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: Syeda Afroze

Reviewer's report:

In this manuscript entitled “Deficiency of Endothelial Nitric Oxide Synthase Exacerbates Early-stage Non-Alcoholic Fatty Liver Disease Pathogenesis by Changing the Fat Distribution” submitted by Nozaki et al, the authors aimed to demonstrate the role of endothelial nitric oxide synthase (eNOS) derived nitric oxide (NO) in the pathogenesis od NAFLD / NASH. There are several factors or molecules are responsible for the pathogenesis of non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH), but the role of eNOS derived NO in the pathogenesis of NAFLD / NASH is not yet well defined. Therefore to investigate their hypothesis authors performed experiments in systemic eNOS-knockout mice with high-fat diet. Four groups of mice were used in this study. Those mice were (a) wild type eNOS+/+ with basal diet (BD), (b) wild type eNOS-/- with basal diet (BD), (c) wild type eNOS+/+ with High fed Diet (HFD), (d) wild type eNOS-/- with High fed Diet (HFD). To explore this study the authors performed measurement of different biochemical markers in plasma and serum, liver triglyceride content, liver/spleen ratio of CT values and visceral fat volume as well. The authors determined the insulin tolerance test (ITT), liver MTP activity assay and hepatic tissue blood flow. CT scan has also been performed. The authors conducted several experiments as well, like as histopathological study on liver and immunohistochemical study as well. The authors also performed qPCR for evaluating at these genes expressions (SREBP-1c, PPAR-#, nNOS, iNOS). It is an interesting study. As a whole the expression of this manuscript is well described. So before going for final submission of this manuscript, it is recommended to take care of this manuscript in terms of the following minor revision. Minor concerns are mentioned below:

Minor Concerns:
1. The manuscript should be carefully checked and need to follow the manuscript format criteria for this journal.
2. It is good to revise by Scientific English editor.
3. The Title could be more firm such as “Deficiency of eNOS Exacerbates Early-stage NAFLD Pathogenesis by Changing the Fat Distribution”
4. Materials and Methods section need to be organized in order of experiment, for an example all the measurements need to be one after another then all the assays and then histological study and then qPCR (gene expression study).
5. Figure 4-1 is not clear enough, because all the lines for different groups are
overlapped. Even though the error bar as well. So it is good to use different color line for each group. So the figure should be clearly visible. Or the figure could be represented by bar graph either way.