Format for ANSWERING REVIEWERS

December 14, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: WJH-13545.doc).

Title: Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver

Author: Elmar Aigner, Günter Weiss, Christian Datz

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 13545

Regarding the analysis of overlap with published literature we would like to remark that the relevant overlaps reported are with our own published literature on related subjects. However, we have performed re-wording of the relevant paragraphs without changing the content and the literature cited since both the content and the summary of other authors' publications are still considered timely and correctly cited. We hope that you will be able to follow this argumentation in the re-analysis of the revised version as we hope that you will find it suitable for publication in the "World Journal of Hepatology" and await your response.

The changes and rewriting of the affected passages and the reviewers comments have erroneously not been performed in the track-changes mode. The three passages that have been added have therefore been marked in red.

The manuscript has been adapted according to the suggestions of reviewers: and Revision has been made according to the suggestions of the reviewer.

Comments of Reviewer 1(02860874): This is a very interesting review article, in my opinion, it summarizes clearly the most important aspects concerning to iron and copper as factors which are involved in the pathophysiology of NAFLD and NASH. The figures are very understandable and presented in a didactic way. However, this paragraph: "From a practical point of view, these studies suggest that iron depletion therapy via phlebotomy may represent a safe add-on therapy for NAFLD patients with elevated ferritin. We have adopted the practice to perform biweekly phlebotomies in these subjects until serum ferritin concentrations are between 50 and 100 ng/L. In contrast to patients with hemochromatosis, biweekly phlebotomies are generally performed in NAFLD patients. As NAFLD patients have impaired iron mobilisation from storage sites they may develop anemia in response to phlebotomy treatment", seems to be structured as a conclusion, in my opinion this is not entirely correct, then I suggest it must be restructured.

Re: The paragraph has been restructured as follows:

As far as practical treatment of iron excess in NAFLD patients with elevated ferritin is concerned, available data suggest that iron removal may thus be beneficial in addition to weight loss, diet and lifestyle modification or antidiabetic medication as indicated in an individual patient. We have adopted the practice to perform biweekly phlebotomies in these subjects until serum ferritin concentrations are between 50 and 100 ng/L, however, no evidence-based recommendation for this is currently available. In contrast to hemochromatosis patients, NAFLD subjects have impaired iron mobilisation from storage sites and may therefore develop anemia in response to phlebotomy treatment. We therefore recommend close monitoring of serum ferritin, TfS and hemoglobin at each visit for the period of time while these patients are on phlebotomy treatment.

Comments of Reviewer 2 (02861277): Aigner E. et colleagues well described the physio-pathological factors involved in the iron overload in NAFLD. Moreover, they reported the molecular mechanisms that could explain the liver injury worsening shown in patients affected from iron overload in NAFLD. In addition, the authors described the clinical implications of iron overload and copper deficiency as well as the therapeutic option in NAFLD/NASH patients.

Main observations: As well underlined from the authors there is a strict connection between iron overload, oxidative stress (..and lipid-peroxidation) and liver injury in NAFLD/NASH. They remembered as NAFLD is both a metabolic and an inflammatory disease. On the base of these considerations, I guess that they should include the role of the adaptive immune responses related to the oxidative stress in NAFLD progression (among the other pathological mechanisms).

Re: The following paragraph has been included in the mechanisms section:

Importantly, oxidative stress induced molecules such as malonyldialdehyd and 4-hydroxynonenal may induce the formation of de-novo antigens with subsequent activation of T-lymphocytes and development of immunoglobulin G reactive against these antigens. This response was further enhanced by previous immunization against these antigens with a stimulated M1 macrophage response. Although no studies have been performed, iron may contribute to this process by further augmentation of oxidative stress.

As the authors reported, the iron, at least in part, is stored in the macrophages and given their pivotal role in NAFLD/NASH. I believe that they should include comments about the activation pattern of these myeloid cells in NASH.

Re: We appreciate this comment. We have added a paragraph on this important subject.

Liver macrophages named Kupffer cells, which are an important site of iron storage in NAFLD, are tightly involved in the initiation of the hepatic inflammatory cascade in response to the uptake of oxidized lipoproteins or oxidized phosphatidylcholines. It is well known that macrophage iron status affects their inflammatory response pattern and polarization towards a pro-inflammatory phenotype, however, the particular role of these potential interactions have to our knowledge not been investigated in NAFLD.

Pag 5: The authors reported: "macrophage-derived Kupffer cells". What means? Please clarify. To my knowledge monocytes-derived macrophages and Kupffer cells have a different ontogeny.

Re: This error has been corrected in the manuscript and we only refer to "resident liver macrophages named Kupffer cells" in the manuscript.

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

Sincerely yours,

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