Reviewer's report

Title: Retinal upregulation of inflammatory and proangiogenic markers in a model of neonatal diabetic rats fed on a high-fat-diet

Version: 2 Date: 2 January 2013

Reviewer: Claudio Punzo

Reviewer's report:

BMC Ophthalmology

Review of Manuscript: Retinal upregulation of inflammatory and proangiogenic markers in a model of neonatal diabetic rats fed on high-fat-diet

Authors: Jorge E. Mancini, Gustavo Ortiz, J.Oscar Croxatto, Juan E. Gallo

General Comments:
The work by Mancini et.al., describes the effect of high fat diet in a background of type 2 diabetes. The work is of interest as it addresses the effect of diet on the development of diabetic retinopathy. The authors show that there is a difference between diabetic rats and diabetic rats that are feed a high fat diet (HFD). The manuscript thus merits publication, however there are multiple issues with the current form. The interpretation of the data seems to be sometimes confusing as the authors contradict themselves regarding the phenotypic observations. Graphs in most figures are poorly labeled with respect to their axis and some figure legends need also revisions. Additionally, throughout the manuscript the text could be improved for clarification of the data presentation.

Specific Comments:

Abstract Section:

- Methods:
  - The third sentence:” Each group had a corresponding control of group of non-diabetic rats.” This sentence appears confusing. There seems to be only one control group that is not injected with STZ and not two control groups. Please reword the sentence or clarify what is meant. Additionally, the word control seems to used interchangeably to designate non-STZ or STZ but no HFD. It is important that the authors spell out each time which control they mean.
  - Last Sentence: ”Primary antibodies against ….. and TNF-a were tested for immunohistochemistry and Western Blot (WB).” The second half of the sentence should read more like: “….. and TNF-a were used for immunohistochemistry and Western Blot (WB) analyses.”

- Results
  - Second sentence: “Diabetic animals …. Number of pericytes than controls.”
What controls are meant here? Only non-STZ or also STZ but no HFD. See also comments on Result Section and Discussion Section below. This part of the data presentation seems to be confusing.

Introduction Section:
Third paragraph second sentence: “An increased expression ..... specific disorder of the electroretinogram .....” Second part of the sentence sounds awkward and should be rephrased. Consider: " ..... abnormalities in the electroretinogram …"

Methods Section:
Rat models of diabetes:
• The authors state in this section that the HFD has 25.8% fat and the standard diet has 6.8% fat. The authors refer to tables 1 & 2 for the exact composition of the diets.

Fatty acid profile:
• Here the authors state that the HFD is composed of 24% saturated and 24% polyunsaturated fats while the conventional diet is composed of 46% saturated and 4% polyunsaturated fats. The same numbers are found in table 2. Additionally, table 2 shows that the calorie content of the two diets is 3.2 and 5.6 Kcal/g for regular and HFD respectively.

While the numbers for the calorie content of the food make sense with respect to published literature, the composition of the diets and the resulting calorie numbers are not entirely clear. The carbohydrate and protein composition of both diets is the same thus one would assume that the fat percentage should be roughly the same. It is therefore not clear how the calorie difference comes about. The percentage of total fat presented in table 2 is roughly the same for both diets yet the authors say that the HFD has 26.8% and the regular has 6.8% of fat. Can the authors elaborate on the number in table 2 to clarify exactly in regards to what these percentages are calculated? From table 2 one assumes that the composition of the fat diet changes but not the total amount of fat.

Results Section:
• Second paragraph: The authors state that: “The number of pericytes was found significantly lower in diabetic rats on a HFD than in controls.” Please clarify what controls means, non-diabetic or diabetic. Fig. 1 shows statistically significant changes for both diabetic groups compared to non-diabetic controls but no significant change between the two diabetic groups. This is also what is stated in the figure legend.

• Second paragraph: The subsequent sentence refers to acellular capillaries. Here the authors say in the text that there is a difference between the two diabetic groups. The figure does not show a statistical significance and the figure legend of figure 1 also says that there is no difference. Please clarify the text to address this discrepancy.
• Third paragraph: Authors analyze retinal thickness and number of ganglion cells. The images shown in panel A of figure 3 are not very convincing in terms of a reduction in retinal thickness. Since panel C of the same figure (quantification of thickness) has no labeling with respect to the y-axis it is difficult to appreciate what the difference in thickness is. What does 1-4 stand for? Y-axis needs to be labeled. Similarly in panel D there is no labeling of the y-axis. Although there, it appears clear that the number must reflect the number of ganglion cells. However, there is no mention as to how much retina was analyzed. Is this number the amount of ganglion cells per 100micron of section? How big is the visual field?

• Figure 5: Figure 5 shows the RAGE staining and WB quantification. While the increase in RAGE is very obvious in diabetic rats on HFD as seen in the WB, the control for GFPDH looks very fuzzy and much more intense. The authors say in the Methods section that all experiments were performed in triplicate for the quantification. Do the authors have a better picture for the GFPDH band in the HFD diabetic rats? Also, the y-axis for both graphs, the one in figure 5 and the one in figure 6 need to be labeled. What do these units mean? Additionally, figure 5 & 6 could be combined into 1 figure.

• Paragraph six: Text regarding TNF-a reads confusing. The authors say that the upregulation is seen in both diabetic groups in the text and in the figure legend but the western shows only upregulation in the HFD group. From the images presented there is not much difference in staining between control animals and diabetic animals on normal diet, which seems to be confirmed by the WB analysis in figure 8. Do the authors have a better panel to show the upregulation in diabetic animals on normal diet? Again figure 7 & 8 could be combined into 1 figure. What is concerning about the WB of figure 8 is that the control protein GFPDH is also much higher in the diabetic animals on HFD. Is that difference account for in the quantification? Also, specify units in y-axis.

• Figure 9 & 10 could also be combined and labeling of the y-axis should be included in figure 10.

• Figure 11: The figure legend of figure 11 starts awkward. Please change.

Discussion Section:

• Third paragraph: The authors state that: “In our group of animals on HFD there was a significantly higher frequency of vessel abnormalities in the form of capillary obliterations, loss of pericytes and endothelial cells as well as ganglion cells than in control and diabetic animals fed on normal diet.” This statement is not supported by the data presented in the results section (see comments on second paragraph of result section). Although in the results section the authors state some differences they say that there are no differences in the figure legends. The authors need to be consistent in what they describe.

• Fourth paragraph: “5-LO ARNi” should most-likely read “5-LO RNAi”
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

N/A