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Editors-in-Chief
World Journal of Gastroenterology

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Dear Editors-in-Chief,

Re: Manuscript 24455 – Steatotic livers are susceptible to normothermic ischemia-reperfusion injury from mitochondrial Complex-I dysfunction

The authors would like to thank the Editor and Reviewers for their time and valuable comments. We have responded to all the comments and made the necessary changes to the manuscript as described below.

Response to Editor comments

Comment 1: Please provide a language certificate from a professional English language editing company

Response: With all due respect to the reviewers and editor, this manuscript has been submitted by a native speaker of English. The manuscript has also been reviewed by native speakers of English who were all educated in New Zealand and currently work in New Zealand (including the first author). The "minor language polishing" required involved amending the title and

abstract due to word counts as well as clarifying specific details of the manuscript (from reviewers) and we believed that this does not require input from a professional English language editing company. However, if the editor still requires a language certificate, this will be obtained.

Comment 2: Title should be no more than 10-12 words.

Response: The title has been amended as recommended.

Comment 3: A copy of the full approved grant application form(s) should be provided in PDF format.

Response: The grant application form will be provided in PDF format as requested.

Comment 4: An informative, structured abstract of no less than 246 words should accompany each original article.

Response: The abstract has been amended as recommended.

Comment 5: Please add PMID and DOI citation to the reference list and list all authors.

Response: The references have been amended as recommended with PMID and DOI citations for all available references. For the references without PMID and/or DOI, a file has been provided as requested.

Response to reviewer comments

Reviewer no 1.

Comment 1: **NAS score among various groups needs to be quantified**

Response: The NAS score was not described in our manuscript as there was no evidence of ballooning or inflammation in our model of simple hepatic steatosis; and our manuscript have been amended to clarify this in our results section. As we have used a common clinical grading system for hepatic steatosis as described in the literature, we did not believe adding another grading system score developed for grading livers with suspected steatohepatitis would be

relevant in our manuscript. This was consistent with the histological descriptions provided by other published experimental studies in this field.

Comment 2: **The authors needs to measure (MDA) level, total superoxide dismutase (SOD) activity, and nitricoxide synthase (NOS) in tissue. In addition the level of NO, NOS in serum and in liver tissues needs to be evaluated. Immunohistochemistry/q Rt PCR for TNF alpha and nuclear factor kappa B (NFκB) needs to be done.**

Response: We agree that these tests would have been useful but as detailed in a review performed by our group (*Chu et al. The Open Journal of Transplantation. 2013. 6: 10-32*), these tests have been shown to be either increased (MDA, TNF-α, NOS, NO) or decreased (SOD) in steatotic livers post-IRI. We did not perform these tests as our study was designed to focus on detailed mitochondrial function analysis and these additional tests, while good to have, may have detracted the manuscript from its primary focus of mitochondrial function analysis. That being said, we believe that these additional tests would be the topic for future and much more complicated set of studies which go well beyond the scope of this manuscript and project.

Comment 3: **It has been mentioned the rats were enrolled at 3 weeks old. Please clarify whether the animals are weaned are kept with mother.**

Response: The rats were weaned at 3 weeks and removed from the mother at that stage. The manuscript have been amended to clarify this.

Comment 4: **In abstract it has been mentioned 3 gps and in materials and methods it has been 6 Gps .Please clarify.**

Response: In the abstract, the description was of 3 groups for each dietary state (high-fat/high-sucrose or control diet) which would be equivalent of the 6 groups described in the materials and methods section.

Reviewer no. 2

***Comment 1*: Please describe the fat and carbohydrate contents in the control diet. What is the fat in high fat diet (saturated or unsaturated fat, etc.)?**

Response: The dietary composition of the diets has been added to the manuscript as recommended. With regards to the high-fat/high-sucrose diet, the fat content were made up of 87.7% lard and 12.3% from soybean oil, which was similar to the 45% high-fat diet from the same company (ResearchDiet, D12451).

***Comment 2:* The histology of necrosis (Fig. 2) is not very convincing. Moreover, Fig. 2D has much less fat than B and F.**

Response: We apologize that the wrong image was previously formatted onto the figure and Figure 2D have been amended to the correct image. We believe that the current image better show the degree of inflammation and steatosis.

***Comment 3*: This study measured respiration using liver homogenates but not isolated mitochondria. Some of the oxygen consumption may be not completely related to mitochondrial respiration but linked to other pathways (e.g. proxisomal oxidation of fatty acids). What percentage of the respiration is cyanide inhibitable?**

Response: Liver homogenates were chosen over isolated mitochondria as liver homogenization allowed avoidance of mitochondrial selection bias and were more physiological. It also permitted shorter processing time with rapid measurement of the entire mitochondrial population (*Mittal et al. HPB 2011; 13:332-341*) and has been considered to be superior to mitochondrial isolation (*Pecinova et al. Mitochondrion, 2011; 11(5): 722-8*). We agree that it is possible that oxygen consumption may be linked to other pathways but as discussed, it has been shown that liver homogenates are better representation of mitochondrial function analysis. The mitochondrial assay did not utilise cyanide as this was not the standard chemical used in our lab but we used antimycin-a as inhibitor of Complex III to measure so we are unable to comment on cyanide-inhibitable respiration. However, there was minimal residual respiration after addition of antimycin-a, indicating minimal non-mitochondrial respiration.

Comment 4: **How IPC decreased I/R injury in steatotic livers? Did IPC decrease reactive oxygen species formation in this study?**

Response: As discussed in our manuscript, we were not able to determine an exact mechanism of the protective effect of IPC in steatotic livers. We have demonstrated that IPC did not affect mitochondrial function or key mitochondrial enzymes, which was the first time in dietary-induced hepatic steatosis and in a detailed fashion. We believe that this is an advance on our understanding of the interaction between steatosis, IRI, IPC and mitochondrial function. We do agree that changes in reactive oxygen species formation may be a possible mechanism and would be an interesting topic for future studies which are beyond the scope of this project.

Once again, thank you for the time in reviewing this manuscript. We think we have now corrected the manuscript in line with the comments and it is from our point of view a much improved manuscript. If there are any further corrections or changes that need to be done, please do not hesitate to contact us.

Kind regards,

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