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Letter to the Editor

What to eat or what not to eat—that is still the question

See the editorial by Rieger and Steinbach, volume 18, issue 8, 2016 on pages 1035–1036.

See also the article by De Feyter et al., volume 18, issue 8, 2016 on pages 1079–1087.

In the August issue of *Neuro-Oncology* in 2016,[1] Rieger and Steinbach provide an excellent editorial about 2 different rat glioma cell lines to address 2 crucial questions: (i) why RG-2 and 9L glioma cells do not substantially metabolize the ketone body beta-hydroxybutyrate (BHB) *in vitro*, as is demonstrated in *in vivo* rat xenograft models[2] using ^{13}C-labeled BHB infusion and sophisticated MRI spectroscopy; and (ii) why the ketogenic diet (KD) used in the study they evaluate[2] did not slow the growth of orthotopic 9L or RG-2 tumors.

A closer look suggests that the first question may be answered by the culturing conditions. The 9L and RG-2 cells were maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum.[2] This medium contains 4500 mg/L (450 mg %) glucose, which is over three-fold the physiologic concentrations of circulating glucose in blood of rodents. In addition, this media has high ketogenic amino acid content that would hamper the uptake of additional ketone substrates without further induction of monocarboxylic acid transporters with their downstream intracellular catabolic enzyme channels. The working 9L and RG-2 experimental cell stocks from American Type Culture Collection were created at an early passage (between 4 and 7) without conditioning them to the high BHB substrate environment. Therefore the lack of BHB uptake in cultured glioma cells is the result of excessive ketogenic amino acid substrate availability that can certainly compromise bidirectional monocarboxylic acid transport in the *in vitro* experiment according to the Randle cycle as shown by ^{13}C-glucose guided metabolomics.[3]

The more challenging question is the finding that the KD did not inhibit tumor growth in rats, although it strongly reduced plasma glucose levels, which is in contrast to the majority of mouse xenograft models. Furthermore, the cell lines studied *in vitro* readily take up BHB and metabolize it to citrate, then oxidize it to glutamate *in vivo* in a way comparable to that of contralateral brain areas in the healthy cortex. The explanation offered by the authors[1]—the species differences among mouse, rat, and man—certainly plays a role. However, a more mechanistic explanation requires us to consider the quality and source of the dietary fat used in the diets in order to translate the *in vivo* experiment for the clinics. This relates to metabolic water production by complete mitochondrial ketogenic substrate oxidation to CO_2 and H_2O,[4] which is a critical factor in nutritional and/or metabolic ketosis studies as the downstream effect of ketogenic substrates. Mitochondrial matrix water is produced in 1.1 excess weights in comparison with that of dietary fat, while the same ratio for carbohydrates is reduced matrix water by a factor of 0.55.[4] More importantly, the heavy hydrogen isotope (ie, the deuterium content of dietary fat) strongly influences the therapeutic value of ketogenic diets through reducing intracellular compartmentalized water with particular deuterium loads, whereby deuterons pose significant kinetic isotope effects.[5] Metabolic water's deuterium load regulates cellular and mitochondrial functions, including interfacial protein interactions, energy production via ATP synthase's proton transfer velocity, molecular crowding and cell growth,[6] as shown in [1,2-$^{13}C_2$]-D-glucose guided metabolic water cycling single tracer experiments.

De Feyter et al[2] placed animals on a KD containing 91% fat and 9% protein using the Harlan Teklad #TD96355 protocol. Each kilogram of this formula contains 173.3 grams of casein; 2.6 grams of DL-methionine; 586.4 grams of Crisco vegetable shortening, which is artificially hydrogenated; and 86.2 grams of corn oil, besides the other ingredients of vitamin and mineral mixtures. In contrast, the standard diet (Harlan Teklad #2018) contained 17% fat (ie, 6.2 grams of ether extracted fat with 3.1 grams linoleate [C18:2ω6] as its main fat component). Due to the artificial hydrogenation of Crisco oil in the KD with unknown protium and deuterium ratios but the organic ether extraction of vegetable oil in the control diet, the study by De Feyter et al[2] has limited translational values.

We herein recommend that ketogenic dieting experimental protocols use fat sources with known deuterium content and ratios,[7] and only when natural deuterium depleting potential of fatty acids upon oxidation of the β carbon is established should the study be introduced into the literature as such. This is important as the deuterium depleted water yield of ketogenic substrates is strictly dependent on mitochondrial functions and oxygen delivery, which may be limited in primary brain tumors and experimental xenografts, whereas deuterium depleted water for cytoplasmic hydrogen exchange reactions needs to be incorporated into dieting protocols to achieve more favorable clinical outcomes based on deuterium depleting biological principles currently entering contemporary medicine.[5,8]

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