Reviewer's report

Title: Maternal exercise does not impact offspring exercise or body composition in mice

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Reviewer: Hui Hui Chen

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The manuscript by Kelly and colleagues investigated the impact of maternal voluntary running wheel exercise from prior to or during gestation on the exercise behavior in the offspring. Although the levels of exercise in the offspring correlate with those in the mothers, the authors did not find any changes in body weight, nor lean and fat tissue compositions. The negative results on body composition are against some previous studies; however it is important to report such finding. There are several aspects in this study that require further clarification.

Major Compulsory Revisions

1. Please provide information on the ethics approval for this study.
2. Please justify the rationale of using Hsd:ICR mice? What is special about this strain?
3. Please described in details how the body composition was measured? DEXA or CT?
4. Please review the hippocampal genes in the offspring that were changed due to maternal exercise in the previous studies. On page 8, the authors reported the significant correlation between maternal wheel running speed and distance with those in the offspring. However, there are no measures or discussion on the possible mechanisms for this only positive finding in this study. Would the same hippocampal genes in the literature be involved in the maternal-offspring correlation found in this study? Would any epigenetic changes be involved? Will an environment insult in postnatal life, such as high-fat diet consumption, make a difference in the body composition and metabolic disorders in such offspring?
5. It is well known that the hypothalamus is critical for energy homeostasis. Why the changes in the hypothalamic gene expression by maternal exercise were not discussed?
6. Were the birth weights different between the G2 groups due to maternal exercise?
7. It has been found in the other studies that metabolic markers can still be improved even when body weights are not changed. Were the blood levels of glucose, insulin, and lipids different between the G2 groups?
8. What are the average starting body weights of the three G1 groups at 3 weeks of age?
9. How many G2 litters were used from each G1 group?
10. Please provide information on end-point euthanasia and results on postmortem measurement of body length, weights of different fat pads and organs, including skeletal muscles.

11. There were no details on the statistical method used to analyze the differences between the maternal and offspring groups. According to the second last line of the Statistical analysis section, it seems that the authors have used student t test, which is not suitable for this type of study with more than two experimental groups. T test can significantly increase the error. The two factors, maternal exercise and offspring exercise, need be analyzed together. Two-way ANOVA should be performed to analyze the interactions between the two factors, followed by Bonferroni or Tukey post hoc test if the interaction is significant. As such, the statistics needs to be re-performed for all the data.

12. There are several number errors in the Supplementary table 4. This table needs to be carefully revised.

There are no values on the % of fat and lean masses for 7 weeks of age.

The values of the female body weight were between 1.3-1.54 g at 4 - 9 week of age. It is too small for a mouse.

The values of the male body weight were between 8000-8400 g at 8-9 weeks post-exercise. It is unlikely that a mouse can grow to this size.

The values of the % of lean mass at 6 weeks, 8-9 weeks prior to and after exercise are reported as 10 powered by 9 - 11.

13. In the literature, the mean fat mass % of a male mouse is 26% and that for the female is 22% (Reed et al. Forty mouse strain survey of body composition. Physiol Behav. 2007;91:593-600.) Is there any particular reason that the average % of fat mass in the Hsd:ICR mouse is only between 1-3%?

Minor Essential Revisions

14. Page 11, last line, it reads ‘These breeding pairs were utilized to generate 15 females.’ However, in the method, 15 G1 females were assigned to each group, in total 45 G1 breeders. Is this a typo?

15. Page 14, second last line of the Statistical analysis section: it should read ‘Statistical significance was judged at P< 0.05 and all P-values …’.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.