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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 13763
Title: Ezetimibe improves hepatic steatosis in relation to autophagy in obese and diabetic rats
Reviewer code: 02539944
Science editor: Su-Xin Gou
Date sent for review: 2014-09-01 11:23
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[Y] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

The manuscript by Chang et al. describes the effect of Ezetimibe on hepatic steatosis in a rat model of obesity and type II diabetes. Major conclusion of the manuscript is the induction of autophagy in the liver by application of Ezetimibe and, therefore, a reduction of hepatic steatosis. The idea of Ezetimibe as inducer of autophagy in the liver/hepatocytes is in line with a previous report by a different group earlier this year. There are several points that need to be addressed. Major points: 1. Figure 2A: The authors need to show that the expression of the housekeeping gene is not changed. With a rather mild elevation of the 3 investigated genes, small changes in housekeeping gene expression could have a major impact. 2. The reason, why these 3 ATG genes were studied and no other autophagy-related genes remains unclear. 3. LC3 is not a simple marker of autophagosome formation. It can also indicate a block of autolysosome formation. Therefore, it cannot be used as sole indicator of autophagy induction. The authors calculate the ratio of LC3-II and LC3-I, however the Western Blot in Figure 2B has a poor quality. Statistical analysis based on n=2 are not allowed. 4. Besides increasing the number of tested rats, the authors need to consider at least 1 additional method to convincingly show the induction of autophagy by Ezetimibe in vivo. 5. How does the suggested effect look like, when only LC3-II is analyzed by Western Blot rather than calculating the ratio of LC3-II/I? 6. In Figure 3C there is no obvious change in LC3-II levels among the groups. Instead the authors again calculate the ratio of LC3-II/I. Looking at the band intensities, the ratio of

LC3-II/ in controls (“Con”) should be higher than calculated by the authors, since the band of LC3-I is weaker compared to, e.g. “Ez”. It appears, there is a mistake in the quantification. 7. It is a far-fetched interpretation when the authors call the application of PA in cell culture as “induction of hepatic steatosis”. 8. For autophagic flux experiments the degradation of p62 should be investigated. Minor points: 1. What was the vehicle to apply Ezetimibe in rats? 2. In Table 2 the liver tissue weight is measured in “%”? Footnotes are lacking to explain superscript letters. 3. The letters in Figure 3A are not explained.

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[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

1. First, the significant deficiency for this manuscript is sample size. The authors do not introduce the theory-guided, especially for the LETO control group (just 3 rats). Obviously, the conclusion is quite unpersuasive. Additional experiments should be conducted to confirm that difference between two groups is statistically significant but not sampling error. It cannot be accepted before more data is added. 2. In Discussion section, deeper discussion should be added. The author just give a quick glance. Many factors is not clearly illustrated. Such as how does autophagy regulate hepatocyte lipid metabolism and hepatocellular injury and death? There are potential beneficial effects of a therapeutic increase in hepatocyte autophagic function: 1) Decrease triglyceride and cholesterol accumulation. 2) Improve insulin signaling. 3) Prevent cellular injury from oxidative stress. 4) Block TNF and Fas death receptor-mediated liver injury. 5) Reduce endoplasmic reticulum stress and the resultant cellular damage and insulin resistance. 6) Prevent hepatocellular carcinoma development. In Table 2 some difference such as insulin resistance are mentioned. But the author do not explain this difference in the discussion. 3. The authors want to prove that Ezetimibe improves hepatic steatosis in relation to autophagy in obese and diabetic rats. Some fundamental serum index such as LDL-C and HDL-C is not shown. 4. In Table 2, superscript letters a, b, and c is not clear, and they should be noted under the Table. 5. Four Figure legends are so long, and they should be compressed. 6. Why the author just select male rats? Please explain this.