Author's response to reviews

Title: Alteration of renal respiratory complex-III during experimental type-1 diabetes

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Author's response to reviews:

November 10, 2008

Dear Editor,

We are grateful to the reviewers for noting the specific strengths and weaknesses of our research article titled “Alteration of respiratory complex-III during experimental type-1 diabetes”. It was clear from both reviewers that they considered the research article to be of high importance and provided novel insights in understanding the involvement of mitochondrial dysfunction in the pathogenesis of diabetic nephropathy. Our responses to each reviewer and the resulting changes in the manuscript are listed on the following page.

Thank you for your help in improving the quality of this manuscript.

Sincerely,

Lee Ann

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RESUBMISSION MODIFICATIONS

Comment 1: “It has been reported that in STZ diabetic rats the kidney
mitochondrial ATPase and respiratory activity with selected substrates increased (Ref. 9 in the manuscript). The authors should have discussed increased ATP content in this context. Increase in ATPase per se does not increase ATP content; increased respiratory activity provides the driving proton motive force”.

Response: We apologize for not correctly acknowledging the importance of reference 9 to our study findings. We have added several sentences in the background and discussion as shown below:

Background (page 4):

• Katyare et al. showed that respiration rates and ATPase activity were elevated in diabetic renal mitochondria. This study also demonstrated that renal mitochondria were tightly coupled during diabetes (Ref. 9).

Discussion (page 14):

• Interestingly, a study by Katyare et al. (Ref. 9) also demonstrated that respiration and ATPase activity were increased in diabetic renal mitochondria. Thus, it is possible that the increased respiration leads to increased proton motive force and hence increased ATP levels which may serve to fulfill the increased energy demands of the kidney during diabetes. Increased respiration would likely result in more superoxide (oxidant) generation which could lead to altered Complex III.

Comment 2: “It has also been reported that the cytochrome aa3 contents increased in STZ diabetes (Ref. 9 in the manuscript). However, the authors did not find changes in complex IV. This discrepancy should have been discussed”.

Response: Despite the findings by Katyare (Ref. 9) which showed increased cytochrome aa3 contents in renal mitochondria during diabetes, we did not detect any significant changes in complex-IV activity in our rat model of diabetes. It is also important to note that the authors could not correlate the changes in cytochrome content with the altered respiration rates observed during diabetes. In addition, Katyare et al. did not measure complex IV activity and we did not measure cytochrome aa3 contents so a direct comparison of these studies is difficult to assess. One important difference between the two studies is the source of renal mitochondria used: we used whole kidney mitochondria, while Katyare et al. used cortical mitochondria (please see comment 3 below). In the revised manuscript, we have incorporated these statements in the discussion section.

Comment 3: “The authors used whole kidney for isolating mitochondria. If medulla is not removed this can vitiate the results. It would have been desirable to use cortex for isolation of mitochondria. Then one gets clean results”.

Response: Although the renal cortex has been primarily considered to be the energetically active segment of the nephron, several studies suggest that the medullary thick ascending limb (mTAL) is an active site for reabsorption in kidney
and thus might play a major role in mitochondrial superoxide production. In addition, increased oxidant production has been observed in both the cortical and medullar regions of the kidney. Thus, we felt it was important to include both cortex and medulla (whole kidney) for mitochondrial isolation and study the net effect of diabetes on the renal mitochondria.

Comment 4: “In one or two places the sentences are incomplete. Manuscript needs to be thoroughly and carefully checked for such errors/omissions”.

Response: We apologize for any such errors. We have carefully checked our manuscript in the revised version.

Comment 1: “In particular the authors indicate that the levels of complex III are decreased on BN-PAGE, yet a complex III immunoprecipitation brings down more complex III subunits than in control. The authors indicate that there could be an assembly defect/intermediate yet they do not show this. It is therefore important that they verify that the complex III seen on BN-PAGE is in fact complex III (e.g. by western blotting) and also showing that they can detect such intermediates (e.g. by BN-PAGE followed by SDS-PAGE in the second dimension and then blotting for Rieske and Core 2)”.

Response: As suggested by the reviewer, we have performed additional experiments to prove that complex III in the BN-PAGE was in fact complex III. These included western blotting for Rieske and Core 2 proteins after BN-PAGE (new Figure 4 A & B) as well as BN-PAGE followed by SDS-PAGE in the second dimension (new Figure 4 C & D). These data clearly demonstrate that the band identified as complex III contains both the Rieske and Core 2 subunits. If the reviewer intended for these studies to be included for review purposes only, then we can modify the revised manuscript accordingly.

Comment 2: “The authors should also show the entire lanes on Figure 2A. If there are other differences observed between the control and diabetic lanes, these must be mentioned and interpreted. It may be important to quantify the levels of complex III and perform a statistical analysis”.

Response: Figure 2A does show the entire lanes of control and diabetic mitochondria. The only differences observed were a decrease in complex-III and an increase in complex-V levels. As suggested by reviewer 2, we have quantified the levels of complex-III by densitometry and included this new data as Figure 2b in the revised manuscript.

Comment 3: “In discussion, change the text “(decreased expression on BN-PAGE)” to “(decreased levels on BN-PAGE)”.

Response: We apologize for the confusion, and the text was corrected from “decreased expression” to “decreased levels” in the revised manuscript.

Comment 4: “How do the authors reconcile a decrease in complex III
levels/activity with the observed increase in ATP production? The suggested change in complex III appears to indicate that this is already in response to mitochondrial hyperpolarization and so ATP synthesis should be already affected. Might the complex V assay not be sufficiently sensitive and/or lack physiological relevance?”

Response: We apologize for the confusion with this point and have added a paragraph in the discussion which offers our explanation of these findings. A study by Katyare et al. using STZ-diabetic rat kidneys has showed that respiratory activities for selected substrates as well as ATPase activity were increased during diabetes (Ref 9 in manuscript). These data correlate well with our findings with complex V activity and ATP levels. Thus, it is possible that the increased respiration leads to increased proton motive force (hyperpolarization) and hence increased ATP levels which may serve to fulfill the increased energy demands of the kidney during diabetes. Increased respiration would likely result in more superoxide (oxidant) generation which could lead to altered Complex III. A paragraph has been added in the discussion (page 14) to better address the apparent contradiction with regard to complex III and complex V activity.