Response to the comments

Round-1

Dear editors and reviewers:

Thank you for allowing us to revise the manuscript. We are very grateful for the constructive comments that have helped us improve the manuscript. We have provided a point-by-point response to each of the reviewer's comments below. The revisions in the revised manuscript have been marked in red.

We look forward to hearing from you soon.
Sincerely
Benjie Zhou

Answer to the reviewers

Reviewer 1:

1) Abstract – "Diabetes mellitus (DM) is one of the biggest public health concerns worldwide and includes type 1, type 2, gestational, and other rare forms." – avoid referring to other types of diabetes when the review should be centered on T2DM

Answer: *Thank you for the suggestion. We have modified the sentence in an appropriate form.*

2) Abstract – a large part of the abstract is rather introductory and provides very limited indices for the potential reader, which would motivate he/she to open the full-text. Rewrite the abstract in a way that it is more focused and avoids the textbook-like phrases and superficially covered facts.

Answer: *Thank you for the suggestion. We have modified the abstract. The rewritten part was marked in red.*

3) Abstract – check the manuscript for the presence of typos, like “timethylamine”

Answer: *Thank you for reminding. We have corrected it.*

4) Core tip – “However, because the composition of natural products is so complex that gut microbiota may also influence the metabolism of natural product in host, further studies should focus on the metabolism of natural products and their bioactive components by gut microbiota” – it is unclear why it would be surprising that the microbiota is affected by the consumed food. Specify whatever is related to T2DM.

Answer: *Thank you for the suggestion. We have rewritten it.*

5) Introduction – “which is rising more rapidly in middle- and low-income countries[1,2]” – this could be true but check where the diabetes prevalence is already high

Answer: *Thank you for the suggestion. We have checked it. It was mentioned in the original that the dominant risk factor of DM is becoming more prevalent over time in both developed and developing regions.*

6) Introduction – “According to the classification of the International Diabetes Federation (IDF) in 1997,” – refer to the most recent ADA guidelines published in Diabetes Care instead of this several decades old source.

Answer: *Thank you for the suggestion. We have upgraded it.*

7) Alternation… - “indicating that these bacteria were more specific to T2DM than obesity was[22].” – ref. #22 is a review. Replace it with primary reference(s). Double-check, whether the respective sentence tells what you aimed to tell – the fact that the bacteria segregated more with glucose levels than with obesity does not mean that

obesity was not specific to T2DM.

Answer: *Thank you for the reminding. We have checked the original reference and modified sentence.*

8) Metabolites… – "mouse70 and bovine71 cells" – the meaning of the numbers is unclear

Answer: *Thank you for the reminding. We have corrected it.*

9) Metabolites… - "Peptide YY (PYY) is released into the circulation" – the respective subchapter is about SFAs, so avoid starting the para with the peptide YY. It is necessary to make the text fluent and to guide the reader through the topics covered.

Answer: *Thank you for the reminding. We have modified it.*

10) Metabolites… - "inulin propionate ester supplementation at 10 g/d when compared with 10 g/d inulin alone" – do you mean that the mice (20g-weighing animals) were fed with 10g inulin daily? That is irrelevant. The mouse typically eats 3-5 g of food per day.

Answer: *Thank you for the reminding. We have rechecked the reference. The subjects for the article were overweight adults. We have modified it.*

11) Metabolites – "Hattori et al showed that acetate administrated orally, in comparison to distilled water, increased energy expenditure and lipid oxidation[44]. Similar to the above results, acetate (5.2 mg/kg)" – when claiming that something was administered orally, always make sure how exactly was it administered, whether in form of some solution, capsules, gavage, etc. By the way, which acetate? There are many acetates…

Answer: *Thank you for the suggestion. We have rechecked the references. In the first*

referene, 1.5% AcOH (diluted in water) was administered with a stomach tube. And for the second reference, the acetate was not specified in the original research. In my opinion, the specific type of SCFAs is more important and adequate to imply its function.

12) Branched… - "Inflammatory factor signaling pathways, including the nuclear factor (NF)-κB pathway and mammalian target of rapamycin complex 1 (mTORC1), might be candidate therapeutic targets" – please specify why do you consider mTORC1 to be an "inflammatory factor signaling pathway" in a context of the gut environment. mTOR is a major nutrient sensing pathway and is important across multiple cell types.

Answer: *Thank you for the question. The research of BCAAs metabolism and mTOR has so far mainly focused on mTORC1. The role of mTORC2 in BCAAs metabolism is rarely known. However, Zhao H and his colleagues (**Diabetes. 2020 Jun;69(6):1164-1177**) found mTOCR2 also plays an important role in this process, which display an opposite trend of expression after BCAAs supplemention, compared with mTORC1.*

13) Fig. 2 – the bottom half of the figure needs to be improved in order to avoid the current (likely unnoticed) interpretation that the discussed pathways are affected in blood vessels

Answer: *Thank you for your reminding. We have modified the Figure 2.*

14) Metabolic endotoxemia – "The amount of Bifidobacterium is significantly and negatively correlated with high portal plasma levels of LPS in HFD-induced models[86]. However, by using a broad-spectrum antibiotic (ampicillin and neomycin), metabolic endotoxemia and cecal content of LPS in ob/ob mice are dramatically reduced, and then the glucose intolerance, inflammation and body weight are also improved[81]." – these two sentences should not be interconnected, as the two mentioned antibiotics are not selective for Bifidobacterium and therefore the causative link cannot be established with this argument.

Answer: *Thank you for your suggestion. We replaced the reference with another one, and adjusted the order to make the content more coherent.*

15) Interactions… - "Clinical data prove that BAs induce metabolic disorders, such as disorders of lipid, glucose and energy metabolism, as well as inflammatory cytokine generation, which are closely related to T2DM[100]." – this is not true. The dysregulation of bile acid homeostasis could cause that but do not attribute this effect to a simple presence of bile acids.

Answer: *Thank you for your suggestion. We have modified the sentence into "Clinical data prove that dysregulation of bile acid homeostasis and dysbiosis can induce metabolic disorder, such as disorders of lipid, glucose and energy metabolism, as well as inflammatory cytokine generation, which are closely related to T2DM".*

16) Table 1 – "Increasing the ratio of Bacteroidetes and Firmicutes in intestine" – should "and" be replaced with "to"?

Answer: *Thank you for your suggestion. We have modified the sentence into "Increasing the ratio of Bacteroidetes/Firmicutes in intestine".*

17) Table 1 – check, whether all the claims are correct. For example, the claim "abundance of Bacteroidetes, Prevotella and Deltaproteobacteria" does not make sense because Prevotella is part of Bacteroidetes.

Answer: *Thank you for your suggestion. We have rechecked the reference. Actually, as far as I know, 16S sequencing analysis does not guarantee that all sequences identified at the same level of biological classification (phylum, class, genus or species). The updating of the database and the depth of the sequencing, as well as the types of samples influence on the level of biological classification. Therefore, in my opinion, it is reasonable to exist at different levels of classification for all identified sequences.*

18) Table 1 – references should be in the same format as in the rest of the text.

Answer: *Thank you for your suggestion. We have corrected it.*

19) Many recent references point to journals with questionable peer-review practices, like Theranostics. Please make sure that all the claims are supported by trusted references.

Answer: *Thank you for your reminding. We have rechecked the reference. The original description is as follows: "after oral administration, the blood level of BBR in hyperlipidemic patients was higher than that in healthy individuals (*P<0.05)". We have modified it so that make it more close to the meaning of the original text.*

20) The formatting of references is inconsistent. Check, for example, the presence/absence of capital letters in "Nature reviews Endocrinology"

Answer: *Thank you for your comment. We have corrected it with Online reference automatic editing system.*

21) The current figures are fine but there is no figure, which would focus on the bacteria diversity and changes in bacteria diversity with respect to diabetes

Answer: *Thank you for your suggestion. This review is mainly focused on the mechanisms of the gut microbiota in T2DM, including short-chain fatty acids, biosynthesis and metabolism of branched-chain fatty acids, trimethylamine N-Oxide, bile acid signaling, endotoxin leakage and gut permeability (presented and discussed with each subchapter). Changes in bacteria diversity on T2DM subjects is not key content of paper. However, we have enriched the information of Fig 3. Please checked*

it.

22) Polyphenols – “Resveratrol attenuates HFD-induced NASH ameliorates the intestinal barrier dysfunction and inflammation in rats[125].” – check the dose used, it was far above the “safe” dose for humans. Check also other compounds mentioned in this manuscript for the same issue.

Answer: *Thank you for your suggestion. We checked it carefully, the dose of resveratrol, which were used in the cited reference [125] was not mention in the paper.*

Reviewer 2:

1) The English need improvement since there are some grammatical and syntax errors in the manuscript (For example, the words “Methanismsand” may be as “Mechanisms and” as “”; “” as “- in the title”; “overall” as “the overall”; “examination” as “the examination”; “in host” as “in the host”; “of Human” as “of the Human”; “host are” as “host is”; “expenditure were” as “expenditure was”; “than cause” as “than a cause”; “progression” as “the progression”; “have low” as “have a low”; “to high” as “to the high”; “to improvement” as “to the improvement”; “expression” as “the expression”; “in intestinal” as “in the intestinal”). The grammar mistakes which are not mentioned here also to be checked and corrected properly.

Answer: *Thank you for your suggestion. We have* checked and corrected the paper properly.

2) There are a few typing mistakes as well, and authors are advised to carefully proof-read the text (For example, the words “microbiotaas” may be as “microbiota as”; “(T1DM),gestational” as “(T1DM), gestational”; “(IDF),latent” as “(IDF), latent”; “maturity onset” as “maturity-onset”; “includestype” as “includes type”; “showsthat” as “shows that”; “inthis” as “in this”; “Thereis” as “There is”; “timethylamineN” as

“time thylamine N”; “anddescribeshow” as “and describe show”; “canimprove” as “can improve”; “willrise” as “will rise”; “prevalenceof” as “prevalence of”; “causingmultiple” as “causing multiple”; “dysfunction,inflammatory” as “dysfunction, inflammatory”; “havestarted” as “have started”; “andprovidessignals” as “and provides signals”; “[22].Further” as “[22]. Further”; “haslower” as “has lower”; “tissue,pancreas” as “tissue, pancreas”; “glycemia regulation” as “glycemic regulation”; “multidimensional,including” as “multidimensional, including”; “oxidation,as” as “oxidation, as”; “[42] ,” as “[42],”; “Jockenet” as “Jocken et”; “andinsulin” as “and insulin”; “BCAAahave” as “BCAAs have”; “andmammalian” as “and mammalian”; “affecthost” as “affect host”; “causingenergy” as “causing energy”; “andinduces” as “and induces”; “andoriginates” as “and originates”; “oxidizesTMA” as “oxidizes TMA”; “TMAO.There" as “TMAO. There”; “shownthat” as “shown that”; “todecrease” as “to decrease”; “andincrease” as “and increase”; “promotesnormal” as “promotes normal”; “arestill” as “are still”; “leadingto” as “leading to”; “whichis” as “which is”; “humans,and” as “humans, and”; “bilesalthydrolase” as “bile salt hydrolase”; “millimolarconcentrations” as “millimolar concentrations”; “competesfor” as “competes for”; “regulatinglipid” as “regulating lipid”; “gutmicrobiota” as “gut microbiota”; “Thisemphasizes thatchanges inthe” as “This emphasizes that changes in the”; “intestine,FXR” as “intestine, FXR”; “host.The” as “host. The”; “abundanceof” as “abundance of”; “,increase” as “, increase”; “inplants” as “in plants”; “areinversely” as “are inversely”; “activityof” as “activity of”; “increasesabundance” as “increases abundance”; “decreasesthe” as “decreases the”; “improvingIR” as “improving IR”; “isrelated” as “is related”; “largeheterogeneous” as “large heterogeneous”; “toall” as “to all”; “arealso” as “are also”; “stress;inhibition” as “stress; inhibition”; “);promotion” as “); promotion”; “ofpolyphenols” as “of polyphenols”; “[125].Quercetin” as “[125]. Quercetin”; “hasa” as “has a”; “andprotease” as “and protease”; “thealkaloids” as “the alkaloids”; “chinensisFranch” as “chinensis Franch”; “thegut” as “the gut”; “shownthat” as “shown that”; “ofthe” as “of the”; “feces[” as “feces [”; “ofBCAA” as “of BCAA”; “ofthe” as “of the” ; “ofgut” as “of gut”; “products,such” as “products, such”;

“hostmetabolism” as “host metabolism”; “Thisis importantfor” as “This is important for”; “onhostmetabolism” as “on host metabolism”; “Ofgut” as “Ofgut”). The typos not mentioned here also to be checked and corrected properly.

Answer: *Thank you for your suggestion. We have checked and corrected errors in the text.*

3) The keywords that are not in the title should also be included in the other parts of the manuscript. The keywords should assist computer searches to find your specific article.

Answer: *Thank you for your suggestion. The key words listed in the article are the high-frequency words that appear many times in the text.*

4) The databases or search engines used (like PubMed, ScienceDirect, Google scholar etc.,) for collection of this information with the keywords (in the text) used should be mentioned.

Answer: *Thank you for your suggestion. We have added the information in the* **Introduction** *part.*

5) Check the abbreviations throughout the manuscript and introduce the abbreviation when the full word appears the first time in the text and then use only the abbreviation. And it should be in both abstract as well as in the remaining part of the manuscript.

Answer: *Thank you for your reminding. We have checked and modified it.*

Reviewer 3:

Although the article is good, but blanks are left which makes barriers to follow the authors. The gut microbes look to play controversial roles, a matter that is good to be

understood and further studied more than to give final images. I agree with the authors about the difficulties in targeting the study subject, but how these issues can be transferred into facts. diabetes has been known for more than 3500 years, and I think that it is a developmental disease. It is good if the author adds a new paragraph in the developmental aspects of diabetes in response to gut microbes. Regarding the figures, I do not know if the figures are produced by the authors or copied from secondary sources. the authors have to frankly answer this question, and if they are copied, is/are there any permission/s?

Answer:

*Question 1: Thank you for your suggestion. This is a really tricky problem. As far as I know, there is not a standard for evaluating the development of diabetes. Clinically, it is mainly evaluated by the duration of disease and the severity of complications. Therefore, it is difficult to accurately describe the developmental aspects of diabetes in response to gut microbes, especially for the role of gut microbes in each stage of diabetes. However, with the understanding of the relationship between gut microbes and diabetes. People are more focused on the role of metabolites of gut microbiota on the diabetes. For example: by using MGWAS analysis, people found that T2DM patients were characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some SCFA-producing bacteria (such as Roseburia intestinalis and Faecalibacterium prausnitzii), and an increase in various opportunistic pathogens, as well as an enrichment of other microbial functions conferring sulphate reduction and oxidative stress resistance (**Nature. 2012 Oct 4;490(7418):55-60, Gut. 2014 Sep;63(9):1513-21**). Moreover, endotoxaemia and abnormal amino acid metabolism (BCAAs, tryptophan) are also significant correlated with the development of diabetes. Therefore, in my opinion, establishing a characteristic database of gut microbiota and metabolites of diabetic population and new monitoring techniques/methods is a good strategy to clarify this problem.*

Question 2: All Figures were created by the first author Fan Xia with Microsoft PowerPoint software. We have upload the file.

Round-2

Answer to the reviewers

Reviewer1: The provided comments were reflected to a sufficient extent. - Language check is necessary, there is a typo already in the manuscript title. - There are still unnecessary parts of the text left. These include, for example: "According to Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes in 2020, there are three main types of DM, including type 1 DM (T1DM), type 2 DM (T2DM) and gestational DM (GDM). However, there are some other types of diabetes, such as autoimmune latent diabetes in adults (LADA), pancreatic diabetes, and maturity onset diabetes of the young (MODY)1–3[3]." - these textbook-like facts can easily be deleted and the manuscript trimmed accordingly. - Many of the relevant recent references are uncited. These include, for example, Acta Diabetol. 2021 May 10. doi: 10.1007/s00592-021-01727-5. Diabetes Metab Syndr Obes. 2021 Apr 28;14:1855-1869. Clin Transl Med. 2021 Apr;11(4):e326. PeerJ. 2021 Apr 1;9:e11128. Gut. 2021 Mar 30:gutjnl-2020-323617. and many others. - There is no method disclosed concerning how the reviewed papers were selected. However, given that the recent papers are nearly completely absent, it is necessary to check the major abstract databases for relevant papers, read through the full-texts and extract relevant information into this review. This could be a time-demanding task, as the review would be virtually rewritten in many of its parts. - Many concepts are developed here based on old models. For example, the authors state that "Transplantation of microbiota from genetically obese to germ-free mice caused a significant weight increase compared with germ-free mice that were transplanted with microbiota from thin mice[19]." However, the authors completely avoid informing the reader that fecal transplantation was applied in numerous studies targeting obesity in humans. Many other such examples exist through the manuscript.

Question 1: - Language check is necessary, there is a typo already in the manuscript title.

Answer:

Thank you for your careful examination of the manuscript. We have corrected the typo

in the title.

Question 2: - There are still unnecessary parts of the text left. These include, for example: "According to Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes in 2020, there are three main types of DM, including type 1 DM (T1DM), type 2 DM (T2DM) and gestational DM (GDM). However, there are some other types of diabetes, such as autoimmune latent diabetes in adults (LADA), pancreatic diabetes, and maturity onset diabetes of the young (MODY)1–3[3]." - these textbook-like facts can easily be deleted and the manuscript trimmed accordingly.

Answer:

Thank you for your remaindering. We have deleted the sentence and modified it.

Question 3: - Many of the relevant recent references are uncited. These include, for example, Acta Diabetol. 2021 May 10. doi: 10.1007/s00592-021-01727-5. Diabetes Metab Syndr Obes. 2021 Apr 28;14:1855-1869. Clin Transl Med. 2021 Apr;11(4):e326. PeerJ. 2021 Apr 1;9:e11128. Gut. 2021 Mar 30:gutjnl-2020-323617. and many others.

Answer:

Thank you for your suggestion. Because the searchable date of publication of these new references are concentrated after March. Our submission date is Jan 28 2021. Therefore these new references were not cited in our manuscript. We have updated some references (Ref 29, 113).

Question 4: - There is no method disclosed concerning how the reviewed papers were selected. However, given that the recent papers are nearly completely absent, it is necessary to check the major abstract databases for relevant papers, read through the full-texts and extract relevant information into this review. This could be a time-demanding task, as the review would be virtually rewritten in many of its parts.

Answer:

Thank you for your question. We have disclosed how the reviewed papers were selected

at the end of the INTRODUCTION part. Because the manuscript, we submitted, belong to descriptive review paper, rather than a systematic review paper. There are no strict criteria for including or excluding references. The only purpose of each citation is to be as complete and comprehensive as possible.

Question 5: Many concepts are developed here based on old models. For example, the authors state that "Transplantation of microbiota from genetically obese to germ-free mice caused a significant weight increase compared with germ-free mice that were transplanted with microbiota from thin mice[19]." However, the authors completely avoid informing the reader that fecal transplantation was applied in numerous studies targeting obesity in humans. Many other such examples exist through the manuscript.

Answer:

Thank you for your question. We have modified it. As far as I know, although Transplantation of microbiota (FMT) have been carried out in a number of hospitals of the world. But more or less present as the characteristic of a new technology, an exploratory experimental. It is far from being a routine technique in clinical practice. There are still many challenges need to be solved in clinical application of FMT. For example: how we ensure that the transplanted flora is from an absolutely healthy individual? Therefore, finding the characteristics of human flora by establishing the database of a large sample of various diseases and healthy human might be an effective measure. Moreover, the provenance and preservation of the donor flora is also a major challenge. At last, the acceptance level of subjects who will treat with FMT is also a big barrier for its application in clinical practice.