Reviewer’s report

Title: Reintroducing testosterone in the db/db mouse partially restores normal glucose metabolism and insulin resistance in a leptin-independent manner

Version: 0 Date: 02 Feb 2018

Reviewer: Rowan Hardy

Reviewer's report:
This is an interesting manuscript and an impressive body of work. The authors show that db/db mice have lower circulating testosterone and insulin resistance with high fat exacerbating suppression of testosterone. This looks like it is most likely mediated through gonadal axis suppression in the db/db mouse and should be stated clearly within the abstract and discussion. Normalising testosterone in castrated Db/db and wt animals restored glucose tolerance to all groups suggesting testosterone signalling is central to insulin resistance and glucose metabolism and hepatic steatosis in this murine model. The protective effects of testosterone appeared to be independent of leptin signalling as has been previously suggested. This is an important statement that should be made in the abstract and title of the paper. Interestingly, these effects did not appear to be dependant of test aromatization to oestrogens based on the use of selective inhibitors. However, the inhibitor is not fully validated in the data and needs to be if the authors wish to include it. This study merits publication and is well suited to this journal, however major revisions of the abstract and discussion need to be undertaken for clarity.

Major Comments:

Title:
The title should be changed to better reflect the primary findings of the paper. Namely that reintroducing testosterone in the db/db mouse partially restores normal glucose metabolism and insulin resistance in a leptin independent manner
Abstract:
The abstract should be significantly rewritten for clarity. Throughout it is difficult to follow how experimental groups were set up and how results relate to respective groups. There are a lot of findings to report and this needs to be done with greater transparency and clarification between the conclusions drawn from db/db versus db/wt, NFD versus HFD and non castrated versus castrated.

Minor
Better labelling and description of what db/+ and db/db/ are in reference to WT and leptin receptor KO
"db/db mice (a model of obesity) were used as controls". Controlling for what? Better clarity required

Introduction:
As with the intro, a much better description of the animal groups and how they relate to the questions being asked is required. Please address this better in the final paragraph. The comparisons you are making are fairly complex, involving several different models. Please rewrite and clarify this in the intro. I would suggest going in the order of 1, investigating testosterone and glucose metabolism in the in the db/db mouse relative to WT db/+ controls and assessing NFD and HFD on this. 2, Examining replacement of testosterone in db/db to assess its contribution to glucose metabolism and liver steatosis. 3, examining whether aromatisation is important to the testosterone affects.

Methods
How were plasma samples collected
What were animal numbers in each group
Please provide details on power calculations for animal experiments.
ANOVA where applicable: give details of post hoc test. Are all data normally distributed?

Results
Please state animal numbers per group in fig legend
Please combine data from respective figures together onto one page for better comparison

Fig3b: Magnification does not look completely comparable across all groups and staining intensity appears different in castration testosterone supplemented group. Please include further supplementary representative images to better validate these representative images

Fig 4 a and b require labelling in reference to the tissue type

Fig 4: please include any data supporting an effective functional action of the aromatisation inhibitor anastrozole so that we know it is working. Without this you should not include this data as it may not effectively inhibit at a tissue specific level 100% making conclusions as to the significance invalid

4e: label GTT is missing

Discussion

From the start clarify that leptin deficiency clearly regulates testosterone levels in this model. This is your first finding. It may be direct or indirect. These data cannot differentiate this. Please review the literature on this and discuss. Supports previous human data where leptin affects testosterone in low body weight males: Wabitsch J, Serum leptin, gonadotropin, and testosterone concentrations in male patients with anorexia nervosa during weight gain. Clin Endocrinol Metab. 2001 Jul;86(7):2982-8.

I find the data in figure 5 a little distracting from the main story. Can this be introduced as supplementary material and reduced.

Please update the discussion to reflect changes in the abstract and introduction

Are the methods appropriate and well described?

If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?

If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?

If not, please explain in your comments to the authors.

Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?

If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English

Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

Declaration of competing interests

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