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The role of natural products and intestinal flora on type 2 diabetes mellitus treatment

Dear Editor,

Thank you for giving us the opportunity to submit a revised draft of the manuscript. We appreciate the time and effort that you and the reviewers dedicated to providing feedback on our manuscript and are grateful for the insightful comments on and valuable improvements to our paper. We have incorporated most of the suggestions made by the reviewers. Please see below for a point-by-point response to the reviewers' comments and concerns.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This review mainly briefly describes the role of intestinal flora in type 2 diabetes mellitus, and does not mention type 1. It is suggested to change the title. Is there basic experimental support? The relationship between stool and gut microbiota needs to be described in detail. The intestinal flora mechanisms in the formation and development of type 2 diabetes mellitus is not particularly clear.

Response: Thank you for your comments. We changed the title as you suggested. We have detailed the intestinal flora mechanisms in the genesis and development of type 2 diabetes: 'Short chain fatty acids (SCFAs) are organic carboxylic acids that have 1-6 carbon atoms. They are primarily produced by bacteria in the intestine. Bacteroides, Clostridium, Bifidobacterium, Eubacterium, Streptococcus, Peptostreptococcus, and others are typical SCFA-producing bacteria (3). The most important SCFAs consist of acetic, propionic, butyric, valeric, and caproic acids. Acetate (C2), propionate (C3), and butyrate (C4) are the most prevalent SCFAs in the human body and anions in the colon, respectively. Depending on the fiber content of the diet, the large intestine produces between 500 and 600 mM SCFA daily. Colonic fermentation of fibre into SCFAs decreases pH, increases faecal acidity, and promotes the proliferation and diversity of gut microbiota. SCFAs serve as mediators in numerous pathways involving local, immunological, and endocrine impacts, as well as microbiota-gut-brain interactions. SCFAs are absorbed by colonocytes through either passive diffusion or active transport mediated by H^+-linked monocarboxylate transporters(MCTs),

following breakdown of dietary fibres by intestinal bacteria. At the cellular level, SCFAs regulate the homeostasis and function of intestinal epithelial cells, generating complex and integrated effects (Table 1)(4).

Recent research has revealed that there is an abnormality in the bacteria that produce short-chain fatty acids in person with diabetes, resulting in abnormal short-chain fatty acid formation. Short-chain fatty acids can help the colon's acidic environment, inhibit the growth of harmful bacteria, maintain water and electrolyte balance, and prevent intestinal dysfunction. Disturbance of the intestinal flora results in a decrease in short-chain fatty acids, which results in a decrease in the ability of the intestinal tract to mount an anti-inflammatory response, resulting in the emergence of intestinal inflammation (3).

G protein-coupled receptors (GPR41 and GPR43) activated by short-chain fatty acids (SCFA) have important regulatory properties for fat metabolism and glucose metabolism (8). By activating GPR41 and GPR43, SCFAs can promote the activation of peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) from intestinal enteroendocrine L cells. The neuroendocrine hormone PPY affects food intake and energy balance. Reduced GLP-1 secretion in T2DM results in decreased insulin levels and poor glucose and energy metabolism(Figure 1)(9). Dysbacteriosis causes low SCFA production in the intestinal tract, impaired activation of SCFA receptors, and abnormalities in fat and glucose metabolism, all of which contribute to type 2 diabetes (8).'

'The primary functional components of bile are bile acids. They are produced from cholesterol in hepatocytes via the classic or alternative pathway, stored in the gallbladder, and subsequently released into the small intestine. The cholesterol 7a-hydroxylase (CYP7A1) enzyme is the rate-limiting enzyme in the synthesis of bile acids via the so-called classical pathway. Sterol 27 hydroxylase (CYP27A1) is active in the alternative pathway. 95% of cholic acid (CA) and chenodexycholic acid (CDCA), the two principal bile acids, enter the enterohepatic circulation. The gut bacteria convert 5% of primary bile acids into secondary bile acids. In humans, these are deoxycholic acid (DCA), lithocholic acid (LCA), and ursodesoxycholic acid (UDCA), whereas in mice, they are DCA, LCA, muricholic acid (MCA), hyodeoxycholic acid, and murideoxycholic acid (10-12).They then influence lipid, glucose, and energy metabolism by activating a series of nuclear receptors (farnesoid X receptor (FXR) in liver and intestine, G protein-coupled bile acid receptor 5 (TGR5) in enteroendocrine cells and pancreatic B cells) involved in the production of liver bile acids and intestinal bile acid reabsorption (Figure 2) (13).

Intestinal flora disruption reduces secondary bile acid production and bile acid receptor activation, resulting in impaired glucose metabolism and T2DM(3). GLP-1 release from L cells is stimulated by the activation of TGR5 by secondary BAs, which increases insulin secretion and glucose tolerance. As a result of the decreased TGR5 stimulation with the changing BA content, the released GLP-1 decreases. This causes insulin resistance and increased glucose (14). Bacterial diseases result in decreased bile acid activation and diminished FXR activation, which contribute in a variety of ways to the development of T2DM. It decreases insulin sensitivity and glycogen synthesis, while hepatic gluconeogenesis and blood sugar increase. Fibroblast growth factor 15 (FGF15), FGF21, FGF 19 and energy consumption decrease, body weight increases and insulin sensitivity decreases. It decreases the expression of Krueppel-like factor (KLF11), a kind of transcription factor involved in glucose regulation, hence diminishing KLF11's ability to support insulin gene transcription and resulting in a lower insulin level (Figure 3) (3, 14).'

'Through the upregulation of inflammatory signalling pathways and proinflammatory cytokine production, a high quantity of LPS generated in the gut (metabolic endotoxemia) may induce chronic low-grade inflammation in T2DM patients(14).'

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Indeed, a good topic selected for review. The present review focused on the role of natural products and intestinal flora on diabetes mellitus treatment. The findings reported the short-chain fatty acids theory, the bile acid theory, and the endotoxin theory are all potential methods by which intestinal flora contributes to the establishment and progression of type 2 diabetes. According to research, regulating intestinal flora can improve insulin resistance, increase insulin production, and play an important role in blood sugar regulation. The present review suggested the use of Natural products (bioactive compounds) helps in the modulation of intestinal flora and can be used in the treatment of metabolic diseases such as diabetes and obesity. Key problems are not mentioned and the tentative solution. In upcoming years use of natural products to can be used for diabetes treatment that regulates intestinal flora and this could be an innovative step in the prevention and treatment of diabetes.

Response: Thank you for your comments.

Reviewer #3:

Scientific Quality: Grade D (Fair)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Rejection

Specific Comments to Authors: In recent years, the research on the intestinal microbiota has been continuously deepened, and more and more studies show that the microbiota may play an important role in the development of various diseases in the human body. This paper combines the two research topics of diabetes and intestinal microbe, and summarizes the relationship between diabetes occurrence and development and intestinal microbiosis and the treatment of diabetes through the treatment of intestinal microbial dysbiosis. The review content has great guiding value for clinical practice, and the direction selection is also relatively novel. But there are still the following problems： 1. It is suggested to add a subheading to the paragraphs of the three mechanisms of short-chain fatty acids, bile acids and endotoxin to make the article more organized. 2. The content of the three theoretical mechanisms is less. Please continue to consult the relevant literature in the past three years, enrich the content, and add some mechanism maps appropriately. 3. The overall content of the article is slightly less, with only 18 references. Please continue to consult the literature and supplement the relevant content.

Response: Thank you for your comments. In line with your suggestion, we added a subtitle to the paragraphs of the three intestinal flora mechanisms. We increased the content of the three theoretical mechanisms and added some mechanism maps accordingly. We increased the number of references by referring to the literature.

‘Short chain fatty acids (SCFAs) are organic carboxylic acids that have 1-6 carbon atoms. They are primarily produced by bacteria in the intestine. Bacteroides, Clostridium, Bifidobacterium, Eubacterium, Streptococcus, Peptostreptococcus, and others are typical SCFA-producing bacteria (3). The most important SCFAs consist of acetic, propionic, butyric, valeric, and caproic acids. Acetate (C2), propionate (C3), and butyrate (C4) are the most prevalent SCFAs in the human body and anions in the colon, respectively. Depending on the fiber content of the diet, the large intestine produces between 500 and 600 mM SCFA daily. Colonic fermentation of fibre into SCFAs decreases pH, increases faecal acidity, and promotes the proliferation and diversity of gut microbiota. SCFAs serve as mediators in numerous pathways involving local, immunological, and endocrine impacts, as well as microbiota-gut-brain interactions. SCFAs are absorbed by colonocytes through either passive

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'Through the upregulation of inflammatory signalling pathways and proinflammatory cytokine production, a high quantity of LPS generated in the gut (metabolic endotoxemia) may induce chronic low-grade inflammation in T2DM patients(14).'

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Dear Authors, Your review is an interesting summary on current literature about the role of intestinal flora on diabetes, primarily type 2 diabetes, prevention and potentially treatment. I would suggest to leave out the classification of diabetes in 3 types. In addition, do not use term diabetics but rather person with diabetes or a T2DM patients which ever is more convenient. You should also prepare literature citations within text according to Journal's propositions.

Response: Thank you for your comments. In line with your suggestion, we left out the classification of diabetes into 3 types. We did not use the term diabetics. In addition, we arranged the literature citations in the text according to the suggestions of the journal.

Reviewer #5:

Scientific Quality: Grade E (Do not publish)

Language Quality: Grade B (Minor language polishing)

Conclusion: Rejection

Specific Comments to Authors: The manuscript is very short (~ 3 Word pages) with only 18 references, and lacks any in-depth details about the topic. In its current form it is more like an informative online article than a true scientific review article. My recommendation: Reject.

Response: Thank you for your comments. In line with your comment, we increased the references we used while preparing the review article and enriched its content. We've added mechanism diagrams and content-related tables.

(2) Company editor-in-chief:

I recommend the manuscript to be published in the World Journal of Clinical Cases.

Response: Thank you for your comments.