Reviewer’s report

Title: Non Alcoholic Fatty Liver disease and eNOS dysfunction in humans

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Reviewer: Yasuo Terauchi

Reviewer's report:

Although eNOS derangement has been demonstrated in experimental models, no studies have directly shown that eNOS dysfunction is associated with NAFLD in humans. The aim of this study was to investigate eNOS function in NAFLD patients.

Fifty four NAFLD patients underwent clinical and laboratory evaluation and liver biopsy. Patients were divided into two groups by the presence of NAFL or NASH. Vascular reactivity demonstrated a reduced function induced from NAFLD platelets as compared to controls, associated with an impaired p-eNOS in both in platelets and in liver. NAFL showed a higher impairment of eNOS phosphorylation as compared to NASH. In contrast to what observed in vitro, the vascular response by FMD was worse in NASH as compared to NAFL.

The authors concluded that NAFLD patients showed a marked eNOS dysfunction, which may contribute to a higher CV risk and that eNOS dysfunction observed in platelets and liver tissue didn't match with FMD.

Criticisms

1. Simple steatosis (NAFL), which represents the early stage of the disease, seems to be associated to a worse eNOS impairment compared to steatohepatitis (NASH). On the other hand, clinical evaluation of endothelial dysfunction, measured via FMD, has shown to be worse in NASH than NAFL patients. Although the authors speculated the reasons for the discrepancy in Discussion section, it seems to this reviewer that the authors should rather focus on the impacts of their findings on the higher risk of cardiovascular disease in NASH subjects.

2. No statistically significant differences were found between the NAFL group and NASH group for age, sex, BMI, ALT, prevalence of hypertension, diabetes, dyslipidemia, obesity and metabolic syndrome (Table 1). However, there are marked differences were found between the control group and all NAFLD group for the prevalence of hypertension, diabetes, and dyslipidemia. The differences raise a concern that the results obtained from this control group is really adequate for the comparison with the NAFLD group. This reviewer asks the authors to comment on this issue.
3. Six cirrhosis patients are included in the NASH group. This reviewer has a concern that far advanced liver damage cirrhosis has a role on the clinical evaluation of endothelial dysfunction. Therefore, I would like to know the results of NASH subjects excluding cirrhosis (n = 33-6 = 27).

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

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I am able to assess the statistics

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