

Heidelberg, May 3<sup>rd</sup> 2021

Point by point response:

Dear editor,

We would like to thank for considering a revised version of our manuscript to be published in World Journal of Hepatology. We also would like to thank you and the reviewers for their very helpful comments.

Please find below our point by point response to the reviewer's comments. Our response is highlighted in bold text. We have also uploaded a revised version of our manuscript.

Yours sincerely,

Sebastian Mueller

### Reviewer 1

Reviewer Name:	Anonymous
Review Date:	2021-03-12 22:39
Scientific Quality:	Grade C (Good)
Language Quality:	Grade C (A great deal of language polishing)
Conclusion:	Minor revision

Dear Authors, the manuscript Direct modulation of hepatocyte hepcidin signalling by iron submitted by your team is generally worth reaching audience of World Journal of Gastroenterology. The aims and experimental methods were appropriate and the study brings advancement in the field of hepatology research. Nevertheless, I find several problematic aspects of the manuscript and those deserve improvement before the article gets approval for publication. Some of these issues are formal, some relate to the content and message of the manuscript. First, a declaration was submitted along with the manuscript file, that the text was written by a native English speaker. Contrary to that, author contributions paragraph states it was Sebastian Mueller affiliated with University of Heidelberg who wrote and revised the manuscript. This raises doubts about fair presentation of your work. Some language weaknesses throughout the text reveal, that the manuscript was at least written in haste.

Examples include: Why repetitive blood transfusions have been... instead of While...(page 6);

**Response: Text has been corrected according to the reviewer's suggestions.**

... FAC had no significant effect ... in the presence or absence instead of FAC had significant effect neither in the presence nor in the absence... (page 11);

**Response: Text has been corrected according to the reviewer's suggestions.**

Notable instead of Notably (page 11);

**Response: Text has been corrected according to the reviewer's suggestions.**

Both FAC and LDN had no significant effect instead of Neither FAC nor LDN had significant effect (page 12).

**Response: Text has been corrected according to the reviewer's suggestions.**

Besides that some sentences are too long or have unusual and confusing word order rather suggesting the text was not written by a native speaker. Also some facts are handled inaccurately. Alcoholic liver disease is claimed to be the most common human liver disease (page 6) without referencing any data source. This is not true. In fact, it is the non-alcoholic fatty liver disease (NAFLD) holding the position of the most prevalent chronic liver disease worldwide (see e.g. Wessels DH, Rosenberg Z. Awareness of non-alcoholic steatohepatitis and treatment guidelines: What are physicians telling us? World J Hepatol 2021; 13(2): 233-241 URL: <https://www.wjgnet.com/1948-5182/full/v13/i2/233.htm>).

**Response: We have now cited the WHO global burden report.**

Sometimes the text needs providing more details to ease readers' experience. Example of that is on page 4: By binding to and degrading the iron exporter ferroportin 1 (Fpn1) which is localized at the plasma membrane of duodenal enterocytes, macrophages and hepatocytes, hepcidin efficiently blocks iron absorption, iron recycling and iron storage. Clarifying the localisation of Fpn1 to either luminal (apical) or basolateral portion of enterocyte membrane would be very useful.

**Response: We would like to thank for this helpful comment. We have improved the structure of the paragraph and also provide the information of Fpn1 localization.**

Introduction or discussion section would definitely benefit from adding a picture or a scheme of hepcidin interactions.

**Response: We have now included a novel scheme in Figure 7 to better highlight the findings of our work, especially the various signaling pathways causing hepcidin elevation.**

A frequent inaccuracy in the manuscript is the missing specification whether hepcidin transcription, translation or activity is matter of debate. With SMAD6 mRNA and p-SMAD1/5/8 protein expression you succeeded not to omit it. On the other hand, regarding hepcidin - the main factor of interest - the text lacks needed clarity multiple time.

**Response: We are grateful for this comment. It is well established in the field of iron research that, in contrast e.g. to SMAD expression, hepcidin is primarily regulated at the transcriptional level and it is sufficient to study levels of hepcidin mRNA. Hence, no assessment of the propeptide or the secreted**

**peptide is necessary. We have added a comment in the materials and methods section.**

The last formal remark is on using adjectives dramatically and drastic to describe the results. It feels inappropriate in the academic style and I would strongly prefer replacing it by profoundly, markedly or another synonyms.

**Response: The whole manuscript has been corrected according to the reviewer's suggestions.**

Regarding the scientific content I also suggest several improvements. Calling hydrogen peroxide central oxygen metabolite (page 5) is rather weird.

**Response: We have changed the term to "central redox signaling molecule".**

Did the Huh7 and THP-1 cell lines reflect mammalian physiological conditions when cultured at 25 mM glucose concentration? (page 6 and 7)

**Response: Both Huh7 and THP1 cells are standard models to study iron homeostasis in hepatocytes and macrophages and they both mimic important aspects of primary cells e.g. expression of key iron molecules such as hepcidin, ferritin, transferrin receptor etc. in addition, both cells have been established for many years and are used in many laboratories around the globe with DMEM and RPMI-1640 media and the required 25 mM glucose**

What is Ferrlecit (page 7)? It is mentioned without any explanation.

**Response: Ferrlecit (sodium ferric gluconate) is an established and approved intravenously applied drug to treat patients with iron deficiency. This information has been added to the manuscript.**

Hypoxic experiments deserve better description. How readers shall understand 3 minute gas mixture treatment followed by 24 h incubation?

**Response: We have modified the text accordingly to better describe the hypoxia experiments using the hypoxia chamber. Reference 15 is quoted.**

Also note on pathophysiological cell ratio (1:4) of macrophage/hepatocyte to mimic the liver microenvironment (page 12) is not sufficiently clear.

**Response: We would like to thank for this helpful comment. It has been shown in the past by us and other groups that the cell ratio of hepatocytes and macrophages can have an impact on hepcidin regulation. For this reason, we explicitly mention in our manuscript that a pathophysiological cell ratio has been used in contrast to physiological conditions with a lower ratio of macrophages (e.g. ratio of 1:10). The text has been slightly modified accordingly.**

The discussion and conclusion sections show many sentences deserving rephrasing or language polishing to contribute to clear message of the study. For example (page

14): These patients show pronounced hemolytic anemia and require a continuous (rather repeated) blood transfusion.

**Response: Text has been changed according to the reviewers suggestions.**

Patients with severe disease typically show progressive liver disease (rather damage) and cirrhosis due to heavy (rather serious) iron toxicity.

**Response: Text has been changed according to the reviewers suggestions.**

In the figure legends, I strongly recommend adding a space between letters indicating a statistical significance and the P values. In its current wording this looks strange.

**Response: Text has been changed according to the reviewers suggestions. In addition, we have followed the WJG guidelines.**

Editor's comments:

Comments:

1 Scientific quality:

The manuscript describes a basic study of the direct modulation of hepatocyte hepcidin signaling by iron. The topic is within the scope of the WJG.

(1) Classification: Grade C;

(2) Summary of the Peer-Review Report: The authors found that the aims and experimental methods were appropriate and the study brings advancement in the field of hepatology research. However, the questions raised by the reviewer should be answered; and

**Response: All questions raised by the reviewer have been answered and the manuscript was modified/corrected accordingly.**

(3) Format: There is 1 table and 6 figures.

**Response: According to the reviewer, we have now added an additional figure (Fig. 7) resulting in 7 figures and one table.**

(4) References: A total of 48 references are cited, including 5 references published in the last 3 years;

(5) Self-cited references: There are 4 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations that are closely related to the topic of the manuscript, and remove other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated; and

**Response: After the reviewer's suggestions, the manuscript contains now 50 references with a total of 4 self-cited references. Consequently, the self-referencing rate is less than 10%.**

(6) References recommend: The authors have the right to refuse to cite improper references recommended by peer reviewer(s), especially the references published by the peer reviewer(s) themselves. If the authors found the peer reviewer(s) request the authors to cite improper references published by themselves, please send the peer reviewer's ID number to the editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

2 Language evaluation:  
Classification: Grade C.

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, and the The ARRIVE Guidelines. No academic misconduct was found in the Bing search.

4 Supplementary comments: This is an invited manuscript. The study was supported by 1 grant. The topic has not previously been published in the WJG.

5 Issues raised:

(1) The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend:  
<https://www.wjgnet.com/bpg/gerinfo/240>;

**Response: The total manuscript has been carefully reassessed and an English certificate has been uploaded accordingly.**

(2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

**Response: The DFG approval form is provided for DFG grant to Dr. Rausch and Dr. Mueller S (RA 2677/1- 2).**

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and

**Response: Original pictures are now provided.**

(4) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

**Response: The "Article Highlights" has been added at the end of manuscript.**

6 Recommendation: Conditional acceptance.