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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 47797

Title: Trimethylamine N-oxide attenuates high-fat high-cholesterol diet-induced steatohepatitis by reducing hepatic cholesterol overload in rats

Reviewer's code: 03536216

Reviewer's country: Japan

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-03-28 08:17

Reviewer performed review: 2019-03-28 10:01

Review time: 1 Hour

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept (High priority)	Peer-Review:
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Accept (General priority)	[Y] Anonymous
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Minor revision	[ ] Onymous
[ ] Grade D: Fair	[ ] Grade D: Rejection	[Y] Major revision	Peer-reviewer's expertise on the topic of the manuscript:
[ ] Grade E: Do not publish		[ ] Rejection	[ ] Advanced
			[Y] General
			[ ] No expertise
			Conflicts-of-Interest:
			[ ] Yes
			[Y] No

SPECIFIC COMMENTS TO AUTHORS

In general, this manuscript provides the useful information about TMAO modulate steatohepatitis in rats fed high-fat and high-cholesterol diet. However, there are some problems and flaws in presentation. Specifically, the discussion in this MS is

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inadequate. I hope that my comments are very useful for the improvement of this research. (1) The authors should review the statistics. For this grouping, I think a two-way ANOVA is better. (2) It is necessary to consider whether the various effects obtained in this experiment are the effects of TMAO or TMA, that is metabolized from TMAO. (3) Authors showed that TMAO alter the gut microbial profile and restore the diversity of gut flora. But the relationship between the change of gut flora and NASH is not known in the current consideration. Please specifically discuss the relationship between this change in gut flora and NASH. (4)It would be better to measure the hepatic cholesterol level. (5) Authors should measure the fecal cholesterol content. In this study, the intake of TMAO decreased the NPC1L1 and increased the ABCG5/8 levels in intestinal mucosa. These data do not indicate whether cholesterol absorption is inhibited. Cholesterol is absorbed not only by the route via NPC1L1 but also by passive transport in intestine. (6) Authors should describe the conditions of dissection including dissection time, anesthetic, etc. (7) There is no discussion as to whether hepatitis is induced by feeding HFHC diet. Authors should consider this point. I hope that my comments are very useful for the improvement of this manuscript.

INITIAL REVIEW OF THE MANUSCRIPT

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[ ] Duplicate publication

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[Y] No

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 47797

Title: Trimethylamine N-oxide attenuates high-fat high-cholesterol diet-induced steatohepatitis by reducing hepatic cholesterol overload in rats

Reviewer's code: 00241774

Reviewer's country: Japan

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-03-28 09:32

Reviewer performed review: 2019-03-29 00:43

Review time: 15 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection	Peer-Review: [Y] Anonymous [ ] Onymous Peer-reviewer's expertise on the topic of the manuscript: [ ] Advanced [Y] General [ ] No expertise Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Zhao Zh et al. investigated the effects of TMAO on the HFHC diet-induced steatohepatitis in rats. TMAO alleviated inflammation and hepatocyte ballooning, reduced ALT and AST, and decreased ER stress markers. Hepatic and serum cholesterol

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levels were reduced and the expression of NPC1L1 was reduced. TMAO altered the gun microbial profile and restored the diversity of gut flora. Major comments 1. The cholesterol markers, lathosterol and desmosterol, and cholesterol absorption markers, β-sitosterol and campesterol, should be measured to determine whether major target of TMAO is absorption of cholesterol. 2. Since the diversity changes in gut flora may induce the changes in short fatty acids in intestine, the authors should investigate the profile of fatty acids in the feces. 3. Transplantation of feces from TMAO-treated animals ameliorate the steatohepatitis in rats? 4. The bile acids profiling by MS/MS analysis should be performed.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 47797

Title: Trimethylamine N-oxide attenuates high-fat high-cholesterol diet-induced steatohepatitis by reducing hepatic cholesterol overload in rats

Reviewer's code: 00506276

Reviewer's country: Poland

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-03-28 11:56

Reviewer performed review: 2019-04-07 22:10

Review time: 10 Days and 10 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection	Peer-Review: [Y] Anonymous [ ] Onymous Peer-reviewer's expertise on the topic of the manuscript: [Y] Advanced [ ] General [ ] No expertise Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In the present study authors have examined the effect of trimethylamine oxide (TMAO) administered orally for 8 weeks on the progression of high fat diet-induced non-alcoholic fatti liver disease. The results indicate that TMAO improved liver

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histology, reduced plasma transaminase activities, decreased hepatocyte ER stress and hepatocyte apoptosis, decreased the expression of cholesterol-absorbing protein, NPC1L1, and increased the expression of intestinal cholesterol exporters, ABCG5 and ABCG8. These effects correlate with the improvement of plasma lipid profile but are achieved without reduction of body weight or adiposity scores. Finally, TMAO restored diversity of intestinal flora which was restricted in high fat diet-fed rats. The results are of interest and the paper is well-written. Nevertheless, there are some concerns regarding experimental design and data interpretation. 1) The implications of the findings are unclear. TMA is well-known to be involved in the pathogenesis of cardiovascular diseases which often accompany NAFLD in patients with obesity/metabolic syndrome. Therefore, recommending TMAO therapy would be highly questionable. 2) Why this specific dose of TMAO was used? 3) It would be of interest to measure TMA and TMAO levels in animals with NAFLD vs. control group as well as in TMAO-treated rats. 4) It would be of interest to compare the effect of TMAO with its precursor, TMA. 5) What buffer was used for sample homogenization/lysis for Western blot? Were any protease inhibitors added during sample processing? 6) More details about qRT-PCR should be presented according to MIQUE guidelines. Data such as primer sequence, amplification cycle conditions (duration and temperatures of consecutive phases), methods of assessing mRNA quality-quantity and results calculation should be included. 7) The rate of apoptosis was estimated according to cleaved caspase-3 only. JNK phosphorylation may be but is not always associated with apoptosis; the role of this kinase in apoptosis depends on the experimental system. Overall, the experimental approach regarding apoptosis used in this study is insufficient. 8) Page 10: serum level of triglycerides was NOT altered by TMAO; the text needs revision. 9) Why the expression of intestinal cholesterol transporters was measured only at the mRNA but not at the protein level? 10) It would be of interest to discuss how

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much of the effect of TMAO is exerted directly in the liver and how much results from the improvement of plasma lipids, insulin sensitivity, etc.

INITIAL REVIEW OF THE MANUSCRIPT

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