Reviewer’s report

Title: Fructo-Oligosaccharides Ameliorate Steatohepatitis, Visceral Adiposity, and Associated Chronic Inflammation via Increased Production of Short-Chain Fatty Acids in a Mouse Model of Non-alcoholic Steatohepatitis

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Reviewer: Emanuele Albano

Reviewer's report:

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The manuscript by Takai and co-workers investigates the effects of fructo-oligosaccharides (FOS) on steatohepatitis and visceral adiposity in a model of NASH based on the induction of over-feeding by the administration of monosodium glutamate (MSG) to newborn C57BL/6J mice. The authors observed that FOS treatment improved steatosis, inflammation and hepatocyte ballooning in the liver of mice with NASH. These effects were associated with a lowering in the hepatic mRNA expression of fatty acid synthase and glycerol-3-phosphate acyltransferase. Furthermore, FOS inhibited adipocyte enlargement and macrophage recruitment in epididymal fat in parallel with changes in gut microbiota composition and increased fecal concentrations of short chain fatty acids (SCFA) suggesting that FOS action on dysbiosis might influence the development of metabolic derangements leading to steatohepatitis.

The work is interesting and has a potential translational impact. However, additional data are required to support the author's conclusions. The following points need attention:

a) Figure 1 shows that FOS supplementation improves body weight as well as epididymal fat expansion and inflammation. Nonetheless, the effects on adipose tissue require a better characterization from the metabolic and inflammatory point of view by measuring glucose tolerance and insulin response, assessing adipose tissue expression of pro-inflammatory cytokines and the production of adipokines.

b) The characterization of FOS action on NASH is also insufficient. Liver triglyceride content need to be shown along with the liver or circulating levels of pro-inflammatory cytokines.

c) The authors have previously shown that FOS supplementation improves intestinal barrier function in MCD fed mice. It would be important to evaluate how much such an effect accounts for ameliorating NASH in this model. The measurement of circulating LPS levels can address this issue.
d) It is interesting that FOS reduced inflammatory cells infiltration in the adipose tissue, but the data presented are insufficient to substantiate histology. Flow cytometry should be used to show the changes in the prevalence of F4/80+/CD11b+ macrophages and of B and T lymphocytes. Furthermore, M1 macrophages are identified as F4/80+/CD11b+/Ly6Chigh cells. The use of CD11c is not a suitable marker.

e) The overall impact of the work would be increased by investigating whether FOS supplementation is also effective in ameliorating dybiosis and liver alterations with more widely used model of NASH such as the high fat western diet.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
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Yes

Are the conclusions drawn adequately supported by the data shown?
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Yes

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