Alexandra K. Kiemer, Ph.D.
Professor of Pharmaceutical Biology
Saarland University
Campus C2 2
P.O. box 15 11 50
D-66041 Saarbruecken, GERMANY

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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4701-review.doc).

Title: Rapid chromatographic method to decipher distinct alterations in lipid classes in NAFLD/NASH

Author: Stephan Laggai, Yvette Simon, Theo Ranssweiler, Alexandra K. Kiemer, Sonja M. Kessler

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 4701

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

(1) **Reviewer code:** 00069693

> Encourage authors to include a list of abbreviations due to the number of abbreviations used in the text.

We included a list of abbreviations.

> The authors could inform what the software used.

Information about the software we used for statistics is now included. The software to quantify TLC plates was already contained in the original version of the manuscript.

(2) **Reviewer code:** 01806390

> Introduction needs to be re-written. 1. The aim of this study, as stated in the abstract, is at variance with the aim reported in the introduction.

We have completely re-written the introduction. We made the aim in the abstract, the core tip, and the introduction consistent.

> The Authors should be willing to make their submission more consistent by shortly

discussing in the introduction the animal model they're working on.

The used animal models are now discussed in the introduction.

> Always in the introduction, rather than referring to changes in lipid metabolism "in general" (Ref. 12), the Authors might be willing to focus on metabolic changes occurring specifically in hepatocellular carcinoma. Such a topic has extensively been reviewed elsewhere (J Gastroenterol Hepatol. 2012;27:1654-64; J Hepatol. 2012;56:1384-91; Cancer Res. 2013;73:4722-31.).

A short section related to specific metabolic changes in hepatocellular carcinoma is now added to the introduction.

> However, it is obvious that the diagnosis of NAFLD has nothing to do with the follow-up strategy of these patients. Moreover, liver biopsy cannot be proposed in all patients and follow-up strategies aimed at surveiling those individuals prone to develop HCC are still poorly defined (Nascimbeni F, J Hepatol. 2013 Jun 7. doi:pii: S0168-8278(13)00377-2. 10.1016/j.jhep.2013.05.044.).

We have re-written this passage.

> Discussion needs shortening and reworking based on the suggestions appended below.
Start this section by shortly summarizing the chief findings.
Are these novel findings? Are these confirmatory findings alone?

The chief findings and their novelty are now summarized in the beginning of the discussion section.

> The Authors are requested to make clear whether data reported in the present paper may be potentally extrapolated to human disease and whether to simple fatty liver or true NASH. In humans, these 2 conditions are potentially unrelated (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 Nov;36(9):815-23.) and follow a completely different course (Ann Med. 2011 Dec;43(8):617-49.).

We have completely re-written the second part of the discussion and mention the points raised by the reviewer.

> "The better understanding of changes in lipid composition might be helpful for the treatment and diagnosis of NAFLD, NASH, and HCC". Is this statement based on findings reported here ? Has this paper any results concerning HCC?

No, this paper has no results concerning HCC. But this method could also be used for HCC samples. Lipid composition itself might have an impact on HCC development or progression. We have modified our statement.

> REFERENCES Rather than referring to reviews, the Authors might be willing to quote

experimental data. For instance, Ref 9. may be changed with Ricchi M, J Gastroenterol Hepatol. 2009;24:830-40.

Ricchi M, J Gastroenterol Hepatol. 2009;24:830-40 was added to the references; the article describes a quite interesting behavior of fatty acids depending on chain length and saturation. The saturated C16 fatty acid palmitic acid induces apoptosis, whereas the unsaturated C18:1 fatty acid oleic is more steatogenic. We have decided to leave Ref. 9 in the references, because the review describes also effects of different lipid classes.

(3) **Reviewer code:** 01809067

> ITS BETTER TO USE ANOTHER BIOMARKER THAT DIFFERENTIATE BETWEEN NAFLD AND NASH LIKE CYTOKERATINE 18 EXPLAIN WHY THE RATIO OF PHOSPHATIDYLCHOLINE TO PHOSPATIDYLETANOLAMINE DECREASE IN NASH

There are no histological signs of inflammation in the steatotic livers of *p62* transgenic animals as repeatedly confirmed by two independent pathologists (Tybl et al. *J Hepatol* 2011 and Simon et al. *Gut* 2013, in press). This suggests that the model is adequate as a model for simple steatosis. The MCD model is one of the best characterized NASH models and shows all hepatic characteristics of NASH (Wasmuth et al. *Drug Disc Today* 2007 and Simon et al. *Gut* 2013, in press).
The changes in the PC/PE ratio in p62 transgenic animals might reflect a tendency towards a sensitivity towards a second hit. This subject is further discussed in the introduction.

A possible explanation for the altered PC/PE ratio was added into the discussion: PC is synthesized *via* the choline pathway or by methylation of PE *via* phosphatidylethanolamine N-methyltransferase (PEMT). An inhibition of PEMT might be an explanation for the altered ratio, but is merely speculative. A respective statement is given in the discussion.

> SUGGEST TO USE ANOTHER MODEL OF STEATOSIS IN ORDER TO CONFIRM THE RESULTS

Confirmation of the method used was performed by the investigation of known changes in lipid composition in the MCD model, which were described previously in the literature (see introduction). Since the adequacy of the model was confirmed by use of the MCD model we employed the method to study the as yet unknown lipid composition in the *p62* transgenic mouse model. Since a different steatosis model might either show the same or a different lipid composition as p62 animals, we don't feel that the use of another model of steatosis would increase the impact of our results.

3 References and typesetting were corrected

I would also like to confirm that the language quality of the manuscript represents grade A. We therefore choose not to have our manuscript edited by one of the English language editing companies you suggested. Although I am not a native speaker, I have spent several years in English-speaking countries and have daily English correspondence. I have published over 70 papers in English, of which none ever required language polishing.

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

Sincerely yours,

Alexandra K. KIEMER, PhD
Department of Pharmacy, Pharmaceutical Biology
Saarland University
Saarbruecken, Campus C2.2
D-66123-Germany
Fax: +49-681-302-57302
E-mail: pharm.bio.kiemer@mx.uni-saarland.de