Oxidative ketone body metabolism in rat brain tumors and the effect of the ketogenic diet: evidence from *in vivo* ^{1}H-[^{13}C] MRS

Henk M. De Feyter[1], Kevin L. Behar[2], Kevan L. Ip[1], Fahmeed Hyder[1], Lester L. Drewes[3], Robin A. de Graaf[1], and Douglas L. Rothman[1]

[1]*Department of Diagnostic Radiology, Yale University, New Haven, Connecticut, United States,* [2]*Department of Psychiatry, Yale University, CT, United States,* [3]*Department of Biomedical Sciences, University of Minnesota, MN, United States*

Introduction. The ketogenic diet (KD) has been proposed as alternative treatment for the management of brain tumors (1). The KD reduces plasma glucose and increases ketone body levels creating an energetically challenging condition. Brain tumors supposedly lack enzymatic capacity to oxidize ketone bodies (Fig. 1) and could thus be selectively starved. Yet, evidence for the lack of ketolysis in brain tumors is inconclusive. We investigated the metabolic fate of the ^{13}C-labeled ketone body beta-hydroxybutyrate (BHB, Fig. 1) in two rat brain tumor cell lines, 9L and RG2, both *in vitro* and *in vivo* using ^{1}H-[^{13}C] MRS (2). *In vivo* BHB tumor transport and metabolism was compared with non-tumorous brain. In the RG2-bearing rats the effect of a calorie-restricted KD on brain tumor growth was investigated as well.

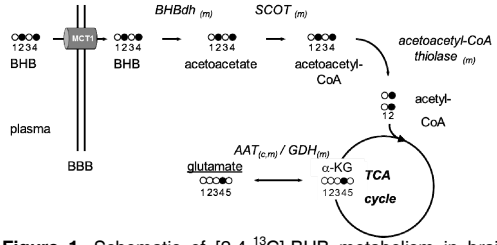

Figure 1. Schematic of [2,4-^{13}C]-BHB metabolism in brain. O=^{12}C, ●=^{13}C. BHBdh: BHB dehydrogenase, SCOT: Succinyl-CoA Acetoacetate-CoA transferase, Co-A: Co-enzyme A, α-KG: α-ketoglutarate, (m): mitochondrial, TCA: tricarboxylic acid cycle, BBB: blood-brain barrier.

Materials and methods. Intracerebral tumors (9L & RG2) were induced in male Fisher344/DuCrl rats by implanting cells in the brain. Animals were randomly put on a calorie-restricted KD (91% fat and 9% protein) started 1 week after RG2 cell inoculation. All *in vivo* NMR measurements were performed using a 9.4T horizontal bore magnet interfaced to a Varian spectrometer. MR images for noninvasive tumor volume measurements (Fig. 2) were acquired in RG2-bearing rats on 3 different occasions using a quadrature ^{1}H radiofrequency surface coil following tail vein injection of T_1 contrast agent (200 µL Magnevist®). A combined quadrature ^{13}C and single loop ^{1}H surface coil set-up was used to acquire ^{1}H-[^{13}C] MR spectra from voxels positioned in tumor and non-tumor brain. A POCE sequence with LASER localization was used (TR/TE: 2500/25 ms) (3) and MR spectra were acquired interleaved from tumor and non-tumor voxels in parallel with the infusion of [2,4-^{13}C]-BHB. At the end of the study the animal was euthanized using focused-beam microwave irradiation (4). RG2 and 9L cells were cultured using high glucose DMEM (25mM) until ~80% confluence. The high glucose DMEM was replaced with DMEM containing 8mM glucose and 4mM [2,4-^{13}C]-BHB for 6 hours. Medium was sampled to quantify BHB and glucose consumption after which cells were harvested while kept at ice or 4°C to halt metabolism. Cells, brain and tumor tissue extracts' powder was re-suspended and ^{1}H-[^{13}C] MR spectra were acquired using a 500 MHz MR spectrometer (Bruker Avance).

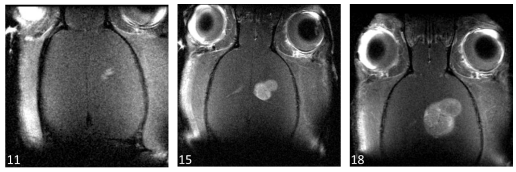

Figure 2. Spin Echo MR images (TR/TE: 500/17ms, 256x256, slice:1 mm) of RG2 tumor following Magnevist injection via tail vein. Numbers refer to days post-inoculation of 1,250 RG2 cells.

	9L	Cortex	RG2	Cortex	RG2	Cortex
Tumor/cortex	normal chow		normal chow		ketogenic diet	
[BHB] (µmol/g)	0.7 ± 0.5	0.2 ± 0.1	0.3 ± 0.2	0.15 ± 0.08[a]	1.4 ± 1.0[b]	0.7 ± 0.4[b]
[Glu] (µmol/g)	10.3 ± 2.3	10.9 ± 2.2	8.2 ± 1.9	12.4 ± 1.3[a]	9.4 ± 1.2	12.1 ± 2.6[a]
Glu F.E. (%)	17.9 ± 4.9	18.0 ± 2.8	18.2 ± 3.3	15.4 ± 1.3	33.9 ± 3.7[b,c]	33.2 ± 2.9[b]
Plasma						
[BHB] (mM)	6.8 ± 1.6		4.0 ± 0.5		10.6 ± 2.8[b]	
BHB F.E. (%)	98.2 ± 3.1		97.8 ± 4.1		84.1 ± 9.3[b,c]	
[AcAc] (mM)	2.3 ± 0.5		1.5 ± 0.3		3.9 ± 1.1	
AcAc F.E. (%)	83.8 ± 3.4		83.8 ± 8.2		70.8 ± 9.9	
[Glucose] (mM)	6.7 ± 0.6		6.0 ± 1.1		6.5 ± 2.1	
Glucose F.E. (%)	2.2 ± 0.6		2.8 ± 0.7		2.9 ± 0.7	

Table 1. Concentrations and fractional enrichments (FE) of metabolites in brain, tumor and plasma at the end of the [2,4-^{13}C]-BHB infusion. Data are presented as mean ± SD. [a] p<0.05 compared to tumor, [b] p≤0.05 compared to RG2 on normal chow, [c] p<0.05 compared to 9L.

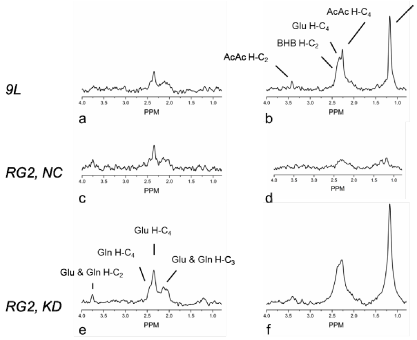

Figure 3. ^{1}H-[^{13}C] edited MR spectra acquired at the end of the [2,4-^{13}C]-BHB infusion in tumors (9L & RG2) and contralateral brain. NC: normal chow, KD: ketogenic diet. AcAc: acetoacetate, BHB: beta-hydroxybutyrate, Glu: glutamate, Gln: glutamine.

Results. *In vitro* RG2 cells showed evidence of BHB uptake from the medium and oxidation (Glu fractional enrichment (FE): 10.9±3.7%). In 9L cells little uptake of BHB was evident and no glutamate was detectable under 8mM glucose and 4 mM BHB culture conditions. Examples of *in vivo* POCE difference spectra are shown in Fig. 3 and tissue extract results are summarized in Table 1. ^{13}C-labeling in glutamate was evident in all brain and tumor tissue and was significantly increased in both cortical and RG2 tumor tissue of animals on the KD. No tumor growth-inhibiting effect was observed in rats on the KD (Fig. 4).

Discussion. We hypothesized that no oxidative metabolism would be present in the 9L and RG2 rat brain tumor cell lines. The *in vitro* results partly agree with this hypothesis as only RG2 cells showed a modest degree of [2,4-^{13}C]-BHB oxidation, but 9L did not. However, in both 9L and RG2 tumors ^{13}C-labeling of glutamate was detected *in vivo* during infusion of [2,4-^{13}C]-BHB (Figure 3, Table 1). Therefore, the premise that brain tumors have limited enzymatic capacity to oxidize ketone bodies does not apply to the tumor models studied here *in vivo*. The KD induced increased transport capacity for BHB in both cortical and RG2 tumor tissue apparently by upregulation of MCT1 expression in tumor cells.

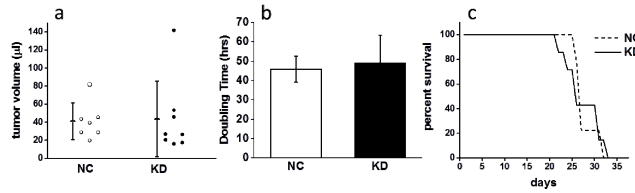

Figure 4. RG2 tumor growth analyzed as volume 18 days post-inoculation (a), doubling time (b) and survival (c). NC: normal chow, KD: ketogenic diet.

Conclusion. Based on these data the foundation for the KD as brain tumor therapy, namely the lack of ketolytic activity in brain tumors, can be challenged. Moreover, the KD could possibly even promote ketone body metabolism in tumor cells by inducing increased transport capacity for ketone bodies. ^{13}C MRS studies in patients are recommended to confirm the effects of the KD on human brain tumor metabolism and tumor growth.

References. (1) Zhou *et al.*, *Nutrition & Metabolism*, 4:5, 2007; (2) de Graaf RA, *In vivo NMR Spectroscopy*, Wiley, 2007; (3) Gruetter R. *Magn. Reson. Med.* 29, 6, 1993; (4) de Graaf *et al.*J Neurochem. 109:2, 2009.