March 27, 2018

Dear Editor and Reviewers:

Please find enclosed the edited manuscript in Word format (file name: 38560-Revised Manuscript.docx).

**Title:** Dynamic alterations in the gut microbiota and metabolome during the development of methionine-choline-deficient diet-induced nonalcoholic steatohepatitis

**Author:** Jian-Zhong Ye, Ya-Ting Li, Wen-Rui Wu, Ding Shi, Dai-Qiong Fang, Li-Ya Yang, Xiao-Yuan Bian, Jing-Jing Wu, Qing Wang, Xian-Wan Jiang, Cong-Gao Peng, Wan-Chun Ye, Peng-Cheng Xia, Lan-Juan Li

**Name of Journal:** World Journal of Gastroenterology

**ESPS Manuscript NO:** 38560

Thank you for your letter and for the reviewers' comments concerning our manuscript. We sincerely appreciate the time and effort you have spent in reviewing our manuscript as well as the opportunity to revise our manuscript. The concerns of the reviewers and their suggestions for improving the manuscript have been carefully studied and addressed. Revised portions of the text are highlighted in yellow in the paper. Below, we provide a point-by-point responses to the comments that we hope will meet with approval.

We are looking forward to your response.

Sincerely,
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**Response to editorial comments:**

1. All of the suggested editorial changes have been made.
2. The requested audio core tip has been uploaded.
3. "ARTICLE HIGHLIGHTS" section has been included.
4. Figure 1, 2, 4, 5 and Table 1 have been modified as the guidelines.
5. Figures without label have been uploaded.
6. Corrigendum: the Science Fund for Creative Research Groups of the National Natural Science Foundation of China has been provided the wrong grant number, and has been corrected in the revised manuscript.
7. Our colleague, Cong-Gao Peng, has conducted a critical revision of the manuscript for important intellectual content, so we would just like to have him added as one of

the coauthor, the copyright agreement has been resubmitted. Please kindly consider our case, thank you very much.

Reviewer #1 (Reviewer's code: 00503536):

The manuscript written by Jian-Zhong Ye et al. describes the dynamic changes in the gut microbes during the development of NASH induced by methionine-choline-deficient diet. The mice fed with methionine-choline-deficient diets develop simple hepatic steatosis after 2 weeks and progress to NASH after 4 weeks, and gut microbiota and metabolome have been changed dynamically during that course. The data suggest that the alteration may be associated with the development of NASH. The data are important and may give new insight on the pathogenesis of NASH. However, there are some concerns that need to be addressed. Major point Even if there are some dynamic changes in the composition of gut microbiota, it is not necessarily mean that the change may contribute the development of NASH. In other words, methionine-deficient diet might induce NASH with no association with changes in gut microbiota. In order to clarify the direct evidence for the contribution, the authors should show that the gut microbiota obtained from methionine-choline-deficient diet at different time points causes the same fatty changes in liver histology if administered to control mice. Alternatively, gut microbiota of NASH mice induced by other methods should be similarly analyzed, and confirm the similar changes in the composition of gut microbiota occur during the development of NASH. The authors should, at least, discuss on that point. 2. The same approach or strategy as #1 should be considered in the metabolites produced by gut microbiota.

Classification: Grade B (Very good)
Language Evaluation: Grade B: minor language polishing
Conclusion: Minor revision

**Response:** We thank the reviewer for his/her important comments and valuable work. Indeed, lack of direct evidences to confirm that the changes in the gut microbiota and metabolites contribute to NASH development is a weakness of our manuscript. The reviewer's suggestion has been discussed in the revised manuscript as "Although our study found an association between altered gut microbiota and metabolism and NASH, a causative contribution of the gut microbiota and metabolism to NASH progression has not been sufficiently documented." ("DISCUSSION" section). Our main purpose in this study was to evaluate dynamic changes in the gut microbiota and metabolism during the progression from simple hepatic steatosis to NASH; the contributing role of the gut microbiota and metabolism in NASH development will be studied in our further work. To avoid over-interpretation, we have deleted the speculative remark "…, which may contribute to NASH progression." throughout the manuscript. However, recent studies may provide some insight: Chiu CC *et al*[1] found that nonalcoholic fatty liver disease is exacerbated in high-fat diet (HFD)-fed germ-free mice by colonization with the gut microbiota from patients with nonalcoholic steatohepatitis. Differences in microbiota composition can determine responses to an HFD in mice[2]. Modification of the microbiota

by treatment of HFD-fed mice with tempol or antibiotics resulted in decreased adverse metabolic phenotypes in nonalcoholic fatty liver disease[3]. These results demonstrate that the gut microbiota contributes to the development of NAFLD independently.

Reviewer #2 (Reviewer's code: 03023823):

The manuscript "Dynamic alteration in the gut microbiota and metabolome during the development of methionine-choline-deficient diet-induced non-alcoholic steatohepatitis" reports the results of an experimental intervention study. Its content is overall interesting and timely. Scope is clearly defined: The purpose of the present study was to investigate the dynamic alterations in the gut microbiota and metabolome during the development of MCD diet-induced NASH. The rationale and the unmet need are not sufficiently justified: "The progression of liver injury in methionine-choline-deficient (MCD) diet-induced rats has been well-characterized[. However, a detailed knowledge of the changes in the gut microbiota and metabolome that occur during NASH development in mouse models is still unknown." Well, where is the rationale? Please, justify links, if any, between the two facets – liver and gut – of your experiment. There is anything that we should accept as a reason for this deprivation model as a mirror of human NASH? There is any analogy of the gut microbiome of rats with the spectrum of human microbiomes? The main limitation is the conclusion toward which all the manuscript runs: conclusion, the MCD diet induced gut microbiota and metabolome deterioration, which may contribute to NASH progression. Even for a conjecture, we need new information using appropriate methods and criteria of rat population selection. The manuscript is written clearly using Standard English English, spelling and syntax of a few sentences need minor reappraisal, and a further professional review could improve style. Overall, the manuscript seems more suitable for a short communication than to a full-length article; this because, among the limitations, several information are not provided. The manuscript should become technically more sound before any further revision.

Classification: Grade D (Fair)
Language Evaluation: Grade B: minor language polishing
Conclusion: Major revision

**Response:** We are grateful for the reviewer's constructive comments regarding our study.

1. We modified the logic regarding the rationale and the unmet need as follows "Despite the importance of the gut microbiota and metabolome in NASH, detailed information regarding changes that occur during NASH development is limited. The purpose of the present study was to investigate dynamic alterations in the gut microbiota and metabolome during the development of methionine-choline-deficient (MCD) diet-induced NASH in a mouse model."
2. The strong anatomical and functional interaction between the gastrointestinal tract and the liver defines the term gut-liver axis, which plays a pivotal role in NAFLD pathogenesis and progression[4]. Under conditions of intestinal dysbiosis and intestinal barrier deterioration, increased influx of harmful substances, such as

lipopolysaccharide (LPS), ethanol, and bacterial DNA, into the liver through the portal vein circulation interrupts immunological tolerance in the liver and promotes inflammation via toll-like receptor (TLR) stimulation, thus constituting the “multiple-hit” hypothesis explaining NAFLD pathogenesis and progression. Consistently, gut microbiota dysbiosis and intestinal barrier impairment were observed in our study, which may contribute to the steatohepatitis and F4/80+ cell infiltration. We added the term gut-liver axis in the revised manuscript (“INTRODUCTION” section, “This intimate connection between the gastrointestinal tract and liver defines the term gut-liver axis.”).

3. The MCD model is one of the best defined models of NASH and is widely used to study NASH[5]. Depending on the time of feeding, the MCD model induces hepatic steatosis, steatohepatitis or fibrosing steatohepatitis and thus simulates the reproducible histological picture of NAFLD with significant inflammation and fibrogenesis.
4. The reviewer may question the similarities between the gut microbiome of **mice** and the spectrum of human microbiomes. The overall phylum-level composition of the mouse gut microbiome was similar to that of the human gut microbiome with Firmicutes, Bacteroidetes and Proteobacteria accounting for more than 70% of the gut microbiota[6-8]. At the genus level, thirteen of the 20 most abundant core genera in mice were also present among the top 20 core genera in humans, highlighting the compositional similarities at higher taxonomic levels. Furthermore, the mouse gut microbiome is functionally similar to its human counterpart, sharing 95.2% of their Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologous groups[8]. In our study, the significantly increased *Enterobacteriaceae*, and significantly decreased *Lachnospiraceae* and *Bifidobacterium* of *Bifidobacteriaceae* in the MCD diet-induced NASH mouse model were also consistently observed in humans with NASH [9]. Therefore, we have reason to believe that studies of the mouse gut microbiome can be used as a model for the human gut microbiome.
5. Lack of direct evidence to confirm that the changes in the gut microbiota and metabolites contributed to NASH development is indeed a weakness of our study, which is discussed in the revised manuscript. Our main purpose in this study was to evaluate the dynamic changes in the gut microbiota and metabolism during the progression from simple hepatic steatosis to NASH; the contributing role of the gut microbiota and metabolism will be studied in our further work. To avoid over-interpretation, we have deleted the speculative remark “…, which may contribute to NASH progression.” throughout the manuscript.
6. We carefully reviewed the entire manuscript to correct the grammatical and language usage errors, and the revised manuscript was further reviewed and polished by American Journal Experts.

Reviewer #3 (Reviewer’s code: 02631746):
Well done

Classification: Grade B (Very good)
Language Evaluation: Grade A: priority publishing
Conclusion: Accept

**Response:** We thank the reviewer for his/her kind comments. As the reviewer has not suggested any revision, we have not highlighted any changes in the revised manuscript.

## REFERENCES


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