

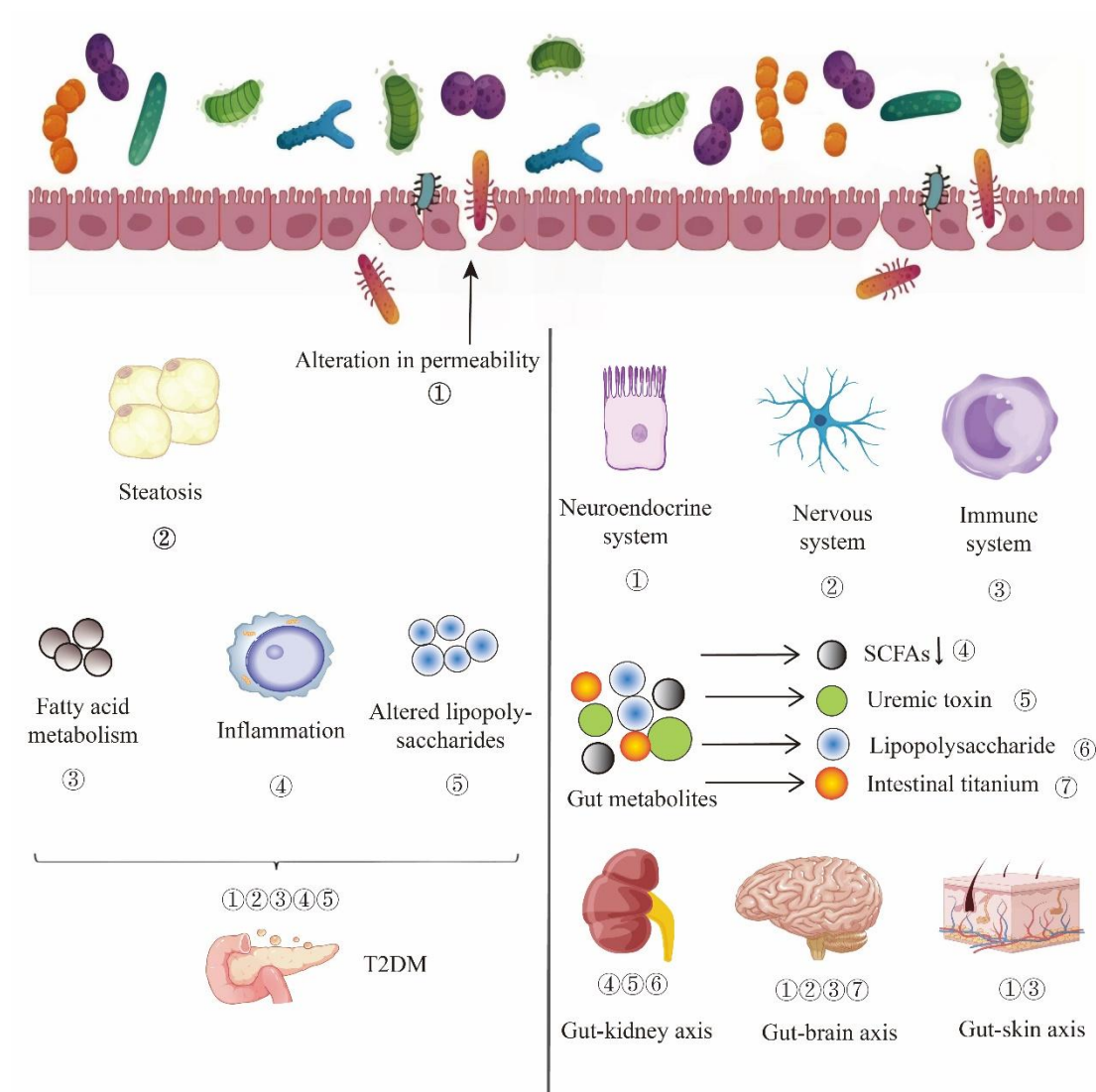
Dear Editor,

Thank you for your letter and for the your and reviewers' comments concerning our manuscript entitled "**Investigating the Influence of Gut Bacteria on T2DM: Mechanisms and Therapeutic Strategy**". We have carefully revised the manuscript according to your and reviewers' suggestion. The point to point responds to the reviewer's comments are listed as following.

Reviewer 1:

Comments 1: In figure (1), there are some overlaps between the words and numbers which make it not clear.

Response: We are very grateful for the question you raised, and we have made corresponding changes in the figure.



Comments 2: There are a lot of abbreviations used with no need, their terms mentioned only one time in the manuscript (eg: BBB, EECs,)

Response: We apologize for not noticing this issue, we appreciate your raising this issue, and we have removed unnecessary abbreviations from the article.

Reviewer 2:

Comments 1 : If possible, include some specific case studies to demonstrate the application of gut microbiota intervention in actual treatment.

Response: Fecal microbiota transplant (FMT) is a groundbreaking approach in recent medical advances for restoring intestinal flora [1]. In 2012, a clinical trial showed that FMT using healthy donor microbiota improved insulin sensitivity and increased butyrate-producing bacteria in metabolic syndrome patients after 6 weeks [2]. Similar results were seen in T2DM patients, with increased butyrate producers in their fecal microbiota [3]. Probiotics offer an alternative to FMT for a healthy gut microbiota. A study found that T2DM patients who took *Lactobacillus casei* daily for 8 weeks had higher SIRT1 levels, lower fetuin-A levels, and improved blood glucose compared to the placebo group [4]. Another trial showed that combining multi-strain probiotics with metformin for 12 weeks increased beneficial bacteria like *Bifidobacterium* and reduced pro-inflammatory bacteria, leading to lower fasting blood glucose and insulin resistance in T2DM patients [5].

[1] LEE C H, STEINER T, PETROF E O, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection: A Randomized Clinical Trial [J]. *Jama*, 2016, 315(2): 142-9.

[2] VRIEZE A, VAN NOOD E, HOLLEMAN F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome [J]. *Gastroenterology*, 2012, 143(4): 913-6.e7.

[3] NG S C, XU Z, MAK J W Y, et al. Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial [J]. Gut, 2022, 71(4): 716-23.

[4] KHALILI L, ALIPOUR B, ASGHARI JAFAR-ABADI M, et al. The Effects of Lactobacillus casei on Glycemic Response, Serum Sirtuin1 and Fetuin-A Levels in Patients with Type 2 Diabetes Mellitus: A Randomized Controlled Trial [J]. Iranian biomedical journal, 2019, 23(1): 68-77.

[5] PALACIOS T, VITETTA L, COULSON S, et al. Targeting the Intestinal Microbiota to Prevent Type 2 Diabetes and Enhance the Effect of Metformin on Glycaemia: A Randomised Controlled Pilot Study [J]. Nutrients, 2020, 12(7).

Comments 2: Further explore the mechanisms by which the mentioned probiotics and Bifidobacterium affect other organs such as the kidneys, muscles, and brain, and provide a schematic diagram of the mechanisms of specific gut microbiota to make the mechanisms clearer and more understandable.

Response: We sincerely appreciate your valuable feedback. We have added mechanisms by which specific gut microbiota such as Bifidobacterium affect organs like the kidneys, skin, and brain, and provided corresponding schematic diagrams to make it clearer and easier to understand.

Probiotics such as Lactobacillus, Bifidobacterium, and Propionibacterium acnes exert antioxidant effects through the action of antioxidant enzymes like catalase[1], inhibit the cleavage of inhibitory molecules like I κ B[2], and reduce the expression of IL-8 to alleviate skin inflammation. Additionally, they can increase the levels of GLP-1 and

insulinotropic hormones, enhancing insulin sensitivity[3], thereby mitigating vascular hardening and improving local ischemia. Probiotic soy milk can ameliorate renal oxidative stress, including urinary protein PRO, SCr, and eGFR, and can also reduce the production of serum p-cresol sulfate (PCS)[4]. Lactobacillus promotes the activation of astrocytes[5], improves the BDNF/TrkB/CREB signaling pathway, and reduces the level of neuronal apoptosis[6, 7]. Lactobacillus and others can increase short-chain fatty acids (SCFAs), which in turn induce the interaction of intestinal hormones with brain receptors.

In contrast, harmful bacteria such as Fusobacterium and Ruminococcus primarily affect the skin, brain, and kidneys by releasing lipopolysaccharides and reducing SCFAs. Ruminococcus may regulate immune responses by affecting gut-associated lymphoid tissue (GALT), thereby influencing the skin's reactivity and inflammatory state in response to external stimuli[8]. The reduction of SCFAs can lead to increased glutathione peroxidase activity[9] and promote the production of transforming growth factor β 1 (TGF- β 1), exacerbating renal fibrosis[10]. SCFAs can also regulate the integrity of the blood-brain barrier (BBB), thereby alleviating neuroinflammation and the maturation of microglia[11].

[1] TAVAKOLI M, HABIBI NAJAFI M B, MOHEBBI M. Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt [J]. Heliyon, 2019, 5(2): e01204.

[2] SHARMA S, SINGH A, SHARMA S, et al. Functional foods as a formulation ingredients in beverages: technological advancements and constraints [J].

Bioengineered, 2021, 12(2): 11055-75.

[3] MOHSENI S, BAYANI M, BAHMANI F, et al. The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial [J]. Diabetes/metabolism research and reviews, 2018, 34(3).

[4] MEIJERS B K, DE PRETER V, VERBEKE K, et al. p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin [J]. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, 2010, 25(1): 219-24.

[5] LI Z H, JIANG Y Y, LONG C Y, et al. The gut microbiota-astrocyte axis: Implications for type 2 diabetic cognitive dysfunction [J]. CNS neuroscience & therapeutics, 2023, 29 Suppl 1(Suppl 1): 59-73.

[6] DAVARI S, TALAEI S A, ALAEI H, et al. Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis [J]. Neuroscience, 2013, 240: 287-96.

[7] MORSHEDI M, SAGHAFI-ASL M, HOSSEINIFARD E S. The potential therapeutic effects of the gut microbiome manipulation by synbiotic containing-Lactobacillus plantarum on neuropsychological performance of diabetic rats [J]. Journal of translational medicine, 2020, 18(1): 18.

[8] MAHMUD M R, AKTER S, TAMANNA S K, et al. Impact of gut microbiome on

skin health: gut-skin axis observed through the lenses of therapeutics and skin diseases [J]. *Gut microbes*, 2022, 14(1): 2096995.

[9] MARZOCCO S, FAZELI G, DI MICCO L, et al. Supplementation of Short-Chain Fatty Acid, Sodium Propionate, in Patients on Maintenance Hemodialysis: Beneficial Effects on Inflammatory Parameters and Gut-Derived Uremic Toxins, A Pilot Study (PLAN Study) [J]. *Journal of clinical medicine*, 2018, 7(10).

[10] ZHANG Y, GAO F, TANG Y, et al. Valproic acid regulates Ang II-induced pericyte-myofibroblast trans-differentiation via MAPK/ERK pathway [J]. *American journal of translational research*, 2018, 10(7): 1976-89.

[11] DU L, CHEN J, YAN J, et al. Lingguizhugan decoction ameliorates cognitive impairment in AD-like mice by influencing the microbiome-gut-brain axis mediated by SCFAs [J]. *Phytomedicine : international journal of phytotherapy and phytopharmacology*, 2024, 133: 155942.

