Author response to Reviewer comments on Manuscript NO.: 82127

Dear Editor,

Please find enclosed the revised version of our manuscript entitled, "Insulin resistance and adipose tissue interactions as the cornerstone of MAFLD pathogenesis and complications". We are sincerely thankful to the Reviewers for their valuable comments and suggestions which strengthened the scientific relevance of our work. All the suggestions raised by the referees have been attended and the changes in the revised manuscript are highlighted in YELLOW color for easy identification. We expect that the revised version fulfills the requirements of the journal and is suitable for publication in *World Journal of Gastroenterology*. Attached please find our feedback on the reviewers' comments.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: This review focuses on insulin resistance of different tissues, together support the epidemiological association between insulin resistance and MAFLD. Somehow, this review provides a valuable point on MAFLD. The discussion is comprehensive, however, some parts might be too brief to bring a persuasive point.

Comment 1. In Page 6, the authors said "the "glucocentric" view of insulin resistance has shifted to the "lipocentric" view. Please discuss more details and add the references.

Reply:

We greatly appreciate your comment, we incorporated the suggestion and elaborated upon the glucocentric vs lipocentric view. The following paragraph was added:

During the past decade, the "glucocentric" view of insulin resistance has shifted to the "lipocentric" view, regarding its pathogenesis and associated mechanisms. We can appreciate how much the focus of IR effect on glucose metabolism prevailed before the year 2000 in any scientific literature, even if we don't look in depth. For instance, IR had been included within a concept denominated the "insulin resistance syndrome", which considered the presence of dyslipidemia, hypertension and impaired glucose tolerance as factors leading to increased cardiovascular risk, however, that's the farthest lipids' involvement got (13).

Chronic hyperglycemia leads to glucotoxicity, directly inducing IR and the IR degree is one of the strongest predictors for T2DM onset in populations at risk (14,15). While all of this is a fact, the role of fatty acid metabolism had been overlooked since in 1965 Randle and colleagues first suggested increased serum free fatty acids (FFAs) as one of the primary causes for decreased glucose oxidation and IR development (16). The last decade has had an increased body of research supporting this fact, centering the role of lipids, along with that of glucose, in the development of fatty liver.

The following references were added accordingly:

13. Rao G. Insulin resistance syndrome. Am Fam Physician. 2001;63(6):1159–63.

14. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism. 2016;65(8):1049–61.

15. Haffner SM. Risk factors for non-insulin-dependent diabetes mellitus. J Hypertens. 1995;13(Suppl 2):S73–6.

16. Randle PJ, Garland PB, Newsholme EA, Halest CN. The glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. Ann N Y Acad Sci. 1965;131(1):324–33.

Comment 2. Browning of adipose tissue showed abundant association with NAFLD, but the discussion in the review is limited. This part could be expanded.

Reply: We greatly appreciate your contribution and thus, we expanded the section on browining of adipose tissue and its relation with fatty liver development. The following paragraph was added:

Briefly expanding upon the importance of BAT, a 2011 paper proved how cold-induced browning of AT in rats controlled triglyceride rich lipoprotein metabolism by boosting their turnover and channeling lipids into AT browning (25). There has been a number of studies proving that the presence of BAT in adulthood is independently associated with lower probability of developing liver steatosis (26,27), for which multiple mechanisms have been uncovered. For instance, the uncoupling protein 1 (UCP-1) expressed specifically in BAT reverses obesity and also antagonizes liver inflammation and pathology (28). Interestingly, uric acid transporters have been seen to influence fatty liver (19); a study carried out by Tanaka and colleagues found that the use of doniturad [uric acic transporter-1 (URAT-1) selective inhibitor] showed amelioration of IR in rats by reducing liver steatosis and promoting rebrowning of AT (29).

The following references were added accordingly:

25. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, et al. Brown adipose tissue activity controls triglyceride clearance. Nat Med [Internet]. 2011;17(2):200–6. Available from: http://dx.doi.org/10.1038/nm.2297

26. Yilmaz Y, Ones T, Purnak T, Ozguven S, Kurt R, Atug O, et al. Association between the presence of brown adipose tissue and non-alcoholic fatty liver disease in adult humans. Aliment Pharmacol Ther. 2011;34(3):318–23.

27. Ahmed BA, Ong FJ, Barra NG, Blondin DP, Gunn E, Oreskovich SM, et al. Lower brown adipose tissue activity is associated with non-alcoholic fatty liver disease but not changes in the gut microbiota. Cell Reports Med. 2021;2(9).

28. Mills EL, Harmon C, Jedrychowski MP, Xiao H, Tran N V, Bradshaw GA, et al. UCP1 governs liver extracellular succinate and inflammatory pathogenesis. Nat Metab. 2021;3(5):604–17.

29. Tanaka Y, Nagoshi T, Takahashi H, Oi Y, Yoshii A, Kimura H, et al. URAT1-selective inhibition ameliorates insulin resistance by attenuating diet-induced hepatic steatosis and brown adipose tissue whitening in mice. Mol Metab [Internet]. 2022;55(December 2021):101411. Available from: https://doi.org/10.1016/j.molmet.2021.101411

Comment 3. Insulin signaling promotes DNL, but how IR promotes this process? The relative discussion about the mechanism could be added.

Reply: We clarified that the presence of insulin promotes DNL, and thus, in an IR state with hiperinsulinemia, this process is promoted even further. The following clarification was added:

As we know, increased serum insulin is a compensatory mechanism during IR when there is appropriate endocrine pancreas activity, by having increased insulin release, DNL is stimulated further.

Comment 4. The authors discussed the relationship between MAFLD and CKD, and mainly on the mutation of PNPLA3. Whether other genes involved in the regulation?

Reply: Thank you very much, we added the following paragraph to expand upon the relation with other molecular pathways (if not genes) to enrich the section:

While PNPLA3 gene mutations might be a common factor in the predisposition for both CKD and MAFLD, there's a number of nuclear transcription factors that contribute to both diseases' pathogenesis. These factors include the peroxisome proliferator– activated receptor (PPAR) family, farnesoid X receptor (FXR) and the sterol regulatory element binding protein 2 (SREBP2), which modify their respective molecular pathways and influence the progression of both CKD and hepatic steatosis (54). For instance, PPAR-α,δ,γ downregulation causes a myriad of cellular alterations in the nephron, including increased podocyte apoptosis leading to altered glomerular barrier integrity, increased mesangial cell hypertrophy and enhanced matrix deposition, as well as NF-κB activation with consequent proinflammatory cytokine secretion in the glomerular endothelium (55). These same factors under physiologic circumstances supress fibrogenesis by inhibiting the transforming growth factor β (TGF-β) in stellate cells, lower the M1/M2 Kupffer cell phenotype ratio (thus decreasing inflammatory stimulus in the liver) and increases catalase activity in hepatocytes, among other functions. It is clear how downregulation of the PPAR family of factors hinders these protective mechanisms in the liver and promotes the development of fatty liver, as well as CKD. The same situation of multiorgan damage comes about with decreased

expression of FXR and upregulation of SREBP-2, given that FXR inhibits the SREBP-1c mediated DNL in hepatocytes while decreasing reactive oxygen species formation in mesangial cells and increasing endothelial nitric oxide synthase (eNOS) in the glomerular epithelium (54). Finally, SREBP-2 upregulation leads to increased cholesterol synthesis and decreased excretion in both liver and renal cells (56,57). With this brief compilation of the molecular pathway similarities between CKD and fatty liver development, it would be of no surprise to find in the near future novel discoveries on further overlapping mechanisms and genetic predisposition for both diseases.

The following references were added accordingly:

54. Musso G, Cassader M, Cohney S, De Michieli F, Pinach S, Saba F, et al. Fatty liver and chronic kidney disease: Novel mechanistic insights and therapeutic opportunities. Diabetes Care. 2016;39(10):1830–45.

55. Gao J, Gu Z. The Role of Peroxisome Proliferator-Activated Receptors in Kidney Diseases. Front Pharmacol. 2022;13(March):1–10.

56. Kandasamy N, Ashokkumar N. Renoprotective effect of myricetin restrains dyslipidemia and renal mesangial cell proliferation by the suppression of sterol regulatory element binding proteins in an experimental model of diabetic nephropathy. Eur J Pharmacol [Internet]. 2014;743:53–62. Available from: http://dx.doi.org/10.1016/j.ejphar.2014.09.014

57. Musso G, Gambino R, Cassader M. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. Prog Lipid Res [Internet]. 2013;52(1):175–91. Available from: http://dx.doi.org/10.1016/j.plipres.2012.11.002

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This manuscript provides a review on MAFLD, its pathogenesis and some related mechanisms. It is well written but I have the following suggestios for the authors:

Comment 1. The title of the manuscript did not well cover the content, especially the content about the commodities in MAFLD. Please revise it.

Reply: Thank you for your comment, we interpreted the word "commodities" as "comorbidities" thinking about a possible typing mistake, we hope to have understood correctly. We modified the title to:

Insulin resistance and adipose tissue interactions as the cornerstone of MAFLD pathogenesis and complications

Comment 2. Some descriptions or statements are not accurate. For example, "The same effect is achieved through suppression of adipose tissue lipolysis in insulin resistant states, increasing fatty acid influx into the liver", I think it should be 'impaired suppression'; "For instance, alterations in the peroxisome proliferator-activated receptor α (PPAR-α), which serves as a FFA sensor, leads to decreased fatty acid catabolism and intrahepatic lipid accumulation", what do the alterations mean, activated or inactivated? Please make it clear to the readers as activation of PPAR-α promotes to peroxisomal beta-oxidation of fatty acids by promoting expression of the ACOX1 and P450 genes; "An additional feature of irisin is FFA oxidation, which is a method for lipid removal from ectopic tissue; this will later be explained in the IHTC section".

Reply: Thank you for your valuable review. We modified all of the sections that you suggested, these are laid out below:

The same effect is achieved through impaired suppression of adipose tissue lipolysis in insulin resistant states, increasing fatty acid influx into the liver.

It has been found that stimulation of PPAR-α in mice enhances the expression of Cytochrome P450 4a (Cyp4a) and enhances lipid turnover in the liver, decreasing the risk of developing dietary steatohepatitis (44). Furthermore, PPAR-α activation increases peroxisomal fatty acid β-oxidation by inducing the Acyl-coenzyme A oxidase (Acox1), the rate limiting enzyme in the oxidation of very long-chain fatty acids (45,46). Acox1 is also associated with spontaneous liver damage in humans, as well as spontaneous steatosis, steatohepatitis and hepatocellular carcinoma (HCC) development in mice (45).

The following references were added accordingly:

44. Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARα-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology. 2003;38(1):123–32.

45. Moreno-Fernandez ME, Giles DA, Stankiewicz TE, Sheridan R, Karns R, Cappelletti M, et al. Peroxisomal β-oxidation regulates whole body metabolism, inflammatory vigor, and pathogenesis of nonalcoholic fatty liver disease. JCI insight. 2018;3(6):14–8.

46. Huang J, Jia Y, Fu T, Viswakarma N, Bai L, Rao MS, et al. Sustained activation of PPARα by endogenous ligands increases hepatic fatty acid oxidation and prevents obesity in ob/ob mice . FASEB J. 2012;26(2):628–38.

There's a number of mechanisms involved in FFA oxidation and lipid metabolism. As we briefly touched upon before, irisin is one of the hundreds of exercise-induced myokines secreted by skeletal muscle which plays an important role in the AT-muscle-liver axis, it also regulates the adenosine monophosphate (AMP)-activated protein kinase (AMPK) signalling pathway thus increased FFA oxidation in myocytes (58).

The following reference was added accordingly:

58. Xin C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, et al. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. Vol. 40, International Journal of Obesity. 2016. 443–451 p.

Comment 3. The manuscript needs some editing work. There are some typos or grammatic problems. For example, "unseful" should be "useful". Please carefully check through the manuscript.

Reply: Thank you very much for your observations, Wwe revised the manuscript and corrected the three typing mistakes.

Comment 4. Some abbreviations are not defined. For example, AT, WNT. Please define it before use.

Reply: Thank you for your comment. We revised the abbreviations in the manuscript and corrected the ones you mentioned in addition to a few others.

glucagon-like peptide 1 (GLP-1)

glucose transporter- 4 (GLUT-4)

proinflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

wingless-related integration site (WNT) and the bone morphogenetic protein 4 (BMP-4)

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Thank you for the understandable update and the appropriate format of the review.

Comment 1. Just correct "unseful" to "useful", "preexissting" to "pr-existing" and abbreviation of metabolic syndome to "MetS" instead of "MS".

Reply: Thank you very much for your revision, we corrected the typing mistakes as well as the metabolic syndrome abbreviation.