHA.119.315743

129. Patel VB, Bodiga S, Basu R, Das SK, Wang W, Wang Z, et al. Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the angiotensin II/AT1 receptor axis. *Circ Res* (2012) 110:1322–35. doi: 10.1161/CIRCRESAHA.112.268029

130. Verma A, Shan Z, Lei B, Yuan L, Liu X, Nakagawa T, et al. ACE2 and ang-(1-7) confer protection against development of diabetic retinopathy. *Mol Ther* (2012) 20:28–36. doi: 10.1038/mt.2011.155

131. Covasa M, Stephens RW, Toderean R, Cobuz C. Intestinal sensing by gut microbiota: targeting gut peptides. *Front Endocrinol (Lausanne)* (2019) 10:82. doi: 10.3389/fendo.2019.00082

132. Grasset E, Puel A, Charpentier J, Collet X, Christensen JE, Tercé F, et al. A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. *Cell Metab* (2017) 25:1075–1090.e5. doi: 10.1016/j.cmet.2017.04.013

133. Zhang XY, Chen J, Yi K, Peng L, Xie J, Gou X, et al. Phlorizin ameliorates obesity-associated endotoxemia and insulin resistance in high-fat diet-fed mice by targeting the gut microbiota and intestinal barrier integrity. *Gut Microbes* (2020) 12:1–18. doi: 10.1080/19490976.2020.1842990

134. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiol Mol Biol Rev* (2017) 81:e00036–17. doi: 10.1128/MMBR.00036-17

135. Parkin K, Christophersen CT, Verhasselt V, Cooper MN, Martino D. Risk factors for gut dysbiosis in early life. *Microorganisms* (2021) 9:2066. doi: 10.3390/microorganisms9102066

136. Rowan S, Taylor A. The role of microbiota in retinal disease. *Adv Exp Med Biol* (2018) 1074:429–35. doi: 10.1007/978-3-319-75402-4_53

137. Zhu W, Miyata N, Winter MG, Arenales A, Hughes ER, Spiga L, et al. Editing of the gut microbiota reduces carcinogenesis in mouse models of colitis-associated colorectal cancer. *J Exp Med* (2019) 216:2378–93. doi: 10.1084/jem.20181939

138. Ghosh TS, Rampelli S, Jeffery IB, Santoro A, Neto M, Capri M, et al. Mediterranean Diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. *Gut* (2020) 69:1218–28. doi: 10.1136/gutjnl-2019-319654

139. Liu K, Zou J, Fan H, Hu H, You Z. Causal effects of gut microbiota on diabetic retinopathy: a mendelian randomization study. *Front Immunol* (2022) 13:930318. doi: 10.3389/fimmu.2022.930318

140. Hamady M, Knight R. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome Res* (2009) 19:1141–52. doi: 10.1101/gr.085464.108

141. Morgan XC, Huttenhower C. Meta'omic analytic techniques for studying the intestinal microbiome. *Gastroenterology* (2014) 146:1437–1448.e1. doi: 10.1053/j.gastro.2014.01.049

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Palmitoylethanolamide counteracts high-fat diet-induced gut dysfunction by reprogramming microbiota composition and affecting tryptophan metabolism

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Obesity is associated with gastrointestinal (GI) tract and central nervous system (CNS) disorders. High-fat diet (HFD) feeding-induced obesity in mice induces dysbiosis, causing a shift toward bacteria-derived metabolites with detrimental effects on metabolism and inflammation: events often contributing to the onset and progression of both GI and CNS disorders. Palmitoylethanolamide (PEA) is an endogenous lipid mediator with beneficial effects in mouse models of GI and CNS disorders. However, the mechanisms underlining its enteroprotective and neuroprotective effects still need to be fully understood. Here, we aimed to study the effects of PEA on intestinal inflammation and microbiota alterations resulting from lipid overnutrition. Ultramicronized PEA (30 mg/kg/die *per os*) was administered to HFD-fed mice for 7 weeks starting at the 12th week of HFD regimen. At the termination of the study, the effects of PEA on inflammatory factors and cells, gut microbial features and tryptophan (TRP)-kynurenine metabolism were evaluated. PEA regulates the crosstalk between the host immune system and gut microbiota via rebalancing colonic TRP metabolites. PEA treatment reduced intestinal immune cell recruitment, inflammatory response triggered by HFD feeding, and corticotropin-releasing hormone levels. In particular, PEA modulated HFD-altered TRP metabolism in the colon, rebalancing serotonin (5-HT) turnover and reducing kynurenine levels. These effects were associated with a reshaping of gut microbiota composition through increased butyrate-promoting/producing bacteria, such as Bifidobacterium, Oscillospiraceae and *Turicibacter sanguinis*, with the latter also described as 5-HT sensor. These data indicate that the rebuilding of gut microbiota following PEA supplementation promotes host 5-HT biosynthesis, which is crucial in regulating intestinal function.

KEYWORDS

obesity, gut-brain axis, N-acylethanolamines, serotonin, gut microbiota

1. Introduction

Intestinal homeostasis is preserved by multiple and complex interactions between gut microbiota and host immune system (1). This mutual relationship regulates many physiological functions strictly associated with metabolic and nutritional balance and immune system stimulation. Diets high in fat or sugar, antibiotic administration, and stress are known to induce, at different extents, dysbiosis, loss of gut integrity, and consequently inflammation, and an overall impact on host health. These events contribute to the onset of several diseases, not limited to the gastrointestinal (GI) tract, but involving extra-intestinal peripheral tissues, including the central nervous system (CNS) (2). Over the past two decades, many preclinical studies investigated the role of the gut-brain axis in obesity-induced GI inflammation and behavioral alterations (3, 4). Gut microbiota pivotally plays a role in gut-brain communication through several interrelated mechanisms, including the activation of afferent sensory neurons of the vagus nerve, neuro-immune and neuroendocrine pathways, microbial metabolites, and neurotransmitter release (5). One focal point in this regard is the microbial regulation of circulating tryptophan (TRP) levels, with a potential dual action in regulating serotonin (5-HT) synthesis and kynurenine (KYN) pathway metabolism (6), ultimately affecting both metabolic and neuropsychiatric disorders (7).

Palmitoylethanolamide (PEA), an endogenous lipid mediator belonging to the family of N-acylethanolamines (NAEs), has shown beneficial effects in colonic inflammatory conditions (8–10), and CNS diseases (11, 12). Therefore, the duality of enteroprotective and neuroprotective effects of PEA indicated novel lines of investigation on its potential effect on neurodegenerative and neurodevelopmental disorders through gut-brain axis involvement (13–15). Among CNS disorders, mood and cognitive alterations are often comorbidity during obesity (16). The mechanisms underlying this association involve inflammation, neurotransmitter unbalance, and overactivation of the hypothalamus-pituitary–adrenal (HPA) axis, with the microbiota functioning as a bridge between the brain and intestinal bidirectional communication (17).

We have recently demonstrated that PEA not only counteracts metabolic inflexibility and adipose tissue dysfunction in HFD-fed mice (18, 19) but also limits the depressive- and anxiety-like behaviors and the cognitive decline associated with high-fat feeding. The effects are associated with increased neurogenesis and synaptic strength and reduced neuroinflammation and blood–brain barrier disruption (20, 21). PEA also markedly modulates monoamine levels by decreasing dopamine (DA) levels and increasing DA turnover in the amygdala; by increasing 5-HT levels in the prefrontal cortex (PFC); and by reducing DA and 5-HT turnover in the nucleus accumbens and PFC, respectively (21). These findings, reinforced by the notion that PEA modulates gut microbiota composition and mood disorders (13, 22), prompted us to study the effect of chronic administration of PEA on gut dysfunction, microbiota composition and TRP metabolism induced by fat overnutrition.

2. Materials and methods

2.1. Animals and treatments

Six weeks-old C57Bl/6J male mice (Charles River, Wilmington, MA, USA) were housed in stainless steel cages in a room at 22 ± 1°C with a 12:12 h lights-dark cycle (from 7 a.m. to 7 p.m.). Mice were randomized and sorted into three groups (5/6 mice for each group) as follows: a control group (STD) receiving a chow diet (Mucedola srl, Milan, Italy) and vehicle; high-fat diet (HFD) (Research Diets Inc., NJ, USA) group receiving an HFD having 45% of energy derived from fat and 7% of sucrose vehicle. The exact composition of STD and HFD is summarized in Supplementary Table 1. HFD group treated with ultramicronized PEA (HFD + PEA, 30 mg/kg/die *per os*). The treatments began 12 weeks after HFD consumption and lasted 7 weeks concurrently with HFD. Ultramicronized PEA, provided by Epitech Group Labs (Padua, Italy) was dissolved in carboxymethylcellulose (1.5%) for oral administration. At the end of the study, mice were sacrificed by inhaled enflurane anesthesia followed by cervical dislocation and feces and colons were collected and stored at −80°C. All procedures involving animals complied with the Institutional Guidelines and according to the Italian D.L. no.116 of January 27, 1992 of Ministry of Health under the protocol no. 982/2017-PR, and associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609/ECC).

2.2. Western blot analysis

Colon was homogenized in lysis buffer (20 mM Tris–HCl, pH 7,5, 10 mM NaF, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulphonyl fluoride, 1 mM Na_3VO_4, leupeptin 10 µg/mL, and trypsin inhibitor 10 µg/mL). Total protein lysates were obtained as supernatant by centrifugation at 14,000 × g for 15 min at 4°C. Protein concentrations were estimated by the Bio-Rad protein assay using free bovine serum albumin (BSA) as standard. An equal amount of protein (40 µg) was subjected to SDS-PAGE and electro-transferred onto a nitrocellulose membrane using a Bio-Rad Transblot (Bio-Rad, Milan, Italy). Membranes were blocked at room temperature in milk buffer (1X PBS, 5% w/v non-fat dry milk) and probed with rabbit polyclonal antibody against anti-Toll like receptor 4 (TLR4) (1:1000; Invitrogen, Whaltam MA, USA; 48-2300; AB_2533842), rabbit polyclonal antibody anti-Cyclooxygenase (COX)-2 (1:500; Elabscience, Houston, TX; E-AB-31012; AB_2715578), mouse polyclonal antibody anti-indoleamine 2,3-dioxygenase (IDO) (1:500; Santa Cruz Biotechnology, Dallas, TX, USA; sc-137012; AB_2123436), mouse polyclonal antibody anti-inducible oxide nitric synthase (iNOS) (1:1000; BD Biosciences, Franklin Lakes, NJ, USA; 610432; AB_397808). Western Blot for anti-β-Actin (1:8000; Sigma-Aldrich St. Louis, MO, USA; A5441; AB_476744) was performed to ensure equal sample loading and data were expressed as relative normalized expression. The filter detection was performed by ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. RNA extraction and semi-quantitative real-time PCR (RT-PCR)

Total RNA isolated from the colon was extracted using TRIzol Reagent (Bio-Rad Laboratories, Hercules, CA, USA; 7326890) following the extraction kit's protocol for RNA (NucleoSpin®, Macherey-Nagel GmbH & Co, Düren, Germany; FC140955N). cDNA was obtained using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA; 4374966) from 8 µg total

RNA. RT-PCRs were performed with a Bio-Rad CFX96 Connect Real-time PCR System instrument and software (Bio-Rad Laboratories). The RT-PCR conditions were 15 min at 95°C followed by 40 cycles of two-step PCR denaturation at 94°C for 15 s, annealing extension at 55°C for 30 s and extension at 72°C for 30 s, as previously described (23). Each sample contained 500 ng cDNA in 2X QuantiTect SYBR Green PCR Master Mix (204145) and primers pairs to amplify chymase 1 (*Cma1*; QT0019946), tryptase β2 (*Tpsb2*; QT00252637), IL-1β (*Il1b*; QT01048355), TNF-α (*Tnf*; QT00104006), integrin αX subunit (*Itgax*; QT00113715), EGF-like module-containing mucin-like hormone receptor-like 1 (*Emr1*; QT00099617), corticotropin-releasing hormone (*Crh*; QT00293489), free fatty acid receptor 2 (*Ffar2*; QT00128226) and solute carrier family 16 member 1 (*Slc16a1*; QT00115423) (Qiagen, Hilden, Germany), in a final volume of 50 µL. The relative amount of each studied mRNA was normalized to *Gapdh* (QT01658692) (Qiagen, Hilden, Germany) as a housekeeping gene, and data were analyzed according to the $2^{-\Delta\Delta CT}$ method.

2.4. High-performance liquid chromatography (HPLC) quantifications

Determination of 5-HT, 5-hydroxyindolacetic acid (5-HIAA) and KYN levels in colon were performed by using HPLC coupled with an electrochemical detector (Ultimate ECD, Dionex Scientific, Milan, Italy), as previously described (24). Briefly, colon samples were homogenized and separated by a LC18 reverse phase column (Kinetex, 150 mm × 4.2 mm, ODS 5 µm; Phenomenex, Castel Maggiore-Bologna, Italy). Analytes were detected through a thin-layer amperometric cell (Dionex, ThermoScientifics, Milan, Italy) with a 5 mm diameter glassy carbon electrode at a working potential of 0,400 V (vs. Pd) for 5-HT and 5-HIAA, and 0.750 V (vs. Pd) for KYN, with a flow rate of 0.7 mL/min by using an isocratic pump (Shimadzu LC-10 AD, Kyoto, Japan). A solution of 75 mM NaH2PO4, 1.7 mM octane sulfonic acid, 0.3 mM EDTA, acetonitrile 10%, in distilled water, buffered at pH 3.0, was used as mobile phase. All reagents were purchased from Sigma Aldrich, Milan Italy. Data analysis was accomplished by Chromeleon software (version 6.80, Thermo Scientific Dionex, San Donato Milanese, Italy).

2.5. Microbiota sequencing and data analysis

Fecal microbiota of STD, HFD, and HFD + PEA groups was analyzed by collecting samples from a subset of 4/5 mice/group and immediately stored at −80°C until processed. Bacterial genomic DNA was extracted from frozen fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturer's instructions. Extracted DNA concentration was measured fluorometrically using Qubit dsDNA BR assay kit (Invitrogen) and quality was checked by spectrophotometric measurements with NanoDrop (ThermoFisher Scientific Inc). The V3-V4 region of the 16S rRNA gene was amplified and prepared for sequencing according to the protocol 16S Metagenomic Sequencing Library Preparation for Illumina Miseq System as previously described (25). Sequencing run was performed on the Illumina MiSeq system using v3 reagents for 2 × 281 cycles (Illumina, Inc.). Metataxonomic analysis was conducted using the Quantitative Insights Into Microbial Ecology (QIIME2, version 2021.8) (26). V3-V4 16S rDNA FASTQ paired-end reads were quality filtered, dereplicated, denoised, merged, and assessed for chimeras using DADA2 pipeline (27). Amplicon sequence variants (ASVs) were obtained and filtered out based on a prevalence of at least two samples. Taxonomic assignment was performed utilizing SILVA v138 database, with a classifier trained on the amplified regions (28). Moreover, to achieve a better species taxonomic level resolution, each ASV sequence has also been aligned to a taxonomy classifiers from NCBI Genbank (29, 30). A rarefaction procedure was performed to assess sampling depth coverage and species heterogeneity in each sample. Sample size biases in subsequent analyses were avoided by applying a sequence rarefaction procedure using a depth of 9,151 reads/sample. Alpha diversity within each group was assessed by calculating the following parameters: Observed features, Chao1 (to assess species richness), Shannon's and Simpson (as a measure of species distribution) (31). The statistical significance of alpha diversity was assessed by the Kruskal-Wallis test. Diversity among sample communities was detected by performing beta diversity analysis calculating weighted and unweighted UniFrac distance matrices (32, 33). The statistical significance of beta diversities was assessed on phylogenetic distance matrixes using the ANOSIM method with 999 permutations. Firmicutes/Bacteroidota ratio was also calculated and statistical differences among groups were evaluated through one-way ANOVA followed by Tukey multiple comparison post-hoc tests. The linear discriminant analysis effect size (LEfSe) method was used to identify key species of each group (LDA score > 2; $p < 0.05$).[1]

2.6. Statistical analysis

All data shown are presented as mean value ± SEM. Statistical analysis was performed by one-way ANOVA, followed by Bonferroni *post hoc* for multiple comparisons. Differences among groups were considered significant at values of $p < 0.05$. Analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. PEA reduces immune cells infiltration and stress marker in colon of HFD-fed mice

HFD-fed mice showed a significant increase in protein expression of TLR4, a major player in the immune response, compared with STD and HFD + PEA groups (Figure 1A). Furthermore, PEA reduced the transcriptional levels of *Itgax* (Figure 1B), an integrin mainly expressed by macrophages, monocytes, and NK cells. The levels of *Emr1* (Figure 1C), a murine marker of macrophages markedly upregulated by HFD, were also reduced in the HFD + PEA group. PEA treatment also counteracted HFD-induced mast cell activation, reducing gene expression of murine-specific chymase-1 and tryptase β2 (Figures 1D,E). Moreover, we evaluated *Crh* transcription as a marker of psychogenic stress, showing PEA capability in reducing its expression in the colon (Figure 1F).

1 https://huttenhower.sph.harvard.edu/galaxy

3.2. PEA reduces intestinal inflammation in HFD-fed mice

As shown in Figure 2, HFD feeding caused an increase in the levels of proinflammatory cytokines, such as TNF-α and IL-1β (Figures 2A,B), and the protein expression of iNOS and COX-2 (Figures 2C,D) in colonic tissues. The treatment with PEA dampened gut inflammation, significantly reduced the main proinflammatory factors, and partially lowered TNF-α mRNAs.

3.3. PEA modulates gut serotonin levels and IDO/kynurenine pathway altered by HFD

HFD feeding caused a significant decrease in 5-HT in the colon of obese mice. The high levels of the metabolite 5-HIAA in the HFD group compared to STD suggest an increased metabolism of this neurotransmitter in the gut (Figures 3A,C). Moreover, an increased amount of KYN was measured in colon tissues from obese mice and the induced protein expression of IDO (Figures 3B,E). PEA treatment induced an increase in the levels of 5-HT, paralleled by a reduction of its turnover (Figure 3D) and KYN levels, possibly through the downregulation of IDO protein expression.

3.4. PEA reshapes gut microbiota composition in obese mice

High-throughput sequencing targeting the V3-V4 regions of the 16S rRNA gene was used to examine the effects of PEA treatment on gut microbiota alterations induced by HFD. Following sequence denoising, trimming and chimera picking, 1,166 different features (ASVs with ≥2 counts) were inferred from a total of 301,699 reads. Data were rarefied to the minimum library size of 9,151 reads/sample, a sequencing depth considered adequate as all curves reached ASV detection saturation (data not shown).

Principal coordinates analysis (PCoA) based on weighted UniFrac distances showed that HFD changed the composition of the gut microbiota (ANOSIM HFD vs. STD: $R = 0.92$ and $p = 0.02$) and revealed that the fecal bacterial community of PEA-treated HFD mice diverged from that of untreated HFD mice (ANOSIM $R = 0.29$ and $p = 0.046$). However, a strong difference rather than STD was still observable (ANOSIM $R = 1$ and $p = 0.01$) (Figure 4A).

The gut microbiota composition was then studied at phylum and genus taxonomic levels (Figures 4B–D, 5). For an accurate taxonomic classification and species-level profiling, the representative sequence of each ASV belonging to key genera was re-annotated according to the NCBI taxonomy (Figure 5). At the phylum level, Firmicutes and Bacteroidota phyla dominated the microbial communities of all groups (Figure 4B); however, Firmicutes/Bacteroidota ratio significantly increased in HFD mice principally due to 31.49% of Bacteroidota and 46.37% of Firmicutes compared with 77.72 and 20.07% in STD mice for Bacteroidota and Firmicutes, respectively. Notably, PEA supplementation restored Firmicutes/Bacteroidota ratio to STD levels (Figure 4C). LEfSe algorithm, applied to identify the key feature marking the fecal microbiota of each group, indicated phylum Desulfobacterota significantly enriched in HFD mice and phyla Actinobacteria and Firmicutes in PEA-treated mice. Conversely, Bacteroidota and Verrucomicrobia were reduced in HFD and HFD + PEA groups compared to STD mice (Figure 4D). Furthermore, the statistical analysis highlighted, at the genus level, a selection for

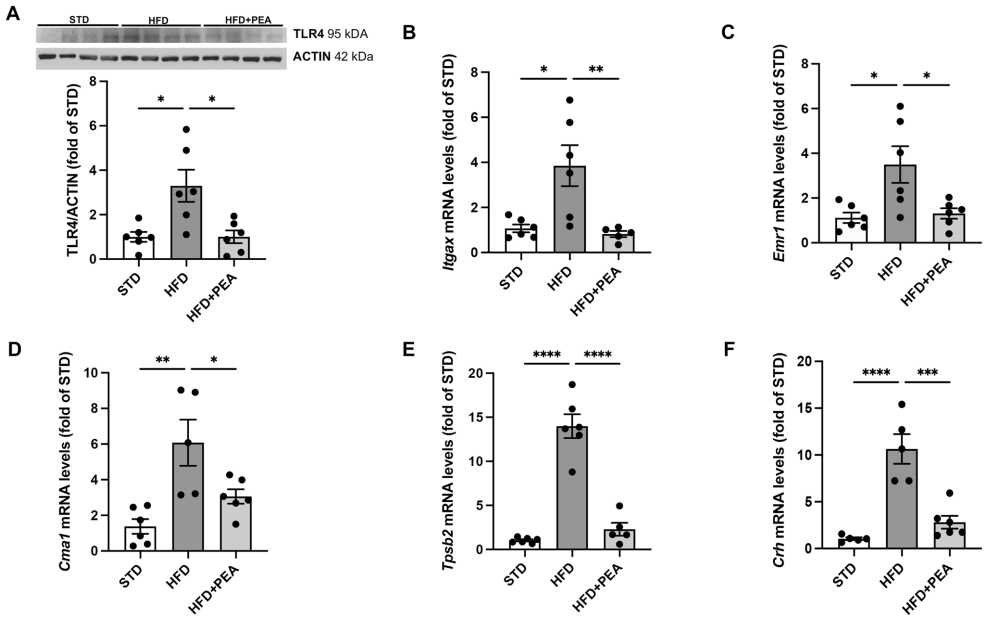

FIGURE 1
PEA counteracts immune cell infiltration caused by HFD overfeeding. **(A)** Protein levels of TLR4 were evaluated by Western Blot analysis. **(B–F)** PEA normalized mRNA levels of *Itgax*, *Emr1*, *Cma1*, *Tpsb2* and *Crh* in the colon of HFD group ($n = 5–6$ each group). A representative Western blot is shown for TLR4. Data are presented as mean ± SEM reaching the significance at $P < 0.05$ (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

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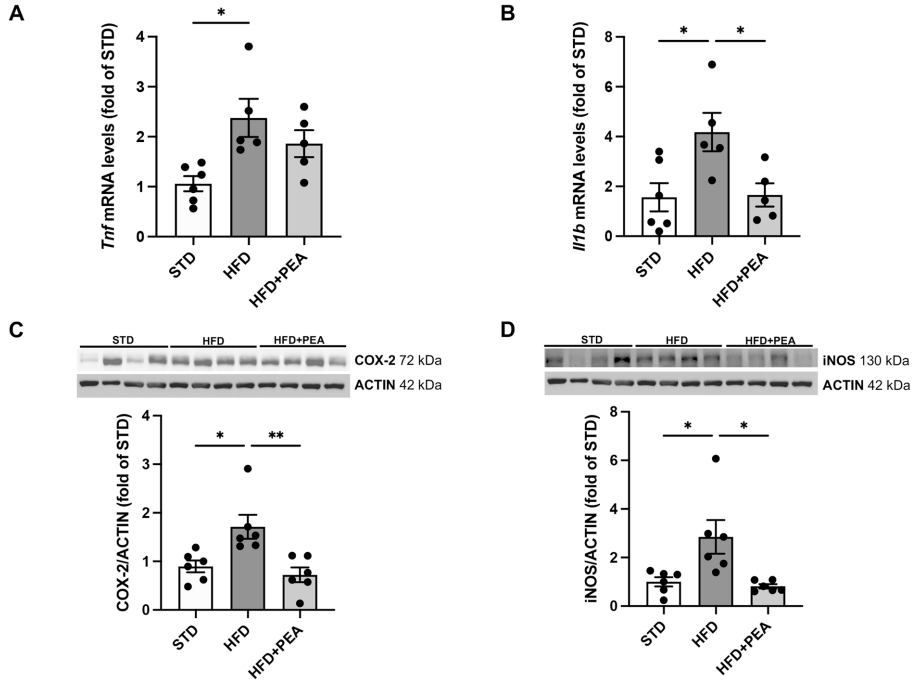

FIGURE 2
PEA lessens gut inflammation enhanced by HFD. **(A,B)** Transcriptional levels of inflammatory cytokines, *Tnf* and *Il1b*, were assessed in colonic tissue. **(C,D)** PEA modulated protein levels of COX-2 and iNOS ($n = 5–6$ each group). Representative Western blots are shown for COX-2 and iNOS. Data are showed as mean ± SEM reaching the significance at $P < 0.05$ ($^{*}P < 0.05$, $^{**}P < 0.01$).

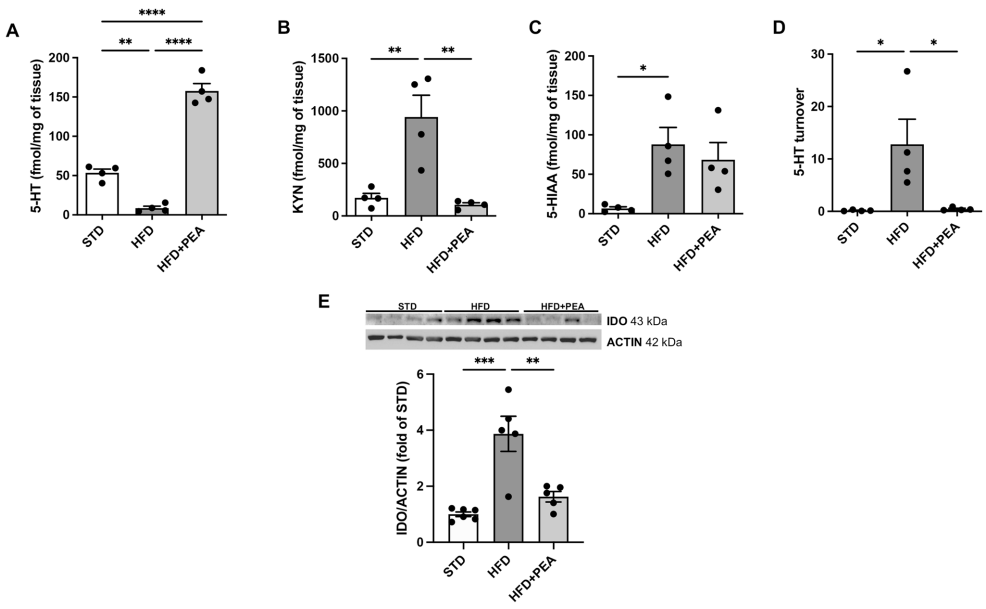

FIGURE 3
PEA increases serotonin levels and modulates IDO/kynurenine pathway in the gut. **(A–D)** PEA increased 5-HT levels in the colon, also modulating KYN, 5-HIAA, and 5-HT turnover ($n = 4$ each group). Data are obtained by HPLC. **(E)** Protein expression of IDO, increased by HFD overfeeding, was normalized by PEA treatment ($n = 5–6$ each group). A representative Western blot is shown for IDO. Data were showed as mean ± SEM reaching the significance at $P < 0.05$ ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$).

members of the gut bacterial communities upon HFD and PEA treatment. Different bacterial genera, namely *Bilophila*, *Desulfovibrio* (*D. fairfieldensis*), *Blautia* (closely related to *Acetatifactor muris*), *Tuzzerella* (*Anaerotignum lactatifermentans*) and an uncultured genus belonging to Lachnospiraceae family (closely related to *Acetatifactor muris*), were significantly increased in the HFD group compared with

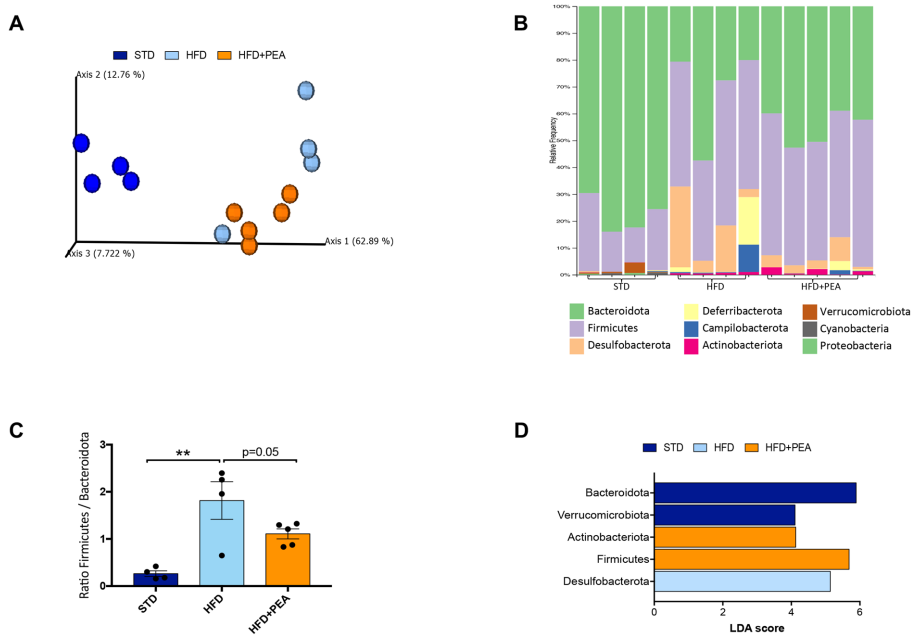

FIGURE 4
HFD mice microbial communities upon PEA supplementation. **(A)** Principal coordinate analysis (PCoA) plot based on weighted UniFrac distance matrix (9,151 reads/sample). **(B)** Stacked bar chart showing the sample relative abundance of all bacterial ASVs taxonomically classified at phylum level. **(C)** Firmicutes to Bacteroidota ratio in each sample group (mean ± SEM, $^{**}P < 0.01$, one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons). **(D)** Gut microbiota differences at phylum taxonomic level based on linear discriminant analysis (LDA) combined with effect size (LEfSe) algorithm ($P > 0.05$ for both Kruskal–Wallis and pairwise Wilcoxon tests and a cutoff value of LDA score above 2.0).

both STD and HFD + PEA groups; in contrast, *Bifidobacterium* (*B. longum*), *Turicibacter* (*T. sanguinis*), *Romboutsia* (*R. timonensis*), *Oscillibacter* (closely related to *Oscillibacter ruminantium* GH1 and *Oscillibacter valericigenes*), and several members of Oscillospiraceae family were significantly enriched in PEA-supplemented HFD mice (Figure 5). Figure 5 also reports the fold change of each key genus in HFD and PEA-treated mice compared to the STD group. Notably, in the HFD + PEA group, it was possible to detect an enhancement of bacteria already increased by HFD (e.g., *Bifidobacterium*, *Rombustia*, and *Oscillibacter*) but mainly a trend of restoration to standard levels of the majority of the bacteria genera affected by HFD (such as *Bacteroides*, *Muribaculaceae*, *Bilophila*).

3.5. PEA induces the receptor (GPR43) and transporter (MCT1) of butyrate in colon of HFD mice

Since the possible modulation of butyrate-producers bacteria induced by PEA treatment, mRNA levels of butyrate receptor (*Ffar2*) and transporter (*Slc16a1*) were evaluated in the colon of mice (Figures 6A,B). After 19 weeks of HFD, obese mice showed a significant reduction of the transcription of both parameters that were increased by PEA treatment.

4. Discussion

In this study, we showed that PEA exerts protective effects in long-term HFD-induced intestinal damage in mice, modulating gut microbiota composition and restoring tryptophan-derived metabolites altered by HFD (Figure 7).

Despite the lack of evidence regarding the effect of PEA on obesity-related gut dysfunction, previous studies have demonstrated its beneficial effects at the GI level (34, 35). Several NAEs, including PEA and oleoylethanolamide, inhibit intestinal hypermotility and attenuate the inflammatory and the immune response through multiple converging mechanisms, e.g., the activation of the peroxisome proliferator-activated receptor (PPAR)-α (36, 37). Moreover, endogenous NAE levels changed in the GI tract in response to several noxious stimuli to regulate food intake, energy balance, and intestinal function (38).

The gut is the earliest and primary source of the inflammatory process triggered by fat overnutrition, based on direct exposure to dietary-derived components (39). The lack of gut barrier integrity, undermined by HFD, induces endotoxemia and contributes to the low-grade systemic inflammation. Thus, TLR4 activation by LPS and fatty acids represents a key link between the inflammatory process and the immune response in the gut during high-fat feeding (40). Indeed, TLR4 knockout mice have attenuated HFD-induced systemic or intestinal inflammation (41). Here, consistently with our previous finding showing that PEA reduces circulating LPS levels in HFD-fed mice (21), we report a decreased protein expression of colonic TLR4, associated with reduced immune cell markers (i.e., *Itgax*, *Emr1*, *Cma1*, and *Tpsb2*). These data indicate that the oral administration of ultramicronized PEA has immunomodulatory effects in obese mice, limiting immune cell recruitment and mast cell activation. Intestinal mast cells represent a crucial neuroimmune defense mechanism at the frontline between the host and the environment (42). Psychogenic stressors, including fat overnutrition, stimulate mast cell

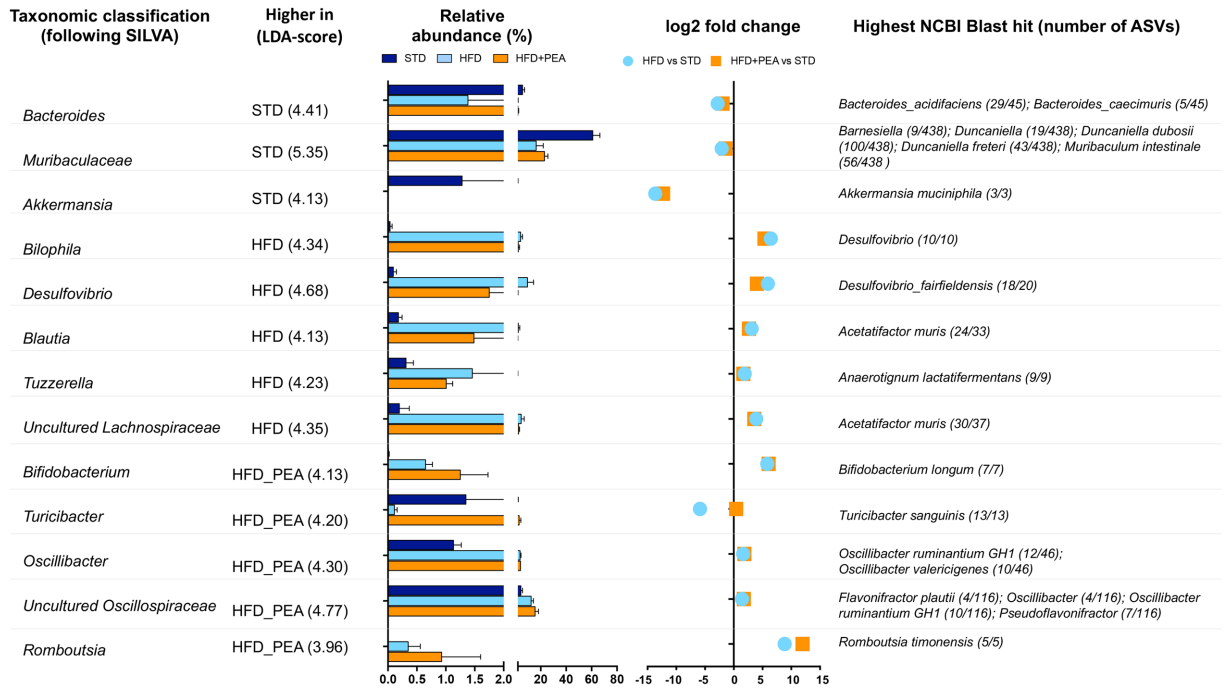

FIGURE 5
Significantly changes genera in HFD and HFD + PEA mice. Significantly changed genera were identified using LEfSe algorithm [alpha values of 0.05 for both Kruskal–Wallis and pairwise Wilcoxon tests and a cutoff value of LDA score (log10) above 2.0]. For each key genus, LDA score, relative abundance (mean and STD err), log2 transformed fold change in HFD and HFD + PEA compared to STD levels, and highest NCBI Blast hits are reported.

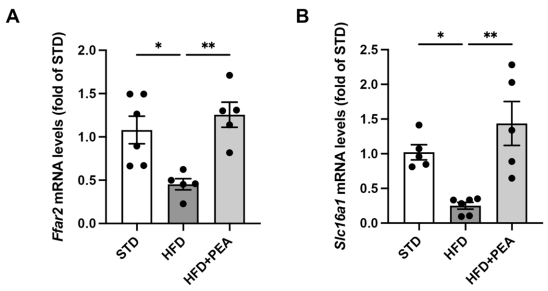

FIGURE 6
PEA induces the transcription of colonic butyrate receptor and transporter. mRNA levels of **(A)** *Ffar2* and **(B)** *Slc16a1* were reduced by HFD and restored by PEA treatment in the colon of obese mice ($n = 5–6$ each group). Data were showed as mean ± SEM reaching the significance at $P < 0.05$ (*$P < 0.05$ and **$P < 0.01$).

degranulation, leading to the release of histamine and tryptase in the gut of both humans and animals (43–45). In our study, PEA administration reduces not only colonic expression of chymase and tryptase, but also *Crh* transcription, indicating its capability to blunt stress-related gut function alterations. Notably, doxantrazole, a mast cell stabilizer, limited CRH-induced hypersensitivity of the colon of maternally separated rats (46), highlighting the detrimental role of mast cell activation at colonic level in stress-related conditions.

The reduced expression of immune cell markers by PEA treatment, particularly regarding macrophages and mast cells, is markedly consistent with the reduction of inflammatory factors in the gut, namely *Il1b* mRNA, and COX-2 and iNOS protein expression.

Proinflammatory factors and cytokines are known to affect tryptophan-kynurenine pathway and its final products. These metabolites sustain local inflammation, foster GI disorders, and may be involved in the pathogenesis of numerous central diseases. TRP metabolism stands in the bridge between the gut and CNS (47). Most of the TRP is oxidized into KYN by the rate-limiting enzyme indoleamine-2-3 dioxygenase (IDO), mainly expressed in the brain, GI tract, and liver, or by tryptophan 2,3-dioxygenase (TDO) explicitly expressed in the liver. KYN can be further metabolized through two divergent pathways associated with the synthesis of kynurenic acid or quinolinic one, whose ratio modulates diverse pathophysiological processes at both central and GI levels. Beyond the IFN-γ-dependent pathway, IDO activity is also synergistically stimulated by the crosstalk among TLR4, IL-1 receptor (IL-1R) and TNF-α receptor (TNFR), increasing the overall KYN levels, which can be transported into the brain and trigger detrimental changes (48). Notably, the enhanced activity of KYN pathway and simultaneous reduction in 5-HT levels have been associated not only with depressive-, anhedonic-, and anxiety-like behavior (49) but also with metabolic dysfunctions related to obesity and insulin resistance (50). Here, we show that PEA re-establishes 5-HT/KYN levels at the colonic level, restoring the altered 5-HT turnover and reducing KYN levels and IDO expression.

It is conceivable to hypothesize that KYN, whose gut levels are increased in HFD mice, reaches the systemic circulation, crosses the blood–brain barrier, and affects brain functions, contributing to behavioral patterns. As already demonstrated, HFD-fed mice show

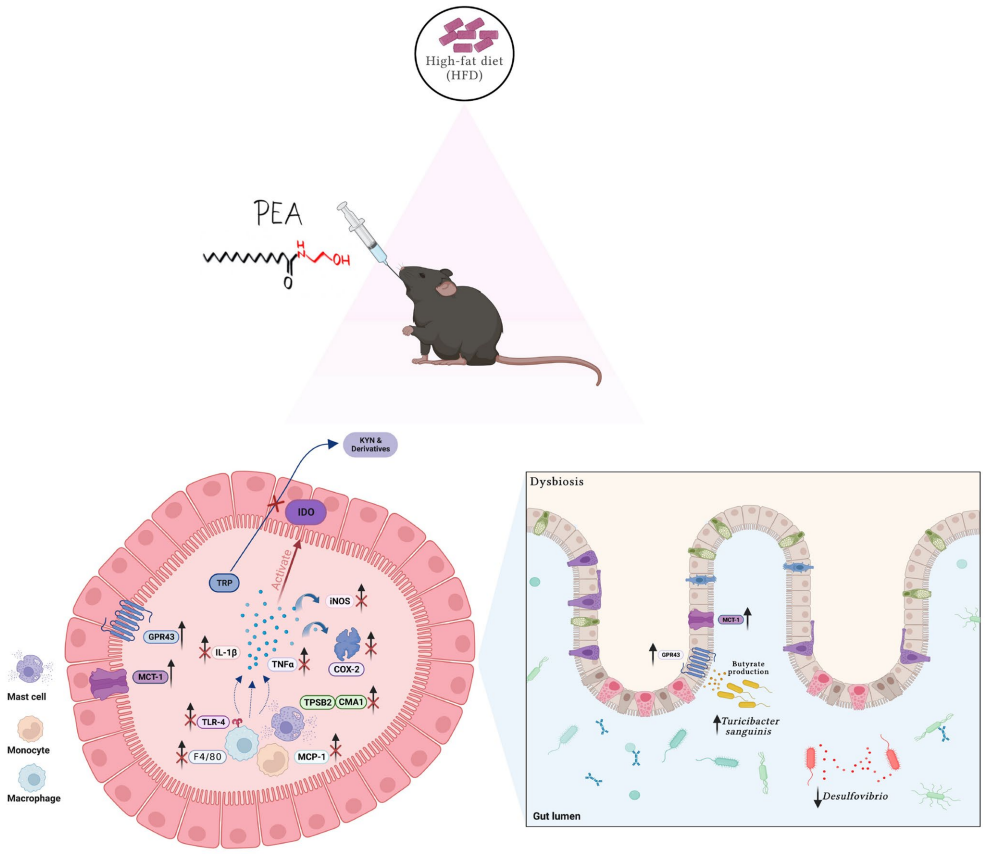

FIGURE 7
Summarizing figure of PEA activity in the colon of HFD mice. Multiple effects of PEA on gut inflammation, TRP metabolites and microbiota composition. Created with biorender.com.

depressive- and anxiety-like phenotypes that are counteracted by administration of PEA (20, 21).

Obesity and associated metabolic disorders show altered gut microbiota composition, impacting on overall human health (51, 52). Thus, microbial reshaping has been proposed as a druggable target. Here, we show that the administration of ultramicronized PEA reprograms gut microbial community assortment. We propose that this event may be considered as a further mechanism in attenuating HFD-induced disorders. According to previous studies, HFD feeding disturbed microbiota homeostasis by increasing Firmicutes/Bacteroidota ratio, a feature associated with obesity and other related metabolic conditions (53). In this study, HFD feeding decreases explicitly the prevalence of the gut barrier-protecting species *Akkermansia muciniphyla* and increases the prevalence of Desulfobacterota, namely *Bilophila* and *Desulfovibrio* genera, opportunistic pathogens, and sources of LPS (54, 55). Desulfobacterota genera are positively related to metabolic disorders, such as type 2 diabetes (56, 57), and their hydrogen sulfide generation might induce intestinal barrier dysfunction and chronic inflammation (58). PEA reduced Firmicutes/Bacteroidota ratio, partially counteracting the HFD-induced increase of specific genera and raising the abundance of those profoundly decreased in the HFD group. PEA treatment also augments the levels of genera already increased by HFD, such as *Bifidobacterium* and Oscillospiraceae members that are potentially butyrate-producing/promoting bacteria (59–63). The further expansion of these bacteria in PEA-treated obese mice could imply beneficial effects on the overall intestinal environment. It may compensate for the loss of beneficial microbes, such as *Akkermansia*, resulting in a healthier gut in terms of mucus layer integrity and reduced inflammation.

Furthermore, PEA increased the relative abundance of *Turicibacter sanguinis*, a spore-forming microbe and short-chain fatty acids (SCFAs)-producer that decreases in obese rodents and alters the expression of gene pathways crucial for lipid and steroid metabolism (64–66). Recently, *T. sanguinis* has been proposed as a serotonin sensor, promoting host 5-HT biosynthesis (66). In this context, *T. sanguinis* could impact the amount of 5-HT synthesized and secreted by enterochromaffin cells throughout its butyrate-generation aptitude. The increased production of SCFAs has been associated with many beneficial effects, including amelioration of obesity and insulin resistance (67). A key role in gut homeostasis has been addressed to butyrate, which is endowed with profound protective effects related to the reversal of obesity and insulin resistance (68). Notably, colon tissue from PEA-treated mice revealed an increase in the expression of genes related to butyrate activity, e.g., MCT1, which mediates butyrate transport into the colonic mucosa, and GPR43 involved in the control of gut inflammation, indicating an increased sensitivity to local butyrate production.

Moreover, in our study, the increased relative abundance of *T. sanguinis* is associated with higher levels of serotonin content in

PEA-treated obese mice. We hypothesize that the reshaping of gut microbiota structure and PEA supplementation might concomitantly promote host 5-HT biosynthesis and rebalance the TRP-KYN metabolism. In conclusion, our results revealed that oral administration of ultramicronized PEA restores colonic homeostasis associated with the reshaping of gut microbiota, the rebalance of TRP-KYN metabolism, and the reduction of the inflammatory response in obese mice, strongly supporting our hypothesis that PEA may have beneficial effects on CNS comorbidities via the gut-brain axis.

Data availability statement

The sequences reported in this study are deposited in the 'European Nucleotide Archive' under the accession number PRJEB61012.

Ethics statement

The animal study was reviewed and approved by Italian D.L. no. 116 of January 27, 1992 of Ministry of Health under the protocol n. 982/2017-PR, and associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609/ECC).

Author contributions

CP, CA, FC, NO, MB, LTu, and AL performed the experiments. CP, LC, and AL performed the research and data analysis. LTr and RM according to their expertise, analyzed data, and reviewed the manuscript. CP, AL, FL, and GM analyzed data and wrote the manuscript. CP, AL, and GM conceived the study design. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

AL declares that he has benefited from a fellowship supported by Epitech Group S.p.A.

The remaining 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1143004/full#supplementary-material

References

1. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol*. (2016) 16:341–52. doi: 10.1038/nri.2016.42

2. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cells*. (2016) 167:915–32. doi: 10.1016/j.cell.2016.10.027

3. Shi H, Wang Q, Zheng M, Hao S, Lum JS, Chen X, et al. Supplement of microbiota-accessible carbohydrates prevents neuroinflammation and cognitive decline by improving the gut microbiota-brain axis in diet-induced obese mice. *J Neuroinflammation*. (2020) 17:77. doi: 10.1186/s12974-020-01760-1

4. Shi H, Yu Y, Lin D, Zheng P, Zhang P, Hu M, et al. Beta-glucan attenuates cognitive impairment via the gut-brain axis in diet-induced obese mice. *Microbiome*. (2020) 8:143. doi: 10.1186/s40168-020-00920-y

5. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol Hepatol*. (2017) 2:747–56. doi: 10.1016/S2468-1253(17)30147-4

6. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology*. (2017) 112:399–412. doi: 10.1016/j.neuropharm.2016.07.002

7. Gao K, Mu CL, Farzi A, Zhu WY. Tryptophan metabolism: a link between the gut microbiota and brain. *Adv Nutr*. (2020) 11:709–23. doi: 10.1093/advances/nmz127

8. Sarnelli G, D'Alessandro A, Iuvone T, Capoccia E, Gigli S, Pesce M, et al. Palmitoylethanolamide modulates inflammation-associated vascular endothelial growth factor (Vegf) signaling via the Akt/Mtor pathway in a selective peroxisome proliferator-activated receptor alpha (Ppar-alpha)-dependent manner. *PLoS One*. (2016) 11:e0156198. doi: 10.1371/journal.pone.0156198

9. Borrelli F, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, et al. Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. *Br J Pharmacol*. (2015) 172:142–58. doi: 10.1111/bph.12907

10. Esposito G, Capoccia E, Turco F, Palumbo I, Lu J, Steardo A, et al. Palmitoylethanolamide improves Colon inflammation through an enteric glia/toll like receptor 4-dependent Ppar-alpha activation. *Gut*. (2014) 63:1300–12. doi: 10.1136/gutjnl-2013-305005

11. Avagliano C, Russo R, De Caro C, Cristiano C, La Rana G, Piegari G, et al. Palmitoylethanolamide protects mice against 6-Ohda-induced neurotoxicity and endoplasmic reticulum stress: In Vivo and in vitro evidence. *Pharmacol Res*. (2016) 113:276–89. doi: 10.1016/j.phrs.2016.09.004

12. Mattace Raso G, Russo R, Calignano A, Meli R. Palmitoylethanolamide in Cns health and disease. *Pharmacol Res*. (2014) 86:32–41. doi: 10.1016/j.phrs.2014.05.006

13. Cristiano C, Pirozzi C, Coretti L, Cavaliere G, Lama A, Russo R, et al. Palmitoylethanolamide counteracts autistic-like behaviours in Btbr T+Tf/J mice:

contribution of central and peripheral mechanisms. *Brain Behav Immun*. (2018) 74:166–75. doi: 10.1016/j.bbi.2018.09.003

14. Cristiano C, Lama A, Lembo F, Mollica MP, Calignano A, Mattace RG. Interplay between peripheral and central inflammation in autism spectrum disorders: possible nutritional and therapeutic strategies. *Front Physiol*. (2018) 9:184. doi: 10.3389/fphys.2018.00184

15. D'Antongiovanni V, Pellegrini C, Antonioli L, Benvenuti L, Di Salvo C, Flori L, et al. Palmitoylethanolamide counteracts enteric inflammation and bowel motor dysfunctions in a mouse model of Alzheimer's disease. *Front Pharmacol*. (2021) 12:748021. doi: 10.3389/fphar.2021.748021

16. Martins LB, Monteze NM, Calarge C, Ferreira AVM, Teixeira AL. Pathways linking obesity to neuropsychiatric disorders. *Nutrition*. (2019) 66:16–21. doi: 10.1016/j.nut.2019.03.017

17. Wachsmuth HR, Weninger SN, Duca FA. Role of the gut-brain axis in energy and glucose metabolism. *Exp Mol Med*. (2022) 54:377–92. doi: 10.1038/s12276-021-00677-w

18. Annunziata C, Lama A, Pirozzi C, Cavaliere G, Trinchese G, Di Guida F, et al. Palmitoylethanolamide counteracts hepatic metabolic inflexibility modulating mitochondrial function and efficiency in diet-induced obese mice. *FASEB J*. (2020) 34:350–64. doi: 10.1096/fj.201901510RR

19. Annunziata C, Pirozzi C, Lama A, Senzacqua M, Comella F, Bordin A, et al. Palmitoylethanolamide promotes white-to-beige conversion and metabolic reprogramming of adipocytes: contribution of Ppar-alpha. *Pharmaceutics*. (2022) 14:338. doi: 10.3390/pharmaceutics14020338

20. Lama A, Pirozzi C, Annunziata C, Morgese MG, Senzacqua M, Severi I, et al. Palmitoylethanolamide counteracts brain fog improving depressive-like behaviour in obese mice: possible role of synaptic plasticity and neurogenesis. *Br J Pharmacol*. (2021) 178:845–59. doi: 10.1111/bph.15071

21. Lama A, Pirozzi C, Severi I, Morgese MG, Senzacqua M, Annunziata C, et al. Palmitoylethanolamide dampens neuroinflammation and anxiety-like behavior in obese mice. *Brain Behav Immun*. (2022) 102:110–23. doi: 10.1016/j.bbi.2022.02.008

22. Minichino A, Jackson MA, Francesconi M, Steves CJ, Menni C, Burnet PWJ, et al. Endocannabinoid system mediates the association between gut-microbial diversity and anhedonia/amotivation in a general population cohort. *Mol Psychiatry*. (2021) 26:6269–76. doi: 10.1038/s41380-021-01147-5

23. Lama A, Annunziata C, Coretti L, Pirozzi C, Di Guida F, Nitrato Izzo A, et al. N-(1-Carbamoyl-2-Phenylethyl) butyramide reduces antibiotic-induced intestinal injury, innate immune activation and modulates microbiota composition. *Sci Rep*. (2019) 9:4832. doi: 10.1038/s41598-019-41295-x

24. Bove M, Lama A, Schiavone S, Pirozzi C, Tucci P, Sikora V, et al. Social isolation triggers oxidative status and impairs systemic and hepatic insulin sensitivity in normoglycemic rats. *Biomed Pharmacother*. (2022) 149:112820. doi: 10.1016/j.biopha.2022.112820

25. Coretti L, Cristiano C, Florio E, Scala G, Lama A, Keller S, et al. Sex-related alterations of gut microbiota composition in the Btbr mouse model of autism spectrum disorder. *Sci Rep*. (2017) 7:45356. doi: 10.1038/srep45356

26. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. (2019) 37:852–7. doi: 10.1038/s41587-019-0209-9

27. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. Dada2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. (2016) 13:581–3. doi: 10.1038/nmeth.3869

28. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The Silva ribosomal Rna gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. (2013) 41:D590–6. doi: 10.1093/nar/gks1219

29. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. Blast+: architecture and applications. *BMC Bioinformatics*. (2009) 10:421. doi: 10.1186/1471-2105-10-421

30. Robeson MS 2nd, O'Rourke DR, Kaehler BD, Ziemski M, Dillon MR, Foster JT, et al. Rescript: reproducible sequence taxonomy reference database management. *PLoS Comput Biol*. (2021) 17:e1009581. doi: 10.1371/journal.pcbi.1009581

31. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16s rRNA gene database and workbench compatible with arb. *Appl Environ Microbiol*. (2006) 72:5069–72. doi: 10.1128/AEM.03006-05

32. Lozupone C, Knight R. Unifrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol*. (2005) 71:8228–35. doi: 10.1128/AEM.71.12.8228-8235.2005

33. Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl Environ Microbiol*. (2007) 73:1576–85. doi: 10.1128/AEM.01996-06

34. Couch DG, Tasker C, Theophilidou E, Lund JN, O'Sullivan SE. Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. *Clin Sci*. (2017) 131:2611–26. doi: 10.1042/CS20171288

35. Couch DG, Cook H, Ortori C, Barrett D, Lund JN, O'Sullivan SE. Palmitoylethanolamide and cannabidiol prevent inflammation-induced hyperpermeability of the human gut in vitro and in vivo-a randomized, placebo-controlled, Double-blind controlled trial. *Inflamm Bowel Dis*. (2019) 25:1006–18. doi: 10.1093/ibd/izz017

36. Capasso R, Orlando P, Pagano E, Aveta T, Buono L, Borrelli F, et al. Palmitoylethanolamide normalizes intestinal motility in a model of post-inflammatory accelerated transit: involvement of Cb(1) receptors and Trpv1 channels. *Br J Pharmacol*. (2014) 171:4026–37. doi: 10.1111/bph.12759

37. Lama A, Provensi G, Amoriello R, Pirozzi C, Rani B, Mollica MP, et al. The anti-inflammatory and immune-modulatory effects of Oea limit Dss-induced colitis in mice. *Biomed Pharmacother*. (2020) 129:110368. doi: 10.1016/j.biopha.2020.110368

38. Borrelli F, Izzo AA. Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance. *Best Pract Res Clin Endocrinol Metab*. (2009) 23:33–49. doi: 10.1016/j.beem.2008.10.003

39. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol*. (2010) 299:G440–8. doi: 10.1152/ajpgi.00098.2010

40. Malesza IJ, Malesza M, Walkowiak J, Mussin N, Walkowiak D, Aringazina R, et al. High-fat, western-style diet, systemic inflammation, and gut microbiota: a narrative review. *Cells*. (2021) 10:3164. doi: 10.3390/cells10113164

41. Kim KA, Gu W, Lee IA, Joh EH, Kim DH. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the Tlr4 signaling pathway. *PLoS One*. (2012) 7:e47713. doi: 10.1371/journal.pone.0047713

42. Wood JD. Serotonergic integration in the intestinal mucosa. *Curr Pharm Des*. (2020) 26:3010–4. doi: 10.2174/1381612826666200612161542

43. Zhang JD, Liu J, Zhu SW, Fang Y, Wang B, Jia Q, et al. Berberine alleviates visceral hypersensitivity in rats by altering gut microbiome and suppressing spinal microglial activation. *Acta Pharmacol Sin*. (2021) 42:1821–33. doi: 10.1038/s41401-020-00601-4

44. Meira de-Faria F, Casado-Bedmar M, Mårten Lindqvist C, Jones MP, Walter SA, Keita ÅV. Altered interaction between enteric glial cells and mast cells in the colon of women with irritable bowel syndrome. *Neurogastroenterol Motil*. (2021) 33:e14130. doi: 10.1111/nmo.14130

45. Burns G, Carroll G, Mathe A, Horvat J, Foster P, Walker MM, et al. Evidence for local and systemic immune activation in functional dyspepsia and the irritable bowel syndrome: a systematic review. *Am J Gastroenterol*. (2019) 114:429–36. doi: 10.1038/s41395-018-0377-0

46. van den Wijngaard RM, Stanisor OI, van Diest SA, Welting O, Wouters MM, de Jonge WJ, et al. Peripheral alpha-helical Crf (9-41) does not reverse stress-induced mast cell dependent visceral hypersensitivity in maternally separated rats. *Neurogastroenterol Motil*. (2012) 24:274–e111, e111. doi: 10.1111/j.1365-2982.2011.01840.x

47. Roth W, Zadeh K, Vekariya R, Ge Y, Mohamadzadeh M. Tryptophan metabolism and gut-brain homeostasis. *Int J Mol Sci*. (2021) 22:2973. doi: 10.3390/ijms22062973

48. Savitz J. The kynurenine pathway: a finger in every pie. *Mol Psychiatry*. (2020) 25:131–47. doi: 10.1038/s41380-019-0414-4

49. Davidson M, Rashidi N, Nurgali K, Apostolopoulos V. The role of tryptophan metabolites in neuropsychiatric disorders. *Int J Mol Sci*. (2022) 23:9968. doi: 10.3390/ijms23179968

50. Cussotto S, Delgado I, Anesi A, Dexpert S, Aubert A, Beau C, et al. Tryptophan metabolic pathways are altered in obesity and are associated with systemic inflammation. *Front Immunol*. (2020) 11:557. doi: 10.3389/fimmu.2020.00557

51. Xu Z, Jiang W, Huang W, Lin Y, Chan FKL, Ng SC. Gut microbiota in patients with obesity and metabolic disorders - a systematic review. *Genes Nutr*. (2022) 17:2. doi: 10.1186/s12263-021-00703-6

52. Xiao L, Sonne SB, Feng Q, Chen N, Xia Z, Li X, et al. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. *Microbiome*. (2017) 5:43. doi: 10.1186/s40168-017-0258-6

53. Stojanov S, Berlec A, Strukelj B. The influence of probiotics on the Firmicutes/Bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. *Microorganisms*. (2020) 8:1715. doi: 10.3390/microorganisms8111715

54. Zhuang P, Zhang Y, Shou Q, Li H, Zhu Y, He L, et al. Eicosapentaenoic and docosahexaenoic acids differentially alter gut microbiome and reverse high-fat diet-induced insulin resistance. *Mol Nutr Food Res*. (2020) 64:e1900946. doi: 10.1002/mnfr.201900946

55. Wu Z, Du Z, Tian Y, Liu M, Zhu K, Zhao Y, et al. Inulin accelerates weight loss in obese mice by regulating gut microbiota and serum metabolites. *Front Nutr*. (2022) 9:980382. doi: 10.3389/fnut.2022.980382

56. Song Y, Wu MS, Tao G, Lu MW, Lin J, Huang JQ. Feruloylated oligosaccharides and ferulic acid alter gut microbiome to alleviate diabetic syndrome. *Food Res Int*. (2020) 137:109410. doi: 10.1016/j.foodres.2020.109410

57. Yan Z, Wu H, Yao H, Pan W, Su M, Chen T, et al. Rotundic acid protects against metabolic disturbance and improves gut microbiota in type 2 diabetes rats. *Nutrients*. (2019) 12:67. doi: 10.3390/nu12010067

58. Zhao J, Wang L, Cheng S, Zhang Y, Yang M, Fang R, et al. A potential synbiotic strategy for the prevention of type 2 diabetes: Lactobacillus paracasei Jy062 and exopolysaccharide isolated from Lactobacillus plantarum Jy039. *Nutrients*. (2022) 14:377. doi: 10.3390/nu14020377

59. Alcon-Giner C, Dalby MJ, Caim S, Ketskemety J, Shaw A, Sim K, et al. Microbiota supplementation with Bifidobacterium and Lactobacillus modifies the preterm infant gut microbiota and metabolome: an observational study. *Cell Rep Med*. (2020) 1:100077. doi: 10.1016/j.xcrm.2020.100077

60. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from Enteropathogenic infection through production of acetate. *Nature*. (2011) 469:543–7. doi: 10.1038/nature09646

61. Falony G, Vlachou A, Verbrugghe K, De Vuyst L. Cross-feeding between Bifidobacterium longum Bb536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. *Appl Environ Microbiol*. (2006) 72:7835–41. doi: 10.1128/AEM.01296-06

62. Gophna U, Konikoff T, Nielsen HB. Oscillospira and related bacteria – from metagenomic species to metabolic features. *Environ Microbiol*. (2017) 19:835–41. doi: 10.1111/1462-2920.13658

63. Tanca A, Palomba A, Fraumene C, Manghina V, Silverman M, Uzzau S. Clostridial butyrate biosynthesis enzymes are significantly depleted in the gut microbiota of nonobese diabetic mice. *mSphere*. (2018) 3:e00492-18. doi: 10.1128/mSphere.00492-18

64. Liu W, Crott JW, Lyu L, Pfalzer AC, Li J, Choi SW, et al. Diet- and genetically-induced obesity produces alterations in the microbiome, inflammation and Wnt pathway in the intestine of Apc(+/1638n) mice: comparisons and contrasts. *J Cancer*. (2016) 7:1780–90. doi: 10.7150/jca.15792

65. Jiao N, Baker SS, Nugent CA, Tsompana M, Cai L, Wang Y, et al. Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. *Physiol Genomics*. (2018) 50:244–54. doi: 10.1152/physiolgenomics.00114.2017

66. Fung TC, Vuong HE, Luna CDG, Pronovost GN, Aleksandrova AA, Riley NG, et al. Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nat Microbiol*. (2019) 4:2064–73. doi: 10.1038/s41564-019-0540-4

67. McNabney SM, Henagan TM. Short chain fatty acids in the colon and peripheral tissues: a focus on butyrate, colon cancer, obesity and insulin resistance. *Nutrients*. (2017) 9:1348. doi: 10.3390/nu9121348

68. Mollica MP, Mattace Raso G, Cavaliere G, Trinchese G, De Filippo C, Aceto S, et al. Butyrate regulates liver mitochondrial function, efficiency, and dynamics in insulin-resistant obese mice. *Diabetes*. (2017) 66:1405–18. doi: 10.2337/db16-0924

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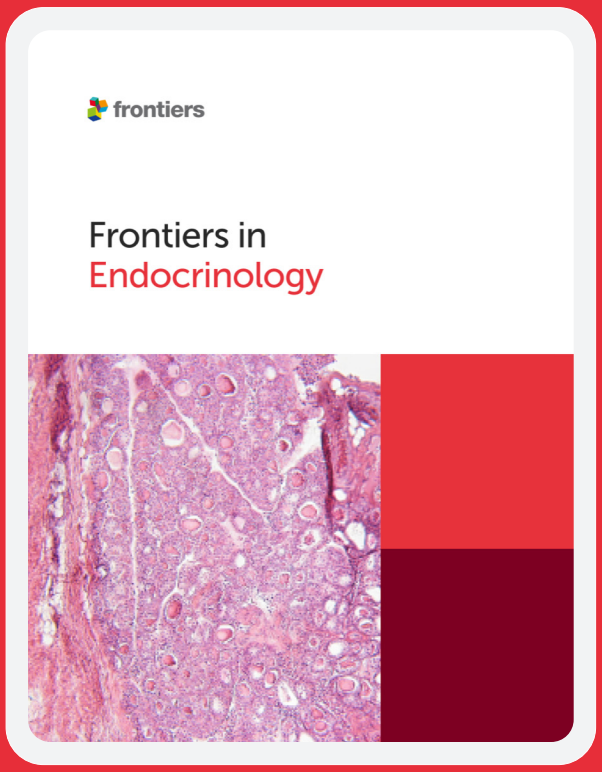

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