Author's response to reviews

Title: Growth of human gastric cancer cells in nude mice is delayed by a ketogenic diet supplemented with omega-3 fatty acids and medium-chain triglycerides

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Author's response to reviews: see over
Cover letter with a point-by-point description of the changes made

First, we would like to thank the reviewers for their comments and suggestions that will improve the quality of the manuscript.

Reviewer 1 - Thomas Seyfried

1. The reviewer’s comment that we presently cannot show which components of the diet (ketone bodies, MCT or omega-3 fatty acids) are responsible for the delayed tumour growth is fully justified. Further studies will focus on this. We agree with the reviewer that ketogenic diets do not necessarily reduce tumour growth. He is also right that we cannot state whether the effects observed are due to fish oil (or vegetable oils with elevated levels of omega-3 fatty acids used in our study) or MCT. We have no results on this at present. Further experiments are in preparation but are not subject of the present study. Therefore, we added a sentence at the end of the first paragraph of the discussion that the “components” of the diet (ketone bodies, MCT or omega-3 fatty acids) should analysed concerning the anti-proliferative effect.

The aim of this study was to test the ketogenic diet low in carbohydrates and high in fat enriched with omega-3 fatty acids and MCT on tumour growth in a mouse model. We can show that the diet had a retarding effect on tumour growth and resulted in larger necrotic areas within the tumours in comparison to the standard diet high in carbohydrates used in this study. The ketogenic diet corresponds to a diet which is currently being tested in a clinical study at the Department of Obstetrics and Gynaecology of the University of Würzburg Hospital. In this study, patients with very advanced tumour growth of different entities received a complex diet low in carbohydrates and high in oil and protein. In these incurable patients we wanted to see the impact of the diet on tumour cachexia, quality of life, and tumour progress. We inserted a short explanation with the link to the ongoing clinical trial into Material and Methods.

To prevent any misunderstandings that we showed experimentally a link between observed tumour delay and single supplements in the diet, we have revised the manuscript. We deleted following sentences from the revised version: “Our data suggest that caloric restriction in metabolic tumour therapy can be compensated by an adequate supply of lipid supplements” (conclusions of the abstract); “Furthermore, caloric restriction in metabolic tumour therapy may be compensated by the correct choice of lipid supplements” (second paragraph of the discussion); “It is unclear whether this effect is due primarily to the absence of postprandial glucose peaks, to direct interference with carbohydrate and lipid metabolic pathways, to lipid-based signalling, or to a combination of these factors” (conclusions).

2. We thank the reviewer for his interesting and detailed comments on basic aspects of tumour metabolism. In our last reply we argued that respiration may not be completely defective in tumour cells quoting Otto Warburg’s work. Indeed the reviewer is right, that Warburg examined preferentially whole tissue sections, and therefore the observed respiration within a tumour could be caused by stromal cells as well as tumour cells. We further agree with the reviewer’s remarks on the Crabtree effect and that Zu and Guppy don’t mention this possibility. We also agree with the reviewer’s remarks on glutaminolysis. Glutaminolysis seems to be an important, and until now more or less disregarded, metabolic process to produce lactate and stimulate protein synthesis. With this in mind, the data from Rossignol et al. should perhaps be viewed critically. To date we do not have our own data on this. However, the reviewer’s comments do not challenge any statements made in the manuscript. They refer to our last reply and we will be sure to consider his comments in our further experiments.
3. The mouse body weights are calculated as means and not medians. This was, unfortunately, false in the text. We corrected this in the revised manuscript. Please excuse this mistake.

4. Since no benign cells corresponding to the tumour cell line used in this study were available we used HUVEC cells as control cells. We mentioned this in the revised manuscript (Materials and Methods).

5. A representative H&E histology was added to the manuscript (Fig. 5).

**Reviewer 3 - W. Elaine Hardman**

1. Since no benign cells corresponding to the tumour cell line used in the study were available we used HUVEC cells as control cells. We mentioned this in the revised manuscript (Materials and Methods).

2. The lactate production values in the text were corrected and now coincide with the data shown in Fig. 1D. The measurement for the lactate production in the text and figure is now shown as mg/ml. Thank you for pointing this out and please excuse these mistakes. We also reversed the x-axis in Fig. 1D.

3. As suggested, the paragraph “course of body weights and survival” was split into two paragraphs, “course of body weight” and “survival”.

We present data about a ketogenic diet low in carbohydrates and high in fat enriched with omega-3 fatty acids and MCT. An antitumor effect has been demonstrated in patients and experimental models for both omega-3 fatty acids and MCT. In this study we cannot state whether the effects of the diet observed are due primarily to omega-3 fatty acids and MCT, or both. Further studies will focus on this. Since ketogenic diets do not necessarily influence tumour growth it is difficult to interpret the information of contrasting studies. We found an effect with the ketogenic diet used, but no reduced levels of blood glucose or insulin. Fearon et al. found persistently high glucose levels in animals on a ketogenic diet with no reduction in tumour growth. The authors concluded that the reason for this observation was the uninfluenced levels of blood glucose. In contrast, Nebeling described decreasing blood glucose levels for her two patients. This is what we also see in our clinical study (see comments to reviewer 1). Further experiments are in preparation to address the quality of the different components of the diet (ketone bodies, MCT, and omega-3 fatty acids) for both tumour growth and metabolic influence (blood glucose and insulin).