

## Glycemic modulation in neuro-oncology: experience and future directions using a modified Atkins diet for high-grade brain tumors

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Dietary glycemic modulation through high-fat, low-carbohydrate diets, which induce a state of systemic ketosis and alter systemic metabolic signaling, have been incorporated into the clinical management of patients with neurological disease for more than a century. Mounting preclinical evidence supports the antitumor, proapoptotic, and antiangiogenic effects of disrupting glycolytic metabolism through dietary intervention. In recent years, interest in incorporating such novel therapeutic strategies in neuro-oncology has increased. To date, 3 published studies incorporating novel dietary therapies in oncology have been reported, including one phase I study in neuro-oncology, and have set the stage for further study in this field. In this article, we review the biochemical pathways, preclinical data, and early clinical translation of dietary interventions that modulate systemic glycolytic metabolism in the management of primary malignant brain tumors. We introduce the modified Atkins diet (MAD), a novel dietary alternative to the classic ketogenic diet, and discuss the critical issues facing future study.

**Keywords:** cancer metabolism, glioma, ketogenic diet, modified Atkins diet, seizure.

Malignant gliomas (MGs) are the most common primary CNS tumors. Despite the introduction of combination chemoradiotherapy with temozolomide in 2005, treatment options remain limited, and median survival is only 12–15 months for patients newly diagnosed with glioblastoma (GBM).<sup>1</sup> While in general, tumorigenesis is thought to involve genetic, epigenetic, and microenvironmental changes that vary widely between tumor types, the preferential metabolism of glucose through aerobic glycolysis (ie, Warburg effect) is common.<sup>2,3</sup> Recent advances in the understanding of the biochemical pathways underlying this metabolic phenotype may provide novel therapeutic targets for intervention.

### Biochemical Pathways Implicated in the Glycolytic State

Unlike normal glia, glioma cells have both a high glycolytic rate and are dependent on glucose for energy metabolism.<sup>3</sup> Metabolism of glucose is 3-times greater in glioma cell lines as compared with normal glia.<sup>4</sup> Gliomas have been shown to poorly metabolize ketone bodies for energy,<sup>5–8</sup> and withdrawal of glucose has

induced apoptosis at rates dramatically higher than in normal human astrocytes.<sup>9</sup> It appears that this metabolic “addiction” to glucose is in part due to alterations in mitochondrial structure and function that may result from genetic, epigenetic, and enzymatic alterations within cancer cells.<sup>10–12</sup> Glucose not only serves to fuel tumor cell aerobic glycolysis but also results in a proglutamate state that may drive peritumoral glutamate excitotoxicity and subsequent tumor invasion into surrounding healthy brain.<sup>13,14</sup>

Current research suggests that this preferential metabolism of glucose through glycolysis may arise as a selective advantage in the hypoxic conditions experienced during early tumor development.<sup>15</sup> Compared with oxidative phosphorylation, glycolysis not only provides a significantly higher rate of ATP production but also provides an effective mechanism for (i) shunting carbon toward biosynthetic pathways necessary to drive cellular proliferation, and (ii) generating the cellular redox potential necessary to scavenge excess reactive oxygen species (ROS) and ensure cancer cell viability. This cellular reprogramming of glucose metabolism to fuel tumor cell growth is largely thought to be driven by the Akt/phosphoinositide 3-kinase (PI3 K)/mammalian target of rapamycin (mTOR) pathway, which is commonly activated in gliomas

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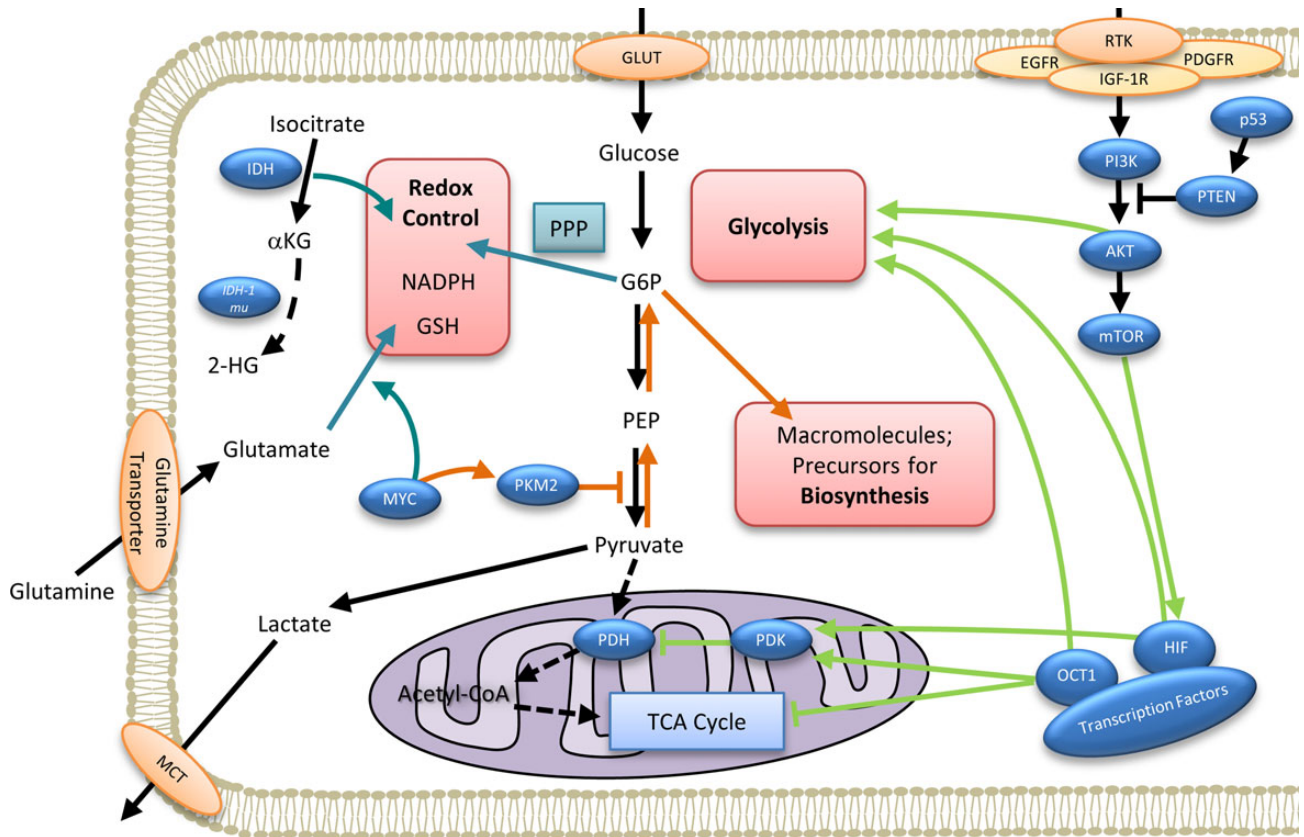
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(Fig. 1).<sup>16</sup> Pathway activation begins with membrane-associated receptor tyrosine kinases such as the insulin-like growth factor receptor (IGFR), epithelial growth factor receptor (EGFR), or platelet derived growth factor receptors (PDGFR) which are frequently over-expressed in gliomas.<sup>17</sup> Stimulation of these receptors results in dimerization, transphosphorylation, and activation of PI3 K. Downstream effects include activation of Akt and mTOR, which activates transcription factors including hypoxia-inducible factor 1 (HIF1). HIF1 stimulates expression of glucose transporters on the cell

surface, thereby increasing cellular glucose influx,<sup>18-20</sup> and shifts metabolic pathways towards glycolysis through activation of inhibitory mitochondrial pyruvate dehydrogenase kinases (PDKs).<sup>21</sup>

Recent research suggests that cytosolic pyruvate kinases appear to provide additional regulation of this metabolic phenotype. The dimeric pyruvate kinase M2 isoenzyme (PKM2) is frequently overexpressed in tumors including gliomas and functionally serves as a negative regulator of glycolysis, resulting in the accumulation of glycolytic intermediates.<sup>22</sup> These intermediary



**Fig. 1.** Molecular mechanisms underlying the oncogenic metabolic phenotype. Oncogenic pathway alterations driving the Warburg effect involve (i) activation of the PI3 K/Akt/mTOR pathway driving a glycolytic state (light green arrows); (ii) pyruvate kinase induced negative regulation of glycolysis, which provides glycolytic intermediates necessary to facilitate macromolecular biosynthesis (orange arrows); and (iii) shunting of glucose through the pentose phosphate pathway to build redox potential (dark green arrows). In the first of these major metabolic alterations (light green arrows), PI3 K activates Akt, which stimulates aerobic glycolysis and enhances transcription factor expression including hypoxia-inducible factor 1 (HIF1) through the action of the mammalian target of rapamycin (mTOR). Expression of transcription factors including HIF1 and Oct 1 further drives a proglycolytic state by enhancing the activity of mitochondrial pyruvate dehydrogenase kinases, which shunt pyruvate back towards glycolysis and away from mitochondrial oxidative phosphorylation. In the second of these major metabolic alterations (orange arrows), C-myc-induced expression of cytosolic dimeric pyruvate kinase M2 functionally negatively regulates glycolysis, shunting free carbons back toward macromolecular biosynthesis, which supports cell proliferation. In the third major metabolic alteration, PKM2-mediated regulation of glycolysis also shunts glycolytic intermediates toward alternative carbon-consuming pathways (dark green arrows) including the pentose phosphate and hexosamine pathways, which generate reducing power in the form of NADPH. C-myc-driven conversion of glutamate to glutathione (GSH) and isocitrate dehydrogenase (IDH) mediated conversion of isocitrate to alpha-ketoglutarate (dark green arrows) also resulting in additional reducing power in the form of NADPH and GSH necessary to scavenge excess reactive oxygen species generation. Abbreviations:  $\alpha$ KG, alpha-ketoglutarate; 2-HG, 2-hydroxyglutarate; AMPK, AMP-activated protein kinase; CHO, carbohydrate; EGFR, epidermal growth factor receptor; G6P, glucose-6-phosphate; GLUT, glucose transporter; GSH, glutathione; HIF, hypoxia-inducible factor; IDH, isocitrate dehydrogenase; IDH-mu, IDH-mutant; IGF-1R, insulin-like growth factor-1 receptor; MCT, monocarboxylic acid transporter; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate; Oct 1, Octamer transcription factor 1; PI3 K, phosphoinositide 3-kinase; P53, tumor protein 53; PDGFR, platelet-derived growth factor receptor; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PEP, phosphoenolpyruvic acid; PKB, protein kinase B, also known as Akt; PKM2, pyruvate kinase-M2 isoenzyme; PPP, pentose phosphate pathway; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; TCA, tricarboxylic acid.

substrates are shunted towards macromolecular biosynthesis and subsidiary pathways of carbon catabolism, which serve to generate cellular reducing power in the form of NADPH. This tightly regulated control of redox potential ensures cancer cell viability within a relative promutagenic environment that may further drive oncogenesis.<sup>22</sup>

## Therapeutic Interventions Targeting Glycemic Modulation

Preclinical studies have shown the antitumor, proapoptotic, and antiangiogenic effects of interrupting these glycolytic pathways in cancer cell lines including gliomas;<sup>23,24</sup> however, no drugs are currently available that can be translated into clinical use now. Alternative therapeutic strategies for targeting glycolytic metabolism in gliomas do exist and include dietary glycemic modulation.

Dietary therapies that modulate glycemic homeostasis and result in a state of systemic ketosis have been termed ketogenic diets (KDs). KDs are high-fat, low-carbohydrate diets that alter host metabolic signaling by reducing serum glucose, driving ketone body production, and decreasing circulating insulin-like growth factor-1 (IGF-1). Such metabolic alterations have been shown in preclinical studies to have anticonvulsant and antitumor effects.<sup>25,26</sup> A mouse epilepsy model study suggested that inhibition of mTOR signaling may underlie the antiepileptic properties of a KD; however, this has not been confirmed, and the exact mechanism of a KD has not yet been resolved.<sup>26</sup> Studies of KDs in malignant glioma xenograft mouse models have demonstrated lowering of plasma glucose levels, reduction in tumor growth by 35%–65%, reduced microvessel density, and improved survival.<sup>25,27–29</sup> Importantly, these studies have shown that inducing ketosis in the absence of calorie restriction does not appear to have this same effect on tumor volume and enhanced survival.<sup>27</sup> Subsequent studies have also reported antiangiogenic,<sup>24</sup>

radiosensitizing,<sup>30</sup> and chemotherapy augmenting<sup>31,32</sup> effects of the diet, which appear to be linked to calorie restriction. Recent animal studies have also investigated the impact of intermittent fasting as a potential alternative mechanism for regulating cell signaling through these metabolic pathways.<sup>33</sup> In these studies, intermittent fasting was found to augment the effects of chemotherapy, enhance response to radiation, and delay tumor progression more effectively than long-term calorie restriction without substantial weight loss.<sup>33,34</sup> In fact, studies have suggested that such an intervention may actually be protective to normal cells, a process that in part may depend on circulating levels of glucose and IGF-1.<sup>33,34</sup>

Clinically, KDs have been safely employed in the management of patients with medically refractory epilepsy since around the 1920s.<sup>35,36</sup> Over this time, several standard therapeutic regimens have been developed and can be characterized by the degree of carbohydrate, calorie, and fluid restriction and by the need for inpatient admission (Table 1).<sup>37–39</sup> Of these, the classic KD was the first to be studied and is characterized by a 4:1 ratio of fat to carbohydrate and protein intake (in grams) with ~90% of calories provided by fat. Patients have traditionally been admitted for initial inpatient fast; calories and fluid intake are strictly monitored and restricted to ensure adequate systemic ketosis as measured by urinary ketones.<sup>38</sup> To date, the conventional KD has been most widely integrated into the clinical management of children with medically refractory epilepsy.<sup>40</sup> In these patients, tolerability and efficacy have been high.<sup>37,38,40,41</sup> Attempts to translate this into adults, however, have been met with some challenge. While efficacy has been similar to children in those able to remain on the diet, compliance with dietary restriction, burden of weighing food, and impact on daily living has been difficult to maintain for adults.<sup>39,42</sup> In an effort to provide a more tolerable alternative, several KD variants have been developed including (i) lower-ratio KDs (primarily 3:1 ratio), (ii) medium-chain triglyceride (MCT) oil diets in which some of the long-chain fatty acids are replaced

**Table 1.** Ketogenic diets and the therapeutic experience in patients with medically refractory epilepsy

Diet Name	Glycemic Intervention ([fat]:[carb + protein] ratio)	Calorie and Fluid Restriction	Inpatient Admission Required	Tolerability <sup>a</sup> (%, range)	Efficacy <sup>b</sup> (%, range)
<b>PEDIATRICS</b> <sup>37,38,40–42,45,46</sup>					
Conventional KD	4:1 ratio	Yes	Yes	83.5% (42–100)	78.0% (35–85)
Conventional KD variants	3:1 ratio or MCT	Variable	Variable	81.0% (78–83)	55.1% (50–64)
MAD	10–20 g net CHO/day	No	No	68.6% (60–80)	50.0% (50)
<b>Adults</b> <sup>39,48,49</sup>					
Conventional KD	4:1 ratio	Yes	Yes	47.0% (22–64)	41.7% (22–55)
Conventional KD variants	3:1 ratio or MCT	Variable	Variable	54.2% (33–75)	27.5% (13–42)
MAD	10–20 g CHO/day	No	No	59.4% (38–78)	29.4% (13–57)

Abbreviations: CHO, carbohydrate; KD, ketogenic diet; MAD, modified Atkins diet; MCT, monocarboxylic acid transporter.

Ketogenic diets employed and studied in the management of children and adults with medically refractory epilepsy include the classic KDs, KD variants, and the modified Atkins diet (MAD). These diets employ varying degrees of carbohydrate, calorie, and fluid restriction that are defined by the ratio of [fat]:[carbohydrate + protein] by weight or the degree of carbohydrate restriction combined with increased fat intake. The major structured dietary therapies include those like the classic KD, which employ strict carbohydrate, calorie, and fluid restriction and require inpatient admission but are more difficult to employ in adults and those like the MAD which employ strict carbohydrate restriction and monitor other dietary parameters but do not require hospitalization and are better tolerated in adults.

<sup>a</sup>Defined as the percent of patients remaining on the diet at predefined study endpoint or final patient follow-up.

<sup>b</sup>Defined as the percent of patients with >50% seizure reduction of those remaining on diet at predefined study endpoint or final follow up.

by MCTs, and (iii) the low glycemic index diet that limits consumption of high glycemic index foods.<sup>43,44</sup> While easier to tolerate, ketosis can be harder to achieve on these diets, and prospective studies are ongoing to address antiepileptic efficacy.<sup>37</sup>

Another alternative, the modified Atkins diet (MAD), is a low-carbohydrate diet that induces ketosis without restricting total calories, fluid intake, or protein consumption and does not require inpatient admission. Patients are prescribed no more than 20 grams of net (subtracting fiber) carbohydrates per day. Studies in adults with intractable epilepsy have demonstrated favorable tolerability, ease of administration, efficacy in seizure reduction, and practicality for adults with medically refractory epilepsy.<sup>47-49</sup> Long term responses have not differed significantly among different ketogenic dietary therapies,<sup>50,51</sup> and reduction in seizures has been similar following transition from the MAD to KD.<sup>52</sup> As such, the MAD has emerged as a safe, effective, and feasible alternative to the classic KD in adults with medically refractory epilepsy.

## Dietary Glycemic Modulation in Neuro-oncology: Challenges to the Classic Ketogenic Diet

In light of the expanding literature on the metabolic implications of KDs, interest in adopting KDs in oncology has increased. To date, 2 prospective studies have investigated the safety and feasibility of KDs for patients with advanced systemic solid tumors (Table 2).<sup>53,54</sup> Tolerability has ranged from 50% at 4 weeks in 10 participants undergoing a carbohydrate-restricted diet (total carbohydrate intake targeted at <5% of total energy)<sup>53</sup> to 31% at 3 months in 15 participants undergoing a 70 g/day carbohydrate-restricted diet.<sup>54</sup> Side effects have included fatigue, constipation, leg cramps, and hunger, with reported weight loss ranging from 2–3 kg. These studies were not powered to determine efficacy, although best responses were reported, with partial response observed in 1 participant and stable disease observed in 10 participants.

In glioma, one prospective human study, the ERGO trial (NCT00575146), enrolled 20 participants with recurrent GBM and prescribed a 60 g/day carbohydrate-restricted KD without calorie restriction (Table 2).<sup>55</sup> Feasibility, the primary outcome measure, was defined as the percent of participants remaining on the diet until disease progression, although participants were permitted to continue the diet until second progression and combine it with cytoactive treatments at progression. Three participants discontinued the diet, all reportedly due to its negative impact on quality of life. The remaining 85% of participants continued for a median of 36 days, with stable ketonuria reported

in 73% of them. A small but significant weight loss of 2.2% was observed. Median progression-free survival was 5 weeks (range: 3–13 weeks), and the investigators concluded that additional investigations into alternative combinations were needed.

In summary, these existing prospective studies have incorporated widely varying degrees of glycemic modulation, limited calorie restriction, varying durations of follow up time, and differing response criteria. Thus, variable tolerability has been observed and conclusions regarding the efficacy of such interventions are limited. Based on the well-defined clinical experience in adult patients with medically refractory epilepsy, it is most likely that a classic, calorie-restricted KD will not be feasible for adult oncology patients. Alternative methods are necessary. Based on the growing literature supporting the MAD as a safe, feasible, and efficacious alternative to the classic KD in patients with medically refractory epilepsy, the MAD may also provide an appealing alternative for glycemic modulation in neuro-oncology.

## Johns Hopkins Institutional Experience with a Modified Atkins Diet in Glioma

The comprehensive Johns Hopkins Adult Epilepsy Diet Center (AEDC) was previously established to manage children and adults with medically refractory epilepsy and has robustly implemented the MAD in the clinical management of these patients. Over the past several years, patients with glioma and epilepsy have been managed in the AEDC. Herein, we review our experience.

From January 2012 through April 2014, patients with glioma and resultant seizure disorders were prospectively observed and their data reviewed retrospectively to evaluate the safety and clinical impact of a MAD. Institutional Review Board approval was obtained and all patients were consented. Patients were excluded from starting diet therapy if there was a history of contraindicated metabolic disorder, significant atherosclerotic disease, acute pancreatitis, or cardiac or renal dysfunction. The MAD protocol is well established and consists of a 20g net carbohydrate (total carbohydrate grams minus fiber grams) per day restriction. During this interval, 7 participants were prescribed the MAD after initial visit to the AEDC, and 1 participant continued a 15 g/day diet begun 5 months before enrollment. High-fat foods were encouraged. Total daily calories and carbohydrate-free fluids were allowed ad libitum. Multivitamin, vitamin D, and calcium supplementation were prescribed. Baseline laboratory assessment included complete blood cell count (CBC), comprehensive metabolic panel (CMP), fasting lipid panel (FLP), urine calcium, creatinine, and human chorionic gonadotropin level (for women). Repeat CBC, CMP, and FLP were performed at 3 and 6

**Table 2.** Prospective studies of ketogenic diets in oncology

Trial	Carbohydrate Restriction	Timeline of Assessment	Tolerability (% on diet)	Weight Loss (mean, kg)	Response
Schmidt et al <sup>54</sup>	70 g/day	3 months	31% (of 16)	2 kg	5 SD, 6 PD, 4 Unknown
Fine et al <sup>53</sup> (RECHARGE)	CHO restriction to 5% of total energy	1 month	50% (of 10)	3 kg	5 SD, 1 PR, 4 PD
Rieger et al <sup>55</sup> (ERGO)	60 g/day	~5 weeks	85% (of 20)	2.2% loss	Median OS 32 weeks

Abbreviations: CHO, carbohydrate; g, grams; OS, overall survival; PD, progressive disease; PR, partial response; SD, Stable disease.

months. Baseline and follow-up imaging were collected as available. Participants were instructed to record daily seizure frequency and weekly weights on a monthly calendar. Urine ketones were initially followed biweekly and then at least weekly after one month. Guidelines for implementation of such a diet have been previously reported, standardized, and generalized in patients with medically refractory epilepsy.<sup>48,49</sup> Baseline and follow-up laboratory and clinical data were compared using Student *t* tests ( $P < .05$ ).

Eight participants with glioma were treated with MAD during the specified interval (Table 3). Six were male (75%), mean age was  $41.5 \pm 10.0$  years, and mean diet duration was  $13.2 \pm 8.0$  months (range: 2–24 months). Mean baseline weight was  $78.8 \pm 18.3$  kg, and body mass index (BMI) was  $25.7 \pm 3.5$ .

At initiation, all participants had a diagnosis of seizure disorder including simple partial seizures ( $n = 2$ ), complex partial seizures ( $n = 5$ ), and generalized tonic-clonic seizures ( $n = 5$ ). Average seizure duration ranged from 1–10 years with mean baseline frequency of  $2.14 \pm 4.0$  seizures per month. On average, participants had previously tried  $2.9 \pm 2.2$  antiepileptic drugs without achieving seizure control. Active AEDs at initiation ranged from 0–4 (one participant elected to use MAD as an alternative to AED although counseled that MAD is typically used as adjunctive treatment).

Tumor types included low-grade oligodendroglioma ( $n = 3$ ), low-grade astrocytoma ( $n = 1$ ), anaplastic astrocytoma (AA,  $n = 1$ ), and GBM ( $n = 3$ ). Of those with GBM, one participant enrolled after initial concurrent chemoradiation, another enrolled at second recurrence, and the last enrolled after secondary progression from AA. Tumor location was frontal ( $n = 2$ ), temporal ( $n = 3$ ), or parietal ( $n = 3$ ) and left sided in all participants. Those with GBM and AA had previously undergone surgery and radiation. Prior chemotherapy included temozolomide (TMZ,  $n = 6$ ), bevacizumab (BEV,  $n = 1$ ), and clinical trial therapies ( $n = 1$ ). After diet initiation, 2 participants were treated with TMZ (one with TMZ and BEV), and the participant with recurrent GBM began clinical trial with BEV and LBH589 (NCT00859222).

At 13.2 months of follow-up (Table 4), 7 of 8 participants remained on MAD; one stopped at 3 months after MAD did not provide adequate seizure control. All participants are living and survival rates for those with progressive GBM are comparable to expected survival: 13 months for the participant with secondary GBM compared with 7.8 months expected<sup>56</sup> and 17 months for the patient with recurrent GBM compared with 7.4 months expected.<sup>57</sup> At analysis, average weight loss was  $3.4 \pm 6.5$  kg. No participant was underweight (BMI range: 20.3–30). During the entire study observation period, a total of 3 participants were on glucocorticoids. At diet initiation, one participant was on dexamethasone (DEX, 2 mg), and one was on hydrocortisone (dexamethasone equivalent dose = 0.56 mg). One tapered off and did not resume glucocorticoids. The second was on a stable DEX dose (2 mg/day) throughout the study. An additional participant was started on DEX (4 mg) after initiating the diet. Mean seizure frequency was  $1.25 \pm 3.3$  per month at 3 months and  $0.4 \pm 0.2$  at 6 months. Active AEDs did not significantly change. At a mean follow up time of 13.2 months, five participants (63%) experienced at least a 50% reduction in seizure frequency. Four were seizure free. Laboratory values were unchanged at 6 months (Supplementary Table S1); lipid values were previously published for a subset of patients.<sup>58</sup>

**Table 3.** Demographic, oncologic, and epileptic characteristics

Patient	Sex	Age at Diet Initiation (years)	Carb Limit (grams/day)	Tumor Type	Tumor Location	Prior Tumor Treatment	Active Chemo During MAD	Seizure Type	Prior AED (no.)	Active AEDs at Initiation
1	Male	30	20	AA	L parietal	Sx, XRT, TMZ	None	CPS	1	LEV
2	Female	54	20	Sec GBM	L parietal	Sx, XRT, TMZ	TMZ, Bev	CPS	5	LEV, PHT, LMT, KLO
3	Male	48	20	LGO	L frontal	Sx	None	SPS, CPS	3	LEV
4	Male	37	15	Rec GBM	L temporal	Sx, XRT, TMZ, Bev, ICT-107, Veliparib	Bev, LBH589	GTC	1	LEV
5	Female	38	20	LGO	L temporal	Sx	None	GTC	1	None
6	Male	28	20	LGO	L temporal	Sx, TMZ	TMZ	GTC, SPS	6	PHT, TPM
7	Female	54	20	LGA	L parietal	Sx, XRT, TMZ	None	CPS, GTC	1	LEV
8	Female	43	20	GBM	L frontal	Sx, XRT, TMZ	None	CPS, GTC	5	LCM, VPA, LEV

Abbreviations: AA, anaplastic astrocytoma; AED, antiepileptic drug; BEV, bevacizumab; CPS, complex partial seizures; CZ, clonazepam; GBM, glioblastoma multiforme; GTC, generalized tonic-clonic seizures; LCM, lacosamide; LEV, levetiracetam; LGA, low-grade astrocytoma; LGO, low-grade oligodendroglioma; LMT, lamictal; no., number; PHT, phenytoin; SPS, simple partial seizures; Sx, surgery; TMZ, temozolomide; TPM, topamax; VPA, valproic acid; XRT, radiation therapy.

**Table 4.** Clinical changes observed during dietary intervention

Follow-up Characteristic	Overall (n = 8)	P value
Duration of diet, months, mean (std)	13.17 (8.0)	
Weight		.48
Baseline weight, kg, mean (std)	78.8 (18.3)	
Follow up weight, kg, mean (std)	72.6 (15.0)	
Weight change, kg, mean (std)	-3.4 (6.5)	
Body mass index		.40
Baseline BMI, mean (std)	25.7 (3.5)	
Follow up BMI, mean (std)	24.2 (3.2)	
BMI change (reduction), mean (std)	0.95 (1.9)	
Seizure frequency		
Baseline seizure frequency, mean per week (std)	0.54 (1.0)	
3-month seizure frequency, mean per week (std)	0.31 (0.83)	.62
6-month seizure frequency, mean per week (std)	0.10 (0.04)	.27
Seizure reduction at follow up		NA
50% seizure reduction (n, %)	5 (63%)	
Seizure free (n)	4	

Abbreviations: BMI, body mass index; kg., kilogram; std., standard deviation.

Seizure reduction at follow-up includes the number and percent of patients with a 50% reduction in seizure frequency or who were seizure free at the time of data analysis (mean follow up  $13.2 \pm 8.0$  months).

In summary, in this series of participants with glioma and seizures treated with a 20 g/day carbohydrate-restricted MAD, the diet was well tolerated without significant toxicity and, although not statistically significant, resulted in improvement in seizure control. All participants are living, and survival rates for those with GBM are comparable with expected median survival for their respective stages of disease. Of the 8 participants treated in this study, only one discontinued the diet (due to lack of anti-seizure efficacy), and the 7 (88%) remaining participants continued the diet for 2–24 months. In comparison, Kossoff et al evaluated 30 adult patients with intractable epilepsy who were treated with a 15 g/day MAD; 26 (87%) remained on the diet at 1 month, 20 (67%) at 3 months, and 14 (47%) completed all prescribed 6 months.<sup>48</sup> Of those who stopped the diet prior to 6 months, 9 (56%) did so due to lack of efficacy, 6 (38%) due to feasibility, and 1 did not start after enrollment. Cervenka et al. evaluated 25 adult patients with intractable epilepsy who were treated with a 20 g/day MAD.<sup>49</sup> Of the 22 who actually initiated the diet, 21 (95%) remained on the diet at 1 month, and 14 (64%) at 3 months. While prospective data are necessary to determine the feasibility and tolerability of the MAD in patients with glioma, these data suggest that the MAD may provide a safe, tolerable, and feasible intervention to explore as a potential method of dietary glycemic modulation and adjunct to standard chemoradiation in glioma. Such an approach also provides an opportunity to pursue novel therapeutic combinations incorporating dietary metabolic regulation with conventional cytoactive treatments and to explore longer-term maintenance approaches

that avoid the myelosuppressive or emetogenic side effects of prolonged chemotherapy.

## Future Directions in Advancing Dietary Glycemic Modulation in Glioma

Moving forward, clinical studies will need to address 4 key issues currently facing the translation of dietary glycemic modulation in neuro-oncology: (i) the lack of a defined nutritional goal or prescribed “dose” of dietary glycemic modulation, (ii) concerns regarding associated weight loss in a potentially “at risk” population, (iii) the need to define optimal endpoints for response and efficacy with such metabolic interventions, and (iv) the safety and efficacy of combination strategies incorporating glycemic modulation into other cytoactive treatment regimens.

### Defining a Dose of Dietary Glycemic Modulation

As with any therapeutic intervention, defining the optimal dose necessary to achieve clinical and biological activity as well as to ensure patient safety and tolerability is important. In a dietary intervention that targets glycemic modulation, dosing considerations include precise definition of daily calorie, carbohydrate (total or net), protein, and fat intake as well as the frequency and duration of these interventions. At present, 6 studies utilizing KDs in patients with glioma are recruiting participants (Table 5). These studies vary in terms of the prescribed dietary intervention, the duration of the study, and the means of calorie restriction. Given the recent preclinical data suggesting that periods of intermittent fasting may provide superior anticancer activity to long-term calorie restriction without substantial weight loss,<sup>33,34</sup> intermittent fasting is potentially an attractive adjunct. Thus in glioma, a diet that combines the tolerability and defined dosing strategy of the MAD with the caloric impact of intermittent fasting may prove optimal.

Strategies for objectively measuring compliance with prescribed diets are also necessary to study feasibility, evaluate tolerability, and ensure generalizability of results. Patient-reported dietary intake through diet records has been well studied and reported to be reliable and valid in nutrition literature.<sup>59,60</sup> However, the subjective nature of self-reported intake presents challenges to ensuring accurate and reliable data.<sup>61–64</sup> Dietary biomarkers (eg, caffeine metabolites for caffeine intake, urine proline betaine for citrus, total resveratrol metabolites for wine) are being evaluated and may aid in evaluating compliance; however, current markers are insufficient to monitor intake comprehensively, and further development is necessary.<sup>65</sup> Serum and urine ketone measurements have been utilized in patients with medically refractory epilepsy with success and have provided a reliable assessment of ketogenic dietary compliance. However, their application in oncology has not been studied, and in fact preclinical studies suggest that they may be less reliable in this setting,<sup>27</sup> particularly with the concomitant use of corticosteroids.

### Mitigating Weight Loss Concerns

Importantly, given concerns of cancer-related cachexia and the importance of maintaining adequate nutrition and caloric intake in advanced cancer patients, care must be taken in applying

**Table 5.** Active studies evaluating ketogenic diets in glioma

	Disease State	Sample Size	Dietary Intervention	Duration of Study	Primary Outcome	Study Status
University of Michigan NCT01535911	Recurrent GBM	12	Total calories 20–25/kg/day	6 weeks ± 6 weeks if response	Tumor response (PET-CT)	Recruiting
Tel-Aviv Sourasky Med Ctr NCT01092247	Recurrent HGG	Unknown	Individualized to patient (details not specified)	1 year	“Tumor progression and patient longevity”	Unknown
Mid-Atlantic Epilepsy and Sleep Ctr/Univ of Pittsburgh NCT01865162	Recurrent GBM	6	Classic KD (4:1 ratio) + 1600 kcal restriction	6 months	Safety	Recruiting
Johann Wolfgang Goethe University Hospitals NCT01754350	Recurrent GBM or gliosarcoma	2-arm: intervention vs standard diet	KD (<60 g/day CHO, 21–23 kcal/kg/day) + intermittent fasting	6 months	6 month PFS	Recruiting
St. Joseph's Hospital and Medical Center, Phx NCT02046187	Newly Diagnosed GBM	Unknown	Classic KD (4:1 ratio)	1 year	Tolerance and compliance (self-report)	Recruiting
Johns Hopkins Hospital NCT02286167	Newly diagnosed GBM	25	MAD + intermittent fasting	8 weeks	Feasibility, biologic activity	Recruiting

Abbreviations: GBM, glioblastoma, HGG, high-grade glioma; KD, ketogenic diet (reference: [www.clinicaltrials.gov](http://www.clinicaltrials.gov). Last accessed: November 17, 2014.); PET-CT, Computed tomographic positron emission tomography.

therapeutics that could negatively impact optimal nutrition. In most studies of KDs, weight loss has been observed though not inherently prescribed. It is not clear whether this observation has resulted from self-selected dietary changes, increased physical activity, or another underlying mechanism. Existing preclinical data suggest that calorie restriction is a critical component to dietary efficacy in oncology<sup>27</sup>; however, recent reports on intermittent fasting suggest that alternative methods for deriving this metabolic “switch” may exist. Limited clinical data do suggest that intermittent fasting may safely augment the effects of dietary intervention in patients with refractory seizures.<sup>66</sup> There is growing interest in supplementing dietary interventions with nutraceuticals, ketone preparations, and other supplements that may also promote alterations in the metabolic phenotype and prevent weight loss; however, further study is required, and initial steps to define precise dosing strategies remain imperative.<sup>23</sup>

### Defining Endpoints for Efficacy

Despite years of research in patients with medically refractory epilepsy, many questions about the cerebral biologic activity of glucose modulation through KDs exist. The current studies of KDs in oncology have added only limited additional data on potential biomarkers of cerebral activity. While systemic markers (eg, fasting glucose or serum ketones) have provided some evidence of systemic biologic activity, particularly in epilepsy, they do not appear to be reliable biomarkers of antitumor response.<sup>27</sup>

Positron emission tomography (PET) has been included in the published studies on systemic malignancy and KDs and is included in at least one currently enrolling study in glioma (NCT01535911); however, given the poor sensitivity and specificity of <sup>18</sup>F-FDG PET in glioma and substantial cerebral background signal, alternative radiographic markers of response will be necessary.<sup>67</sup> Recent studies have highlighted the potential utility of alternative PET radiotracers including <sup>11</sup>C-methionine, <sup>18</sup>F-FDOPA, and recently l-(5-[<sup>11</sup>C]-glutamine,<sup>68</sup> though their utility as a marker of metabolic intervention is unclear.<sup>69</sup> D-glucose is also being investigated as a potential infusible, biodegradable contrast agent in combination with MRI; however, further study is required before clinical application.<sup>70</sup>

Magnetic resonance spectroscopy (MRS) is a safe, feasible, and well-established noninvasive method for evaluating tissue metabolite concentrations. It has been investigated in gliomas for decades. Reliable and valid studies have established concentrations of cerebral metabolites,<sup>71</sup> expected changes in spectral patterns over the course of therapy,<sup>72</sup> and correlation with survival in certain populations.<sup>73</sup> Recent work shows that emerging MRS techniques can be used to reliably evaluate metabolites that were previously difficult to resolve including glutamate (Glu), glutamine (Gln), glycine, and  $\gamma$ -aminobutyric acid.<sup>74–77</sup> Intratumoral Glu concentrations measured by MRS have recently been shown to provide a robust marker of survival in 45 pediatric patients with medulloblastoma (HR 8.0,  $P = .0003$ ).<sup>73</sup> As such, MRS may offer a method of assessing metabolic response to dietary glycemic modulation.

Patient-reported outcome measures, including those assessing both health-related quality of life, and other patient-centered metrics will also be important. Nonvalidated patient-reported outcome measures have been incorporated into existing studies of KDs, but it will be important to determine the potential

generalizability and psychosocial impact of such interventions. In addition, given the reported associations between hyperglycemia and infection, seizure control, and other important medical comorbidities, nononcologic covariates should also be included in future prospective studies.<sup>78–80</sup>

### Combining Dietary Glycemic Modulation With Cytotoxic Therapies

Ultimately, dietary interventions that target the glycolytic dependency of gliomas may prove optimal when combined with additional therapies that target tumor hypoxia, cellular proliferation, or cell signaling pathways. Gliomas are both highly metabolically active and highly mutagenic. Preliminary studies suggest that responses may be improved when KDs are combined with radiation,<sup>81</sup> chemotherapy,<sup>32</sup> and even hyperbaric oxygen.<sup>23</sup> Additionally, current strategies for developing longer-term maintenance therapy for gliomas in general are limited by myelosuppressive and emetogenic risk with chemotherapy as well as the potential for inducing new driver mutations.<sup>82</sup> Additional preclinical work is necessary to determine the impact of KDs on tumor-initiating cells, but some evidence suggests that dietary glycemic modulation may impair growth of these tumor stem cells through reduction in circulating insulin, IGF-1, and other proliferative pathways.<sup>83</sup>

### Conclusion

Knowledge of the metabolic pathways underlying glioma tumorigenesis continues to expand and offers novel therapeutic targets. Pharmacological drug development is ongoing to identify agents that can be successfully delivered to the target tissue and alter key metabolic pathways. Ketogenic diets (classic KD, MAD, and others) offer another readily available therapeutic avenue. Strategies that combine the tolerability of the MAD with intermittent fasting to restrict caloric intake may prove optimal. A systematic approach to developing a clearly defined dose, establishing measures of biology activity, and determining optimal endpoints is critical.

### Supplementary Material

Supplementary material is available online at Neuro-Oncology Practice (<http://neuro-oncology.practice.oxfordjournals.org/>).

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