Format for ANSWERING REVIEWER

January, 14h 2014
Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6509-3rd Submission DEF).

Title: *Translational approaches from fatty liver to non alcoholic steatohepatitis*

Author: *Natalia Rosso; Norberto C. Chavez-Tapia; Claudio Tiribelli; Stefano Bellentani*

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6509

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated
The Title was adjusted at 12 words with no abbreviations.
A running title was added.
Modifications in the affiliations were performed.
The contribution of each author was listed.
The Abstract was modified in no less than 200 words.
A Core Tip was included.
English language was polished.
Figure 1 was changed in decomposable format
Figure 2 and 3 were pasted as enhance metafile (and ppt file will be included in the submission)

2 Revision has been made according to the suggestions of the reviewer
(1) **Reviewer Number 00227342:**
We thank for the suggestions, the added references increased the quality of the contents.
1. In the paragraph entitled "Pathogenesis", the authors might refer to the role of inflammation citing some publications also summarized in the recent review by Braunersreuther V, et al. (World J Gastroenterol. 2012;18:727-35) and Carbone F et al. (Thromb Haemost. 2013;110:940-58). The suggested references have been added
2. The pro-inflammatory role of insulin can be discussed in the paragraph entitled "Pathogenesis" reporting studies investigating the activity of this hormone on inflammatory and vascular cells involved in atherogenesis (Montecucco F, et al. Am J Physiol Endocrinol Metab. 201;300:E681-90; Bunn RC, et al. Cardiovasc Diabetol. 2010;9:73; Gage MC, et al. Atherosclerosis. 2013;230:131-9). Paradoxical effects of insulin can be also described (Tsuchiya K, et al. Cell Metab. 2012;15:372-81). The suggested references have been added and discussed.

(2) Reviewer Number 00503544

1. Several widely used animal models are not mentioned in this paper. The authors should mention KK-Ay mice, high-cholesterol and cholate diet model, and high-fructose diet model. KK-Ay mice and Cholesterol/Cholate (atherogenic diet) were included both in the text and in Table 1. High Fructose diet model was described together with the HFD,

2. Genetic models, dietary models, and combination models are mixed up in Table 1. The authors should

arrange the table more systematically. Table 1 was reorganized in the attempt to present data in a more systematic way. Since many genetically modified animals were also exposed to diet modifications, with different outcomes, we added an extra column “diet modifications” where we described the diet and the obtained results.

3. The quality of English is a little poor. The authors should seek assistance of a native English speaker. We improved the quality of the written English. We trust we succeeded.

4. References and typesetting were corrected
Up-date in the references format was performed including PMID (As requested).

(3) **Reviewer Number 00187937**

1. Authors should explain and discuss the studies about the mitochondrial dysfunction. Because, it is important for hepatocellular injury in patients with NAFLD.
The mitochondrial dysfunction was considered and discussed in the text
2. Authors should also focus on the issue that why some slim shaped patients with NAFLD progress to the NASH and why some obese patients do not?
The presence of NAFLD in non-obese population was considered.

3. What do the authors think about the role of hepatic stellate cell or kuppfer cell (collagen metabolism) for the transition from simple steatosis to steatohepatitis?
The role of Hepatic Stellate Cells (HSC), an extremely interesting issue worth of a whole review, is discussed by the inclusion of a paragraph about the role of HSC in the initiation, perpetuation and progression of fibrosis. The interplay between activated HSC, hepatocytes and activated Kupffer cells was also mentioned.

(4) **Reviewer Number** 02541483

This is an interesting review on translational approaches from fatty liver to non alcoholic steatohepatitis. The article contributes to better understanding of the molecular mechanism associated to the accumulation of fatty acids in the liver cell, what is known and what is still to be discovered on the events related to the accumulation of fat within the liver and the resulting damage. However, I have some remarks:
1. Insulin Resistance (IR) should be written as insulin resistance (IR). This observation was included in the text

2. Who is calculated that in the next 40 years the daily caloric requirements will decrease of 350 calories (reference?) This reference was included in the text: *According to the Food and Agriculture Organization of the United Nations (FAO,* http://www.fao.org/docrep/x0262e/x0262e23.htm *) in the next 40 years the daily caloric requirements will decrease of 350 calories*.

3. In Pathogenesis section insulin resistance should be written as IR. All abbreviations should be explained only the first time they appeared in text! This suggestion was considered and the text modified accordingly.

4. In Pathogenesis section Type 2 Diabetes Mellitus should be written as type 2 diabetes mellitus. It was modified as suggested.

5. Not only MS, but also fatty liver is independently associated with chronic kidney disease and progression of other microvascular complications in T2DM (Targher G, Chonchol M, Zoppini G, Abaterusso C, Bonora E. Risk of chronic kidney disease in patients with non-alcoholic fatty liver disease: Is there a link? J Hepatol 2011; 54: 1020-9; Targher G, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C i sur. Non-alcoholic fatty liver disease in independently associated with an increased prevalence of chronic kidney disease and proliferative/laser treated retinopathy in type 2 diabetic patients. Diabetologia 2008; 51: 444-509). The suggested references were included and discussed in the text.

6. Treatment with GLP-1 agonist exenatide resulted in a reduction of hepatic fat quantity and hepatic biomarkers in T2DM: K. Blaslov, K. Zibar, T. Bulum, L. Duvnjak. Effect of exenatide therapy on hepatic fat quantity and hepatic biomarkers in type 2 diabetic patients. Clinics and Research in Hepatology and Gastroenterology 2013; DOI: 10.1016/j.clinre.2013.10.013. This reference should be added. The reference was added and discussed in the section *"role of incretin hormones*"

7. Authors state that monocytes are activated by conditions of hyperinsulinemia and abnormal levels of FFA encountered in individuals with IR, contributing to the development of complications such as T2DM. Monocytes are also associated with IR in autoimmune type 1 diabetes, and in those subjects IR is independently associated with markers of NAFLD (T. Bulum, B. Kolari?, L. Duvnjak, M. Duvnjak. Nonalcoholic fatty liver disease markers are associated with insulin resistance in type 1 diabetes. Digestive Diseases and Sciences 2011; 56: 3655-3663; The reference was added in the introduction section
T. Bulum, B. Kolari?, L. Duvnjak. Decreased serum monocytes and elevated neutrophils as additional markers of insulin resistance in type 1 diabetes. International Journal of Diabetes in Developing Countries 2013; DOI: 10.1007/s13410-013-0176-5). The reference was added in the text
It think that it would be useful to stress that IR is not only associated with fatty liver in MS related disorders like T2DM, but also in those that are not closely associated with MS like type 1 diabetes, according to mentioned studies. This suggestion was included in the introduction section.

8. In In-vivo and in-vitro Experimental models section metabolic syndrome should be written as MS. The name was substituted by its abbreviation.

9. Figure 1: estimation of the past, present and future of the major etiologies incidence of chronic liver diseases is based on what source? Based on the available data from USA :Kim WR, Brown RS, Jr., Terrault NA, El-Serag H. Burden of liver disease in the United States: summary of a workshop. Hepatology. 2002;36(1):227-42. [DOI: S0270913902000265 [pii];10.1053/jhep.2002.34734 [doi]] and Europe: Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol. 2013;58(3):593-608. [DOI: S0168-8278(12)00924-5 [pii];10.1016/j.jhep.2012.12.005 [doi]]and Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010;28(1):155-61. [DOI: 000282080 [pii];10.1159/000282080 [doi]]
Is this original table from authors as well as Figure 2 and Figure 3? The table and the figures were constructed and designed by the authors of the present review.

(5) **Reviewer Number** 01566349
ENERAL COMMENT The topic is timely and the manuscript is well written. I have just some comments aimed at improving this excellent submission.

SPECIFIC COMMENTS The Authors might be willing to address the following topics:

a) In rodents, given sufficient time, NASH will develop from pure steatosis provided that the offending agent (e.g. High-fat diet) is not removed. This occurs at variance with human disease, where pure steatosis and NASH are probably born as different and "unrelated" disorders (Yilmaz Y. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? Aliment Pharmacol Ther. 2012;36:815-23. Caldwell S et al. Perspectives on cellular dysfunction in nonalcoholic steatohepatitis: a case of 'multiorganelle failure'? Proceedings of a virtual workshop on nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol. 2011;5(2):135-9.). The suggestion was discussed in the section *in-vivo* and *in-vitro* Experimental models and the references were added in the text.

b) Highlight those animal models which more closely resemble human disease (e.g. Kechagias S, et al. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut. 2008;57:649-54. Unfortunately, there is no animal model able to reproduce all the events that occur during the development of human disease. For each model there are advantages and disadvantages that are summarized in Table 1.

c) Are there any relevant differences in NAFLD such as observed in different animal species, e.g. birds, rodents and mammals ? (Insulin resistance in non-alcoholic fatty liver disease: a clinical perspective Carulli N, et al. Leuschner et al Eds- FALK SYMPOSIUM 121, 2001 pages 104-113; Caldwell SH, et al. Has natural selection in human populations produced two types of metabolic syndrome (with and without fatty liver)? J Gastroenterol Hepatol. 2007 Jun;22 Suppl 1:S11-9.; Cohen JC, et al. Human fatty liver disease: old questions and new insights. Science. 2011;332:1519-23.) This topic is extremely interesting, and surely worth to write a review only about this issue. We tried to summarize some of the most interesting facts in this review considering the last two references. Unfortunately we did not have access to the first one.

d) Insulin resistance appears to be a "necessary though not sufficient" trigger in the development of NAFLD (Ratziu V, et al. Insulin resistance in nonalcoholic steatohepatitis: necessary but not sufficient - death of a dogma from analysis of therapeutic studies? Expert Rev Gastroenterol Hepatol. 2011;5:279-89.) The reference was included and discussed in the text.

e) There are two more pathogenic theories that need to be shortly alluded to:
Wanless IR, et al. The pathogenesis of nonalcoholic steatohepatitis and other fatty liver diseases: a four-step model including the role of lipid release and hepatic venular obstruction in the progression to cirrhosis. Semin Liver Dis. 2004;24:99-106.; Caldwell SH, et al. Clinical physiology of NAFLD: a critical overview of pathogenesis and treatment Expert Review of Endocrinolgy and Metabolism 2010). Unfortunately we did not have access to these papers.

f) The different effects of palmitic and oleic acid in hepatocitic cell cultures need to be mentioned (Ricchi M, et al. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. J Gastroenterol Hepatol. 2009;24:830-40.) This reference was added in the indicated section

g) Are there are any particularly relevant animal models to be discussed in some detail ? (E.G. ob/ob and LIRKO) Since Ob/Ob mice have been widely characterized and extensively reviewed by others, a deeper analysis would be redundant. Regarding LIRKO mice, is a valid model for the study of hepatic insulin resistance and the effect of insulin on leptin homeostasis. These animals present abnormal glucose metabolism and progressive liver dysfunction, with some evidence of focal dysplasia and hyper plastic nodules. However the serum TG are decreased and the animals are not obese. We included the LIRKO mice in table 1 and briefly discussed in the text the characteristics of this model.

h) Are the Authors aware of any animal models using mipomersen ? This drug may mimic familial hypobetaliproteinemia. Mipomersen inhibits apolipoprotein B-100 synthesis in the liver, thereby inhibiting the formation and secretion of apolipoprotein B lipoproteins by the liver. Mipomersen has been shown to decrease apoB, LDL-cholesterol and lipoprotein(a) in patients with heterozygous and homozygous FH on maximally tolerated lipid-lowering therapy. Mipomersen thus appears to be a potentially useful agent to lower apolipoprotein B-100 containing lipoproteins and could be used in combination with statin therapy or alone in statin intolerant patients. However, a major concern with therapeutic agents that inhibit hepatic VLDL production is the development of fatty liver. There is no doubt that this is an interesting topic to discuss an to explore, however in the present paper we decided not to include a review about the therapeutic drugs.

i) The sub-title resembles several previously published papers and needs to be changed. The sub-title has

been changed.

j) All English mother tongue Authors invariably use the word “evidence” as a singular (i.e. collective) noun and never as plural such as tpycally used in neo-latin languages. Thank you very much for this useful clarification, the word was changed along the text.

(6) **Reviewer Number** 02861137

Thank you for addressing me this article that aimed to review the in-vivo and in-vitro experimental models of NAFLD and NASH for a better knowledge of its pathogenesis. A better knowledge of this topic is very important to understand the pathogenesis of NAFLD and consequently prevent the evolution from simple steatosis to NASH. The topic was well covered. Major points of the biological pathway and the role of proinflammatory cytokines and atherogenic molecules were well described on the "Pathogenesis" section. The most important in-vivo and in-vitro experimental models were included and essential references were cited.

Minor points:

(i) the abusive use of abbreviation leads to a hard lecture; this point was taken into consideration an modified were was possible to do so.

(ii) few words were not spelled before abbreviation (i.e. FFA on page 6; definition of FFA on page 9); These point was corrected in the text

(iii) authors should at least include an abbreviation section; (iv) English language should be improved. These points were considered and corrected in the text

(7) **Reviewer Number** 00625614

This manuscript deals with an important health issue and therefore is of great interest for medical doctors and scientists. It covers both the pathogenesis and the in vivo and in vitro models of NAFLD and NASH with the aim of developing a translational approach from the lab bench to the clinical practice. Overall, the manuscript is very interesting although it should be better organized in order to facilitate the reader in the comprehension of the different aspects treated.

To this aim, it is advisable to organize the text in distinct paragraphs: we included sub-titles for different sections of the paper.

and to report the results in distinct tables for the in vitro and in vivo studies. We included in the paper a summary for the *in vivo* models, in order to summarized the most relevant information. For the *in vitro* models we refer to previously published data by other groups were complete tables were reported.

In any case, it is important to report the reference for each model listed in Table 1. Due to the big quantity of references in this regard, we included them in the text.

Furthermore, when speaking of high fat diet, other studies could be quoted, such as Ferramosca et al. 2013, Eur. J. Nutr. in press and 2012 PLoS One 7, e38797. In the text was included and discussed the data published by Ferramosca 2013- Eur J. Nutr, since contributed to the characterization of the different types of diets.

Regarding, Ferramosca 2012 PLOSone paper we consider that is an excellent paper, with clear and elegant evidence of the beneficial effect of Krill Oil Supplemented diet on steatotic rats. However our aim for the present review is to report the models that reproduce the human pathogenesis of NAFLD and NASH and not to focus the attention toward the reversion of the pathology, for these reason we decided not to include this outstanding paper.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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