Author's response to reviews

Title: Fast food diet with CCl4 micro-dose induced hepatic-fibrosis - A Novel animal model

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Author's response to reviews:

To,
Editorial Board of BMC Gastroenterology

Dear Editor

Thank you for consideration of our Manuscript entitled “Fast food diet with CCl4 micro-dose induced hepatic-fibrosis – A Novel animal model” (6157822321125312) as an original article in BMC Gastroenterology. Please find the revised manuscript and response to the reviewer’s points

Thank you for the consideration.

Yours sincerely,

Dr. Venkataranganna

Reviewer: Dina Tiniakos

Major compulsory revisions

1. Background, 2nd paragraph: The ALIOS diet (Tetri LH et al, 2008) should be included among the diet modulations producing experimental NASH and compared to the FFD mouse model.

Ans: Reviewer’s suggestion has been considered and the below reference has been incorporated in the revised MS

Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe

2. Methods, Pathology, grading and scoring: The references used do not adequately correspond to the histological methods for grading and staging applied. In the Tables section, Kleiner et al 2005 reference (numbered 31 in this manuscript) is mentioned and this, as well well other more appropriate references, should be included here. When referring to fibrosis the terms "staging" and "stage" is more appropriate than "scoring" and "grade". The method used for assessing fibrosis (Table 1) needs to be referenced. In Table 1, the meaning of the contents in the percentage column is not clear. Does this percentage refer to the extent of fibrosis compared to the whole tissue area? The method of assessing severity of fibrosis needs to be modified according to previously used and widely staging criteria i.e. Kleiner et al, 2005, Brunt et al, 1999. In Table 2, scoring for ballooning is modified and does not correspond to Kleiner et al, 2005. The histological criteria used need to be defined in more detail.

Ans: Reviewer’s suggestion has been considered and one more relevant reference has been incorporated in the revised MS.

As suggested by reviewer, "scoring" and "grade" are replaced with "staging" and "stage" in Table 1.

Table-1: Reference has been included in the revised MS and the numbering has been changed.

Percentage in Table 1 has been modified in the. Fibrosis percentage refers to the extent of fibrosis compared to the total tissue area in 25 microscopic fields in each slide

Table 2, scoring for ballooning is taken form reference - Kawasaki et al…. J Nutr. 2009; 139(11):2067-71 and is listed in reference 33.

3. Results, paragraph 5: The topography of the steatohepatitic lesions is not described nor the presence of Mallory-Denk bodies. The periseptal predominance of lesions in FFD/CCl4 treated animals (Figure 1, 4th row) should be mentioned and adequately explained in the discussion section. The type of inflammatory cells present in the necroinflammatory foci should be described.

Ans: Mallory-Denk bodies are poorly defined in animal models (rodents). No such inclusions could be identified by H&E staining in the present study.

Explanation on type of inflammatory cells present in the necroinflammatory foci is provided in the results section, paragraph 5

4. Results: The metabolic background of this new animal model has to be described and assessed in detail. Its lack of similarity to that of human NAFLD has to be further analysed and a section on the limitations of the model should be added in the discussion.
Ans: Metabolic profile such as fasting and fed state blood glucose levels are mentioned in results section (Results: Paragraph 2) and limitation of this model has been incorporated in discussion (Discussion: Paragraph 4).

5. Results, last page: The authors state that inflammation is indicative of steatohepatitis in their model. Steatohepatitis is diagnosed as a combination of steatosis, hepatocellular ballooning and inflammation and therefore this sentence needs correction.

Ans: Necessary correction is incorporated in results section last page.

6. Discussion: Discussion is repetitive regarding the histology of the model and should be amended accordingly.

Ans: Paragraph 1 and 3 in discussion part has been amended as suggested by reviewer.

7. Discussion, 4th paragraph: Needs rephrasing and attention to the use of English. Progression to fibrosis is not the result of increased fatty acid import or synthesis by hepatocytes as wrongly stated. In addition, the two-hit model of NASH pathogenesis is not supported by current evidence. The authors should revise this part of the discussion and better support their arguments on the pathophysiological mechanisms underlying the development of the new animal model.

Ans: Discussion part has been amended and revised as suggested by reviewer.

8. Figures: Additional arrows needed to point to histological features discussed in the legends. In Figure 2 the arrows do not point to inflammatory foci as stated in the legend. Actually, inflammation is very mild in these photos and not representative of the moderate inflammatory activity that is described in the results. Magnification in Figure 2 is stated as x400 but it looks lower.

Ans: Figure 2 has been modified with better images with arrows pointing to inflammatory foci. Magnification in Figure 2 is x40 and has been corrected

Minor essential revisions

1. Abstract, Results: The acronym TG should be explained here and where it first appears in the text (background, last paragraph)

Ans: Necessary correction is incorporated

2. Abstract, Conclusions: The term "pathologies" should be replaced by "histological features" or "histological lesions". The plural of the word "pathology" although occasionally appears in some scientific texts is not correct and should be avoided. It should be replaced wherever it appears in this manuscript.

Ans: A term "pathologies" has been modified to "histological features" or "histological lesions" as appropriate throughout the manuscript.
3. Methods, Development of animal model, last paragraph: The use of the terms "sacrificing - sacrificed" is recommended instead of "killing-killed"

Ans: The terms "killing" and “killed” are replaced by either "sacrificing" or “sacrificed” in the revised manuscript.

4. Methods: The initials of the pathologist who performed the histological analysis should be included here and he/she should be listed among the co-authors

Ans: Pathologist name has been included in the Methods section who is the coauthor of this MS.

5. Table 2: Change number of reference from 33 to 31

Ans: Necessary corrections are made

6. Tables 1 & 2 (Additional file 1 &2): when referring to microscopic fields please define power (x20, x40 etc)

Ans: : Necessary corrections are made