Reviewer's report

Title: Differential hepatotoxicity of dietary and DNL-derived palmitate in the methionine-choline-deficient model of steatohepatitis

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Reviewer: Joern M. Schattenberg

Reviewer's report:

The authors address an important issue – the role of saturated fatty acids derived from the diet or de-novo lipogenesis in the pathogenesis of NASH. The study of Pierce et al. builds directly on two earlier studies, in which they have shown that enriching the diet with simple sugar enhances steatohepatitis, whereas substituting dietary sugar with complex carbohydrate reduces liver injury (Pickens et al., 2009 and 2010). The question of the present study is well defined, and as a whole contemplated, this manuscript including the single parts of the text (title, abstract, background, methods, results/discussion and conclusion) and figures is proficient, perfectly understandable and clearly structured. The presented data are sound, and well discussed. Nevertheless there are small points of criticism and suggestions for revision. Above all, I would suggest few additional experiments in order to explain the different effects of dietary and DNL-derived saturated fatty acids on liver cells/hepatocytes and to provide mechanistic insights into their metabolic impacts in-vivo. Major comments are listed below.

Major comments

Pierce et al. convincingly demonstrate by appropriate methods (histological stainings, triglyceride and fatty acid analysis) that feeding of MCD diets containing sucrose induced the most pronounced hepatic steatosis after 21 days regardless of the accompanying type of dietary fat. The combination of sucrose and palmitate caused worst steatosis, although all mice fed MCD diets lost comparable amounts of body weight regardless of the macronutrient composition of the diet (Figure 1 and 2). As these weight changes occur universally and are not related to the mechanism observed, this information does not add meaningful and can be omitted.

Moreover, liver injury and accompanying infiltration of inflammatory CD11b+ leukocytes correlated positively with the degree of hepatic lipid accumulation as shown by well done TUNEL and CD11b stainings, serum ALT measurements and qRT-PCR analyses (Figure 3).

At this point it is essential to note, that CD11b is mainly expressed on F4/80+ Kupffer cells/macrophages and infiltrating monocytes, which are known to trigger NASH via secretion of cytokines and chemokines including TNF-#, CCL2, CXCL2 and CXCL10. Therefore, the authors should also investigate the hepatic mRNA expression of the key cytokine TNF-#. Are there differences concerning
the activation of caspases and MAP kinases, esp. JNK? Did the different MCD formulas affect other metabolic parameters of the mice (e.g. glucose levels)?

Furthermore Pierce et al. show, that feeding of MCD diets containing sucrose and/or palmitate caused hepatic palmitate accumulation - preferentially in hepatic triglycerides -, which seemed to correlate positively with the degree of liver injury (Figure 4). However, mice fed MCD sucrose-oleate accumulated no more palmitate than those fed MCD starch-palmitate, although their ALT levels were significantly higher, suggesting that palmitate arising from sucrose in the MCD diet (DNL palmitate) is more hepatotoxic than palmitate itself. Interestingly, the combined feeding of sucrose and palmitate induced more hepatic palmitate accumulation and higher ALT levels than would have been predicted by a mere additive effect of both nutrients. Therefore the authors conclude that dietary sucrose - through DNL conversion to palmitate in the liver - (DNL palmitate) is an important inducer of liver injury in the MCD model and more hepatotoxic than dietary palmitate, which becomes toxic only in combination with dietary sugar. This part is most interesting and I would suggest some further mechanistic analysis as detailed above. Are injurious pathways differentially activated? Does lipoautophagy contribute to the observed differences? These findings also suggests different metabolic fates for DNL vs. exogenous palmitate. Are key enzymes of the hepatic de novo lipogenesis and their regulators regulated differentially in the experimental groups? Are important transcription factors (e.g. PPARs) differently regulated?

Minor essential revisions:
- Please add scale bars to all microscopic images (Figure 2A and 3A).
- How did the authors assess TUNEL+ and CD11b+ cells in figures 3B and D). Please include this information in the Methods/figure legend

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests