

## Response to Review

December 10, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: review\_stea\_WJG-854\_2nd.doc).

**Title:** Strategies to rescue steatotic livers before transplantation in clinical and experimental studies.

**Author:** Qiang Liu, Maria-Louisa Izamis, Hongzhi Xu, Tim Berendsen, Martin Yarmush, Korkut Uygun

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 854

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 reviewer

(1) Reviewers' comment: Abstract: it does not give a clear delineation of the paper; the introduction section better describes the work.

*Authors' reply: Abstract has been revised to clarify as recommended.*

(2) Reviewers' comment: Induction of steatosis in animal models: Cafeteria diet is absent. This is a new robust model that better simulates the human metabolic syndrome and adipose inflammation; recent papers compared it to conventional high fat diets with improved outcomes. CAF diet provides many components associated with Metabolic Syndrome, including fat (saturated and trans-fats), sodium, and cholesterol, plus is low in protective nutrients such as fiber and micronutrients. Metabolomic profiling reveals mitochondrial-derived lipid biomarkers that drive obesity-associated inflammation. Sampey BP, Freerman AJ, Zhang J, Kuan PF, Galanko JA, O'Connell TM, et al. PLoS One. 2012;7(6): e38812. PMID: 22701716.

*Authors' reply: We thank for this reminder. We did not include it in the last version because there are 0 hits on "Cafeteria diet liver transplant" and 0 hits on "Cafeteria diet liver reperfusion" in PubMed. In the 3 hits on "Cafeteria diet steatosis liver" and 16 hits on "Cafeteria diet fatty liver", the studies were on the metabolism and pathophysiology of obesity and steatosis, but not on liver preconditioning before transplantation. Thus we did not initially discuss this model. However, following the reviewer's advice and considering the future potential of this model, we have added a discussion of this model in the current manuscript, in the section "induction of steatosis in animal models".. The new reference papers on this model were carefully selected from the relevant hits aforementioned. They are: Sampey BP et al 2011 and Pasarin M et al 2012 because these papers focused on "NAFLD" and the comparison to high-fat diet model.*

(3) Reviewers' comment: MP preservation: Also the advantages of sub-normothermic MP have been published for DCD models: add this reference.

*Authors' reply: The advantages of sub-normothermic MP on DCD models were actually been mentioned in the*

*previous submitted manuscript. The exact sentence in the text was “Normothermic (32~37°C) and sub-normothermic (20~30°C) machine perfusion (NMP and subNMP) preservation have been reported in experimental studies on livers, but mostly on their advantages for DCD models [141,155-160].”*

(4) Reviewers' comment: Summary and future prospective: The sentence "On the topic of IRI, the commonality of pathologies between steatotic and DCD livers should be kept in mind, and steatosis can be viewed as a factor that amplifies the impact of IRI" should be rewritten. Although the same method could improve both steatotic and DCD livers, the mechanisms involved in the ischemia/reperfusion injury in steatotic and DCD livers are completely different: underline this point. Furthermore, the increased susceptibility to ischemia/reperfusion injury of steatotic and DCD livers is well known.

*Authors' reply: We agree that the mechanisms in the IRI of steatotic and DCD livers are different; the intention was to convey the similarity in “the increased susceptibility to ischemia/reperfusion injury”. It has been revised as suggested.*

(5) Reviewers' comment: Eliminate: “Thyroid hormone (T3) administration on donors, which was effective to prevent non-steatotic rat liver IRI, is another such approach that could also be helpful on steatotic livers”. The effects of thyroid hormone modulation associated with ischemia-reperfusion and cold storage is still controversial (see Potentiation of ischemia-reperfusion liver injury by hyperthyroidism in the rat. Troncoso P, Smok G, Videla LA. *Free Radic Biol Med.* 1997;23(1):19-25)

*Authors' reply: We thank the reviewer's advice; adapted as recommended.*

(6) Reviewers' comment: Table I: n-acetylcysteine in the Antioxidant column for the KH solution should be added and the Glucose concentration (reported in the reference 141) should be modified. Verify all the components of the preservation solutions reported.

*Authors' reply: Upon rechecking the table, we realized that there is a diversity of the exact composition of Krebs-Heseleit solution, e.g. in Bessems M et al in *Transplantation Proceedings* 2006;38:1238-1242; Deenadayalu VP et al 2001 in *Am J Physiol Heart Circ Physiol* 2002;281:1720-1727; and Vairetti M et al in *Liver Transplant* 2009;15:20-29. We have revised the composition as listed in the paper [Vairetti M 2009] in the reference list.*

(7) Reviewers' comment: Table II: It represents only the index of this review.

*Authors' reply: We clarified the table caption to indicate we're limited in scope.*

(8) Reviewers' comment: Tables III: It not appropriately presented: in column II specify the division in genetic and dietary approaches.

*Authors' reply: The table was revised to ensure the genetic and dietary approaches divided clearly.*

3. References and typesetting were corrected

Sincerely yours,

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