Note of Concern

Editor's Note

It has been brought to our attention that some of the data from the article entitled "Oxidative stress-mediated mitochondrial dysfunction contributes to angiotensin II-induced nonalcoholic fatty liver disease in transgenic Ren2 rats," by Wei et al (Volume 174, pages 1329–1337; published in the April 2009 issue of *The American Journal of Pathology*), have been found to be in error. The errors involved published Figures 2G and 5G; corrected figures plus new data appear below. Corresponding author Dr. Jamal A. Ibdah has also provided the following explanation for consideration by the Journal's readers:

To the Editor-in-Chief:

The original experiments for Figures 2G and 5G were performed once (single data point per animal). Because these data were added during the revision process, frozen tissues from various experimental groups were used to generate the data. The error was discovered during a review and comparison of the data in conjunction with discussions within our research group about the limitation of using frozen samples for fatty acid oxidation measurements. A discrepancy was noted between the data collected and the data entered for analysis. The error occurred during data entry for statistical analysis. The error, which was discovered after the article was published in April 2009, was subsequently confirmed and shared with all authors in May and June. We then promptly notified the Editors of the Journal.

To generate the replacement data, we conducted three different experiments using frozen liver tissue from four animals in each experimental group reported in our publication (three data points per animal). We measured both complete oxidation of palmitate to CO₂ as well as total oxidation (palmitate to soluble acid metabolites and CO₂). The three data points from each animal were averaged and entered into the data analysis. The mean ± SEM for each experimental group were calculated and shown in revised Figures 2G and 5G for complete palmitate oxidation and in new Figures 2H and 5H for total palmitate oxidation. Because complete oxidation to CO₂ accounts for only a very small fraction of palmitate oxidation (<2% of total oxidation), we have included comparison of total palmitate oxidation among the various experimental groups. The results show no statistical significance in total palmitate oxidation among various groups (Figures 2H, 5H). However, complete oxidation of palmitate to CO₂ was higher in the Ren2 versus SD animals (1.87% versus 0.84%). Treatment with valsartan reduced complete oxidation (1.27% to 0.76%), while treatment with tempol increased complete oxidation (1.27% to 1.62%) but at levels that were not statistically significant. There was significant variation in data points from one experiment to another that could be attributed to frozen tissue samples.

Extensive studies in our published article, independent of the palmitate oxidation data in question, have documented several novel findings that we believe have contributed significantly to the field. Angiotensin II induced mitochondrial oxidative damage, leading to depletion of mitochondrial genes and proteins, as was

Figure 2. Hepatic fatty acid β -oxidation. **G:** The percentage of palmitate oxidized completely to CO₂. **H:** Total palmitate oxidized (nM/g/hour). The results are mean $\pm$ SEM for four rats for each group. SD, untreated Sprague-Dawley control; RC, Ren2 control; RV, Ren2 valsartan; RT, Ren2 tempol. * $P < 0.05$, Ren2 versus SD.

Figure 5. Effects of valsartan and tempol on β -oxidation. **G:** The percentage of palmitate oxidized completely to CO₂. **H:** Total palmitate oxidized (nM/g/hour). The results are mean $\pm$ SEM for four rats for each group. SD, untreated Sprague-Dawley control; RC, Ren2 control; RV, Ren2 valsartan; RT, Ren2 tempol. * $P < 0.05$ RV versus RC.

evident by reduced expression levels of cytochrome c and other molecular mitochondrial markers. In addition, angiotensin II induced mitochondrial ultrastructural abnormalities, reduced mitochondrial content, and reduced mitochondrial fatty acid oxidation enzymatic activities. We have shown that these mitochondrial abnormalities are mediated via oxidative stress.

We have also documented that all these mitochondrial abnormalities were dramatically attenuated by treatment with angiotensin II receptor blocker valsartan or superoxide dismutase/catalase mimetic tempol, proving a causative effect between angiotensin II and reduced content and function of hepatic mitochondria mediated by oxidative stress.

Unfortunately, our replacement data on palmitate oxidation do not support that fatty acid oxidation is impaired in the Ren2 model as concluded in our published article. However, it is important to interpret the data on palmitate oxidation with caution due to several limitations. First, frozen samples were used; recent work in our laboratory suggests that freezing significantly reduces palmitate oxidation measurements. Second, the measured palmitate oxidation includes both mitochondrial and extramitochondrial oxidation; extramitochondrial fatty acid oxidation pathways are likely to be activated when mitochondrial fatty acid oxidation is impaired. Third, complete oxidation to CO₂ accounts for a very small fraction of total oxidation (<2% in our data); such small fractions may limit reliable comparisons among various groups.

Our studies in the published article provide strong evidence that oxidative stress-mediated mitochondrial abnormalities in content, structure, and function contribute to angiotensin II-induced nonalcoholic fatty liver disease. Our new data on β -oxidation, although having significant limitations, raise the possibility that mechanisms other than impaired hepatic fatty acid oxidation may cause development of hepatic steatosis in the Ren2 model. Future studies are warranted to explore this possibility.

All authors have seen and approved the replacement data and this letter.

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