Reviewer's report

Title: Deficiency of Endothelial Nitric Oxide Synthase Exacerbates Early-stage Non-Alcoholic Fatty Liver Disease Pathogenesis by Changing the Fat Distribution

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Reviewer: Fanyin Meng

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Minor Essential Revisions

In the current manuscript submitted by Yuichi Nozaki et al. the authors aimed to investigate the role of eNOS-derived NO in NAFLD pathogenesis using systemic eNOS-knockout mice fed a high-fat diet. Lipid accumulation and inflammation was more extensive in the liver and lipid accumulation was less extensive in the visceral fat tissue in eNOS-knockout mice, compared with wild-type mice, after 12 weeks of being fed a high-fat diet. While systemic insulin resistance was comparable between the eNOS-knockout and wild-type mice fed a high-fat diet, hepatic tissue blood flow was significantly suppressed in the eNOS-knockout mice, compared with the wild-type mice, in mice fed a high-fat diet. The microsomal triglyceride transfer protein activity was down-regulated in eNOS-knockout mice, compared with wild-type mice, in mice fed a high-fat diet. The overall a deficiency of eNOS-derived NO may change the fat distribution of liver and visceral fat, thereby promoting the progression of disease in an HFD-induced, early-stage NASH mouse model by changing the hepatic tissue blood flow. The current study also examined the fat distribution and the pathogenesis of NAFLD/NASH using an imaging procedure in an NAFLD/NASH mouse model with or without the eNOS gene. It is an interesting manuscript with the innovative concept. The overall findings may provide translational potential to address specific clinical issues related to the pathogenesis of NAFLD/NASH. However, some minor issues should be fixed before further consideration.

Comments:

1) The recent data from Sheldon RD et al (Am J Physiol Gastrointest Liver Physiol. 2015 Mar 15;308(6):G540-9) have demonstrated that systemic NOS inhibition in the obese OLETF rats reduced hepatic mitochondrial respiration, increased hepatic triacylglycerol accumulation, and increased hepatic inflammation. The current studies only have very limited data on hepatic tissue blood flow which may too superficial. Therefore some hepatic mitochondrial respiration markers and hepatic inflammation statues should be verified in the current project to further support the central hypothesis.

2) For hepatic inflammation related studies, neutrophil infiltration detection should be carried out in the liver from eNOS-knockout mice fed a high-fat diet. The presence of neutrophils should be assessed by the measurement of liver tissue
myeloperoxidase (MPO), and the degree of neutrophil liver infiltration should be determined by the naphthol AS-D chloroacetate esterase technique

3) Serum ALT and AST values should be included in Table 2.

4) The fibrosis stage in Table 3 should be verified by Sirius red staining.

5) The relationship between the changes of hepatic blood flow and the degrees of liver injury should be further established.

6) To be consistent with Fig. 2 C&D, Fig. 3 also should include four groups instead of two groups.

7) In “List of abbreviations”, the font and size should be consistent.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I have no conflict of interest.