Dear Professor Ma:

Manuscript NO: 58973

Title: Altered metabolism of bile acids correlates with clinical parameters and the gut microbiota in patients with diarrhea-predominant irritable bowel syndrome

Thank you for your letter and the reviewer's comments concerning our manuscript. These comments are all valuable and very helpful for revising and improving our paper. All the authors have studied the comments carefully and revised the manuscript based on them. According to the suggestions of the science editor, we have added more details in the METHOD section. We also carefully proof-read the language, data, and references in our manuscript. Revised portions are marked in yellow in the revised version of the manuscript.

All the best.

Yours Sincerely,

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Answering Reviewer 02531171

Thank you very much for your comments and suggestions, which are very valuable for us to improve the interpretation of the scientific question in our present study. We have made revisions or explanation point by point.

1. Could the authors postulate as to why more bile acids are spilling over into the colons in IBS-D patients (rather than being recycled in the EHC?)

   Answer: Thank you for pointing out this important problem we neglected to illustrate in our manuscript. On the one hand, there is evidence showing that the synthesis of bile acids in the liver of IBS-D patients may be up-regulated (the detailed discussion of this question is in the answer to question 3 below). On the other hand, whilst the increase of primary bile acids can shorten colonic transit time, bile acid is not the only factor influencing colonic motility in IBS, and the colonic motility can influence luminal bile acids conversely[1, 2]. It has been demonstrated in healthy volunteers that the accelerated transit can lead to more bile acids in the stool[3], which may result from reduced passive absorption of bile acids across the colon epithelium. The discussion on this question has been added in the 2nd paragraph on P 18, highlighted in yellow, and we have replaced the speculative statement of causality in the conclusion that BAs dysmetabolism may promote diarrhea with a description of the correlation.

2. Can the authors elaborate on the impact of BAs (and specific subtypes of bile acids) on microbes and how likely it is that changes in BA spillover is contributing to changes in microbial profiles?

   Answer: Thank you very much for reminding us of this very important issue. It has been demonstrated that bile acids possess antibacterial activity. In vitro studies showed that hydrophobic bile acids (taurodeoxycholic acid and deoxycholic acid) had more significant inhibition on the growth of *Escherichia coli* and *Enterococcus fecalis* when compared with the hydrophilic bile acids (taurocholic acid,

chenodeoxycholic acid, and tauroursodeoxycholic acid)[4], and unconjugated bile acids display more potent antibacterial action on *Staphylococcus aureus* than conjugated bile acids[5]. The discussion on this question has been added in the 3rd paragraph on P 20, highlighted in yellow.

3. Is BA synthesis modified in IBS-D patients?

Answer: Several studies reported that serum 7α-hydroxy-4-cholesten-3-one (C4) was increased in at least a part of IBS-D patients [6-9], which is the precursor of primary BAs and the marker of BAs synthesis rate [10], indicating that the synthesis of BAs may increase in IBS-D patients. It has been interpreted in the 2nd paragraph on P 17, highlighted in yellow. However, no researchers have reported that the synthesis pathway of BAs in IBS-D patients differs from that in healthy populations, and we did not find literature relevant to what may cause the reinforcement BAs synthesis in IBS-D patients.

References:

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