

June 25, 2014

Dear Editor,

We appreciate you for the consideration and the reviewers for their helpful comments. We have carefully considered all the points, and the manuscript has been revised accordingly.

Please find the edited manuscript in Word format (file name: 11495\_revised.docx).

**Title:** Alterations of the gut microbiome and metabolome in alcoholic liver disease

**Author:** Wei Zhong, Zhanxiang Zhou

**Name of Journal:** World Journal of Gastrointestinal Pathophysiology

**ESPS Manuscript No:** 11495

The manuscript has been improved according to the requirements of the journal and the suggestions of reviewers:

1 The title has been abbreviated, a running title and a core tip have been added, and the format of references has been corrected according to the journal requirements.

2 The language has been polished.

3 Figure 1 has been modified. A table (Table 1) has been added.

4 Revisions also have been made according to the suggestions of the reviewers.

#### **Reviewer 1**

(1) In the abstract “These adverse effects ultimately manifest a broad change of gastrointestinal luminal metabolites such as bile acids, short chain fatty acids, and branched chain amino acids.” Need to be edited. It is difficult to understand.

**Response:** We appreciate your evaluation of this work and the comments. The sentence has been changed to “These adverse effects caused by alcohol may ultimately result in a broad change of gastrointestinal luminal metabolites such as bile acids, short chain fatty acids, and branched chain amino acids.” in the revised manuscript.

(2) Why does metronidazole increase anaerobes (reference 19)?

**Response:** Thank you for pointing out the typographical error. It has been changed to “Oral administration of metronidazole, an antibiotic drug, led to high level of intracolonic acetaldehyde by increasing aerobic bacteria and reducing anaerobic bacteria in the intestine.” in the revised manuscript.

(3) “In our study, we found that the hepatic bile salt taurine to glycine ratio was 30:1 in control rats, while the ratio was equivalent in alcohol-treated rats.” What “equivalent” means here?

**Response:** We intended to explain the levels of hepatic taurine-conjugated and glycine-conjugated bile acids were comparable in alcohol-treated rats. The sentence has been changed to “...while the ratio was 1:1 in alcohol-treated rats” in the revised manuscript.

(4) “The possible links between the host, gut microbiota, and gut metabolome will be addressed as well.” Do the authors mean in this review? This should be reworded.

**Response:** The sentence has been deleted to avoid ambiguity.

(5) Wherever the results of reference 68 & 69 is reported, those studies should be cited.

**Response:** Citations have been added in the revised manuscript according to the reviewer’s suggestion.

(6) Based on metabolomics, was there any change in choline and its metabolites (TMA and TMAO)?

**Response:** During the study, first of all, we used HPLC-TOF MS for global metabolite profiling. At that stage, no specific metabolites were explored. Then we used GC-MS and UPLC-TQMS to quantitatively measure short chain fatty acids and branched chain amino acids, and bile acids respectively. As for choline and choline metabolites, we did not detect TMA or TMAO within the GI tract but only choline and acetylcholine. Alcohol exposure significantly and gradually reduced acetylcholine along the intestine (from 0.84 fold of pair-fed in the duodenum to 0.29 fold of pair-fed in the rectum,  $P < 0.05$ ), while no statistical difference of choline were found in the GI tract contents of the alcohol-fed rats compared to the pair-fed rats. An elevated level of TMAO was observed in the serum of alcohol-fed rats (2.54 fold vs. pair-fed,  $P = 0.004$ ) (The data was published on *Am J Physiol Gastrointest Liver Physiol.* 2013; 305(12): G919-932).

## Reviewer 2

(1) Maybe the authors can provide some further details on the approach they have used.

**Response:** The comment is greatly appreciated. More details of the method used in the metabolomics study as well as necessary references have been added in the revised manuscript.

(2) A table to summarize recent studies that have looked into this area is much appreciated.

**Response:** A table summarizing studies exploring the protective role of probiotics and prebiotics has been added to the revised manuscript according to the reviewer’s suggestion.

(3) Rather than a brief mention in the conclusion, I would suggest that authors have another section that describe the potential application of metabolic profiling.

**Response:** The reviewer’s suggestion is highly appreciated. Unfortunately, studies on exploring alcohol consumption-induced gut metabolite alterations are very limited and the results are still preliminary. Stating the potential application of metabolic profiling would be more appropriated until more data, especially those from clinical studies, could be generated.

Thank you again for the reviewers’ comments and your kind consideration.

Sincerely,

A handwritten signature in cursive script, appearing to read "Zhanxiang Zhou".

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