**Reviewer's report**

**Title:** Atkins diet for the developing baby? Effects of a ketogenic diet during pregnancy on embryonic growth in the mouse

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**Reviewer:** Liu Lin Thio

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Sussman et al. examine the effect of the ketogenic diet (KD) on embryonic development in mice. This topic is important because of the growing use of dietary therapies in treating adults with epilepsy and the potential beneficial effects of dietary therapies in other neurological and non-neurological conditions afflicting adults. The authors found an age dependent effect of the KD diet on mouse embryogenesis.

Generally, the manuscript is well written and well organized, though the title requires revision. The goals of the study are clear. The methods are well described, but the study design has some potentially important weaknesses that could influence the results and conclusions. Within the limitations of the methods and study design, the data presented are sound. However, the authors should address these issues and discuss any potential effects they may have on their conclusions. The Discussion also requires some clarification.

**Major Compulsory Revisions:**

1. **Title.** The authors refer to their experimental diet as an Atkins diet in the title, though the 4:1 ketogenic diet used is a classical ketogenic diet. The authors should not use “Akins diet” in the title because they did not examine the effects of an Atkins diet.

2. The pregnant rat has different nutritional requirements than one that is not pregnant (Reeves et al. J Nutr 1993,123:1939-1951). I believe the Standard Diet (SD) used by the authors meets the nutritional needs of a pregnant dam. The authors should confirm that the SD meets these nutritional needs and add a sentence indicating such in the Methods. However, is the KD used in this study designed to meet the nutritional needs of a pregnant dam? The authors should indicate whether the KD meets these nutritional needs in the Methods.

3. **Table 1.** The pregnant dams fed the KD weighed significantly less than those fed the SD at E17.5. The KD embryos also were smaller than the SD embryos at E17.5. These findings may indicate that the KD did not meet the caloric needs of the pregnant dams. Did the authors monitor the caloric intake of the pregnant dams? Did the authors monitor the nutritional status of the dams? If the KD fed dams did not receive their minimal nutritional requirements, the authors’ findings may reflect nutritional deficiencies rather than the effect of ketosis.

4. **Figure 5.** The authors should provide the actual volumes of the organs along
with the percentage of the total volume, so the reader can assess the true differences between the SD and KD embryos and the growth that occurs between E13.5 and E17.5.

5. Figures 6-8. The authors should provide the actual volumes of the structures analyzed along with the t-statistics. Do the authors believe that the statistically different volumes are all biologically significant?

6. Table 2. The comparisons would be more meaningful if the data for the 16 week postnatal mice were presented as percentage volumes.

7. Discussion, 1st page, 3rd paragraph, 4th sentence; Discussion, 2nd page, 3rd paragraph, 3rd sentence; Discussion, 2nd page, 4th paragraph, 1st sentence. The authors refer to “deformations.” I think that the authors are referring to differences revealed by deformation analysis rather than “deformations.” In any case, the authors should clarify what they mean.

8. Discussion, 1st page, 4th paragraph. The arguments in this paragraph need further elaboration. For example, the authors found that the KD slightly reduced triglycerides in the female mice before gestation. This result is similar to that reported by others (Kennedy et al. Am J Physiol Endocrinol Metab 2007,292:E1724-39). In contrast, the KD increases triglycerides in humans (Kwiterovich et al. JAMA 2003,290:912-20). How do the authors reconcile these findings and what are the implications of their findings for humans? In addition, the authors argue that the increased ketones and triglycerides at E17.5 reflect increased nutrient demand by the fetus. However, could the increased ketones and triglycerides reflect decreased ketone utilization?

9. Discussion, 2nd page, 2nd paragraph. The authors argue that glucose is preferentially used at the end of gestation when rapid physiological growth occurs. How do the authors reconcile their findings with those of Cotter et al. (J Biol Chem 2011,286:6902-10) who found that mice lacking succinyl-CoA:3-oxo-acid CoA- transferase (SCOT) die as neonates. SCOT is necessary for ketone oxidation.

10. The authors compare the volume of different anatomical structures in the SD and KD embryos, but they do not provide any information regarding the morphology of these structures. The authors should indicate whether the KD embryos had any malformations or more malformations than the SD embryos at E13.5 and E17.5.

11. Discussion. The authors should discuss the limitations of their methods.

12. The authors should discuss their findings and those obtained by Soares et al. (Nutr Neurosci 2009,12:260-266).

13. The authors should also discuss the potential effects of the type of fat provided in the KD used in their study. Specifically, the authors should consider the effect of polyunsaturated and saturated fats in their KD as discussed by Soares et al. (2009).

Minor Essential Revisions

1. Discussion, 1st page, 2nd paragraph, 1st sentence. “The data reveals...”
should be “The data reveal…”

Discretionary Revisions

1. The significance of the authors’ findings would be strengthened if the authors could provide data regarding the KD embryos after birth. Are the KD embryos viable? Do they feed normally?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.