Author’s response to reviews

Title: A New Method to Induce Nonalcoholic Steatohepatitis (NASH) in Mice

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Author’s response to reviews:

1. Statistically significant results should be described and commented. Non-significant results mean the number is probably to low to highlight a difference, or there is no difference at all. A trend is not informative.

Re: corrected.

2. The paragraph "effect of different diets feeding on liver enzymes" is actually only focusing on transaminases. Such enzymes are an indirect proof of an hepatic damage/necrosis. Fat diet and cigarette smoke are well-know to effect several additional parameters. Functional enzymes such as cytochrome P450 (i.e. 1A2 forms) should be investigated and evaluated. The inducible effect on metabolic enzyme by the diets described by the authors are not leading to any conclusion or relevant observations. Analysis on hepatic parameters, including CYP450 enzymes and phase II metabolism as well as urea cycle enzymatic activity are highly encouraged.

Re: In one hand, according to clinical evidences, the most important biomarker to figure out and diagnosis the liver dysfunction (NAFLD and NASH) is evaluating ALT, and AST. Reviewing the references and guidelines in clinic strongly show that elevation of these enzymes is a strong proof for liver dysfunction.

In the other hand, as the respected reviewers know well, this study submitted over 10 months ago to this journal that implies our survey was finished at least 1 year ago. Thus, how can we perform any new experiment? Definitely, determining the suggested parameters by reviewers (CYP450...
enzymes and phase II metabolism as well as urea cycle enzymatic activity) will give a better insight of the disease induction but performing any experiment is impossible because of finishing the study one year ago.

Notably, we analyzed serum total bilirubin level that could be considered as a marker index of hepatotoxicity especially in conjunction with transaminase elevation¹.


2. histopathological evaluation has been largely described in literature for NASH/NAFLD models. Without any additional biomolecular measurements, there is no way to draw any important and relevant conclusions that has not been described yet.

Re: to our best knowledge, histopathological evaluation is a proof for confirming the induction of NASH/NAFLD which represented the structure of liver. In addition, to evaluate physiological function of this organ, we determined liver functional tests and related biochemical changes. The comments of reviewer is quite suggestion and we added hepatic level of TNF-α as a main proinflammatory cytokine that would confirm histopathological changes.

The authors should consider a short communication in some experimental journal. In particular, due to the fact that the Material and Methods section is so detailed.

Re: Done.

3. Statistical analysis are not clear (how p values are calculated and why SEM instead of SD for a n=8?).

Re: calculation method of p value added in statistical method section. Moreover, the expression of data with SEM exchanged with SD.

Finally, the authors should carefully check and editing the English language.

Re: Done.

Minor issue: all the data should have the same amount of digit after the comma to compare. A graph (histogram with dot plot and mean+SD) could help the reader to understand and the authors to be more organized during the results description.

Re: corrected.
Technical Comments:

1. Please state clearly the role the funder(s) had in your study in the "funding" section of the declarations.

   Re: Done.

2. Please note that the abstract in your manuscript file differs from the title entered in the submission system - please correct so they are consistent with each other.

   Re: Done.