Reviewer's report

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

Version: Date: 28 November 2013

Reviewer: Dina Tiniakos

Reviewer's report:

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.

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.

3. Results, paragraph 5: The topography of the steatohepatitic lesions is not described nor the presence of Mallory-Denk bodies. The perisepal 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.

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.

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.

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

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.

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.

Minor essential revisions

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

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

3. Methods, Development of animal model, last paragraph: The use of the terms "sacrificing - sacrificed" is recommended instead of "killing-killed"

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

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

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

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:

I declare I have no competing interests