Reviewer's report

**Title:** Differential utilization of ketone bodies by neurons and glioma cell lines: a rationale for ketogenic diet as experimental glioma therapy

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**Reviewer:** Thomas Seyfried

**Reviewer's report:**

The authors have evaluated the effects of ketone bodies and the ketogenic diet on the viability of normal cells and tumor cells. Some of their observations support previous findings from other groups, but some of their observations differ from the previous findings of others. Evidence is presented showing that ketone bodies can be used to maintain viability of normal neurons, but cannot be used to maintain viability of the glioma cells in the absence of added glucose. This is novel information that has not been previously reported. Although the results support the conclusions, the authors need to better address why some of their findings differ from previous observations.

**Essential Revisions**

1. Previous studies from the Tisdale, Friedrichs, Zhou groups showed that the genes and proteins for ketone metabolism were lower in tumor cells and tumor tissue than in normal cells and normal tissues; Tisdale et al., British J. Cancer 1983;47(2):293-7; Cancer Biochem Biophys 1984;7(2):101-7; Zhou et al. Nutrition & metabolism 2007;4:5; Fredericks and Ramsey, J Neurochem 1978;31(6):1529-31. The author’s results show that genes and proteins for ketone metabolism are not reduced in their glioma tumors. Were experimental conditions similar or different between the author’s methods and those of previous studies? The authors will need to discuss in more detail why their findings appear to be at odds with the findings of these other groups.

2. If expression of genes and proteins for ketone metabolism is similar in normal neurons and in glioma cells, why are the glioma cells unable to use the ketones for energy like the normal cells? Is it mitochondrial dysfunction that prevents glioma cells from using ketones? The authors cite only a single paper showing that mitochondria are defective in gliomas (Meixensberger et al 1995). How would a defect in mitochondrial respiration prevent ketones from providing energy to the cell? Do other studies suggest that mitochondrial respiration is defective in gliomas or in other tumors?

3. The previous study of Skinner et al show that ketones actually killed neuroblastoma cells in the presence of glucose (Skinner et al., J Pediatr Surg 2009;44:212-6). The authors did not find that ketones alone could kill tumor cells in their studies. It would be important for the author’s to address the findings from the Skinner et al study in relationship to their findings.
4. The authors showed that the ketogenic diet does not have therapeutic effect against brain tumor growth when the diet is fed in unrestricted amounts that do not lower glucose levels. While these findings agree with previous findings of the Seyfried group, they differ from the recent findings of Stafford and colleagues (Stafford et al., Nutrition & metabolism 2010;7:74). It would be helpful for the authors to compare and contrast their results with the findings from these other studies. While they attempted to do this with the Otto et al., study, they should also do this with the Strafford et al., study.

5. The authors should provide a legend for supplementary Figure 1. They should also discuss the interesting finding showing that expression of GLUT1, VEGF, and MCT4 was significantly lower in 21% oxygen than in low oxygen conditions. Did the authors examine lactic acid production or cell viability under these conditions?

6. The authors mention in their discussion (p 18) that calorie restriction and weight loss in association with cancer therapy is undesirable, despite evidence showing therapeutic efficacy of restricted calorie intake on tumor growth. However, many cancer patients suffer significant weight loss and indirect calorie restriction following administration of chemotherapies. Do the authors consider toxicity-associated weight loss less harmful to patients than non-toxic self-induced weight loss? This should be addressed.

Minor issues

1. The authors should mention that the “absence” of ketones in Fig 2A represents the “control” group.

2. The data in Fig 2B are not as clear as the data presented in Fig 2A. Data for at least one glioma line should be presented as the data for normal neurons in Fig 2A. Also, how do the data look for normal neurons when presented on the graph in Fig 2A? These comments are only suggestions in order to more easily compare the differences between normal neurons and glioma cells to the presence or absence of ketones.

Level of interest: An article of importance in its field

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:

I declare that I have no competing interests