The Restricted Ketogenic Diet: An Alternative Treatment Strategy for Glioblastoma Multiforme

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Glioblastoma Multiforme (GBM)

Glioblastoma multiforme (GBM) is considered the most malignant of primary brain cancers with only about 12% of patients living beyond 36 months (long-term survivors).[1,2] GBM is heterogeneous in cellular composition consisting of tumor stem cells, mesenchymal cells, and host stromal cells.[3-6] In addition to the neoplastic cell populations, tumor-associated macrophages/monocytes (TAM) also comprise a significant cell population in GBM sometimes equaling the number of tumor cells.[7-12] TAM indirectly contribute to tumor progression through release of pro-inflammatory and pro-angiogenic factors.[8,10,12,13] It is often difficult to determine with certainty whether cells with macrophage characteristics are part of the stroma or are part of the neoplastic cell population.[14] Many of the neoplastic cells in GBM invade through the neural parenchyma well beyond the main tumor mass making complete surgical resections exceedingly rare.[15,16] Our goal is to review information on the current status of care for GBM and to provide information on how the restricted ketogenic diet (RKD) might serve as an effective alternative to the current standard of care for controlling growth and enhancing survival in patients with GBM. The therapeutic and health benefits of the KD are realized when the diet is consumed in reduced or restricted amounts. Much of the information in this review was compiled from our previous papers and reviews on this subject.[10,17-24]

Ketogenic Diet and Calorie Restriction

The high fat, low carbohydrate ketogenic diet (KD) has long been recognized as an effective non-toxic therapy for reducing epileptic seizures in children.[25] The mechanisms by which the KD manages seizures are linked to shifts in brain energy metabolism.[26-28] Glucose is the sole metabolic fuel used for nearly all brain functions under normal physiological conditions,[29] but the brain will metabolize ketone bodies for energy when access to glucose is limited, as would occur during water-only therapeutic fasting in humans or during calorie restriction in mice.[30-34] It has long been known that water-only fasting or calorie restriction is effective in managing epilepsy in humans and mice.[28,35,36]

The KD was introduced as an alternative to fasting for the long-term management of seizures in humans.[35,37] The efficacy of the classic KD is optimal when the ratio of dietary fats to combined carbohydrate/protein is 4:1.[38] This requires careful attention to diet calculations. Although the safety profile of the KD is favorable when compared to many anti-seizure drugs, adverse effects have been reported in a few patients and a physician's supervision is often necessary while implementing the KD.[25,39] Importantly, the KD is also gaining recognition as a potential therapy for a host of other neurological and neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, traumatic brain injury, and stroke.[25,40-42] When administered in restricted amounts, which do not exceed the individual's total energy needs, the KD can also be therapeutic against malignant brain tumors in mice and humans.[18,19,24,43] Before discussing the role of the restricted ketogenic diet (RKD) as a new treatment strategy for GBM, it would be useful to briefly review information on the current status of treatment for this disease.

Standard of Care for Glioblastoma Multiforme

The current standard of care for GBM includes maximum surgical resection, radiation therapy, and chemotherapy.[1,44] Many GBM patients also receive perioperative corticosteroids (dexamethasone) as part of the conventional treatment, which is often extended throughout the course of the disease.[10,45,46] Despite the best available treatment, prognosis is poor for most patients with GBM.[2,47-49] Indeed, there have been no major advances in GBM management for over 50 years, though use of temozolomide (Temodar) has produced marginal improvement in survival.[1,47]

We recently described how the current standard of care for GBM and other high-grade brain tumors could actually accelerate tumor growth thereby decreasing the probability of long-term patient survival.[10,50] This prediction was based on new information regarding tumor energy metabolism. It is now recognized that glucose and glutamine are the prime metabolic fuels for driving the growth of malignant tumors including brain tumors.[18,22,51-54] Indeed, some glioma cells can become

dependent on glutamine for survival.[55] Ready access to glucose and glutamine will accelerate tumor growth thus enhancing the probability of recurrence and reduced progression free survival.

GBM Energy Metabolism: Role of Glutamine

In contrast to extracranial tissues where glutamine is readily available, glutamine is regulated in the brain through its involvement in the glutamate-glutamine cycle of neurotransmission.[29,56] Glutamate is a major excitatory neurotransmitter that must be cleared rapidly following synaptic release in order to prevent excitotoxic damage to neurons.[56,57] Glial cells possess transporters for the clearance of extracellular glutamate, which is then metabolized to glutamine for delivery back to neurons. Neurons metabolize glutamine to glutamate, which is then repackaged into synaptic vesicles for release.[56] The glutamate-glutamine cycle maintains low extracellular levels of both glutamate and glutamine in normal neural parenchyma. Disruption of the glutamate-glutamine cycle can provide neoplastic GBM cells access to glutamine as we recently described.[18]

In contrast to normal glia, neoplastic glioma cells secrete glutamate. Glioma glutamate secretion is thought to contribute in part to neuronal excitotoxicity and tumor expansion.[57] Neurotoxicity from mechanical trauma (surgery), radiotherapy, and chemotherapy can also increase extracellular levels of glutamate contributing further to tumor progression.[57] How might information on GBM energy metabolism relate to disease progression and to the standard of care for this cerebral neoplasm?

Radiation and chemotherapies are known to induce necrosis and inflammation, both of which conditions will increase tissue glutamate levels.[15,58-60] Local astrocytes rapidly clear extracellular glutamate. The cleared glutamate is metabolized to glutamine for release to neurons. In the presence of dead or dying neurons, however, surviving tumor cells and TAM will use astrocyte-derived glutamine for their energy needs and growth.[10] Besides serving as a precursor for glutamate synthesis in neurons, glutamine might also be used as a fermentable energy metabolite for neoplastic GBM cells through substrate level phosphorylation in the mitochondria.[61,62] Radiation damage to tumor cell mitochondria will hasten the fermentation of glucose and glutamine for their growth and survival.[22,63,64] Indeed, radiation therapy is known to up-regulate the PI3K/Akt signaling pathway, which drives glioma glycolysis and chemotherapeutic drug resistance.[22,65-67]

GBM Energy Metabolism: Role of Glucose

High-dose glucocorticoids (dexamethasone) are generally prescribed to reduce radiation-associated brain swelling and tumor edema. It is well documented that dexamethasone increases gluconeogenesis and blood glucose levels while enhancing apoptosis resistance.[18,24,66,68-70] Glucose fuels tumor cell glycolysis as well as serving as a precursor for glutamate synthesis.[19,21,29,64] Glycolysis is a major energy pathway in malignant gliomas.[21,71,72] Using linear regression analysis, we showed that the growth rate of the mouse CT-2A experimental astrocytoma was directly dependent on blood glucose levels.[19] The higher the glucose levels, the faster the astrocytoma grew. As glucose levels fall, tumor size and growth rate falls. Hyperglycemia not only contributes to rapid tumor cell growth, but also enhances white matter damage in patients receiving radiation therapy.[73] Hyperglycemia was also directly linked to accelerated progression and poor prognosis in humans with GBM.[74,75] In other words, the findings in mice showing that elevated blood glucose accelerates brain tumor growth were also documented in humans with GBM.

Moreover, we found that the expression of insulin-like growth factor 1 (IGF-1) was also dependent on circulating glucose levels.[19,76] IGF-1 is a cell surface receptor linked to rapid tumor growth through the PI3K/Akt signaling pathway.[76,77] The association of plasma IGF-1 levels with tumor growth rate is due primarily to circulating levels of glucose.[19] These findings in animal models and in brain cancer patients indicate that tumor growth rate and prognosis is dependent to a significant extent on circulating glucose levels. Glucose is the prime fuel for glycolysis, which drives growth of most brain cancers.[21,71,72] As long as circulating glucose levels remain elevated, GBM growth will be difficult to manage.

In addition to serving as fermentable fuels for neoplastic tumor cells, glucose and glutamine are also important fuels for cells of myeloid linage, i.e., macrophages, monocytes, and microglia.[13,18,78] These fuels will act synergistically to enhance the energy metabolism and the pro-inflammatory activities of TAM. TAM respond to the local tumor environment as if it were an unhealed wound and thus release pro-angiogenic growth factors.[12,79] Glutamine is an important fuel for macrophages and other cells of the immune system.[78] This is significant, as some neoplastic cells in GBM might actually arise from cells of myeloid origin or from fusion hybrids between macrophages and neoplastic stem cells.[14] Access to glucose and glutamine within the tumor microenvironment provides neoplastic GBM cells and TAM with fermentable fuels necessary for growth and survival in the absence of a supporting vasculature. Moreover, access to these fuels will create escalating biological chaos where the intrinsic properties of TAM to heal wounds will enhance the capacity of neoplastic brain tumor cells to proliferate, invade, and self-renew.[10,12,80] In other words, normally programmed cellular events become counter productive to host survival. High glucose concentrations together with unrestricted glutamine availability will provide the necessary energy metabolites for driving the escalating situation. Figure 1 shows how radiation therapy and dexamethasone treatment can increase availability of glucose and glutamine in the tumor microenvironment.

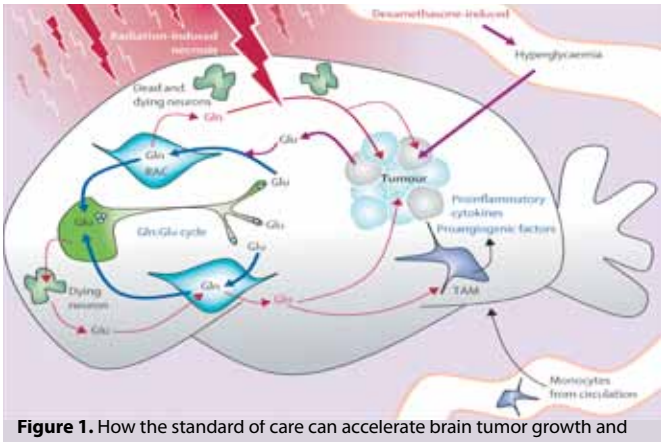

Figure 1. How the standard of care can accelerate brain tumor growth and recurrence. GBM and other high-grade brain tumors consist of multiple neoplastic cell types as well as TAMs, which release pro-inflammatory and pro-angiogenic factors. All these cells will use glucose and glutamine (Gln) as major metabolic fuels for their growth and survival. Increased glutamate (Glu) concentrations will arise after radiation/drug-induced necrosis. Reactive astrocytes (RA) take up and metabolize glutamate to glutamine, whereas hyperglycemia will arise after corticosteroid (dexamethasone) therapy. Together, these standard treatments will provide an environment that facilitates tumor cell growth, survival, and the likelihood of tumor recurrence.[10] With permission from *Lancet Oncology.*

Are Current Treatments the Best Option for all GBM Patients?

Although the existing standard of care for malignant brain cancer will increase patient survival over the short-term (months) compared to the "no therapy" option, we suggest that this therapeutic strategy will eventually accelerate the energy metabolism of surviving tumor cells. Moreover, the malignant phenotype of brain tumor cells that survive radiotherapy is often greater than that of the cells from the original tumor.[66] Treatments that increase tumor energy metabolism will facilitate tumor cell growth and survival. Such treatments will ultimately impede patient survival over the long-term.

Some GBM patients who receive the standard of care are also administered the anti-angiogenic drug, bevacizumab. Although bevacizumab targets the tumor vasculature and may prolong patient survival, these effects are likely related to its anti-edema effect rather than to its anti-neoplastic activity. Bevacizumab exacerbates radiation-induced necrosis and enhances the invasive properties of the tumor cells.[81-83] Recurrent GBM following bevacizumab therapy is almost 100% fatal, as are most recurrent GBMs.[84,85] Glucose and glutamine fermentation could allow some invasive GBM cells to survive in the hypoxic microenvironment.[18] These GBM cell capabilities would render bevacizumab a less than adequate therapy for longer-term GBM management. Due to a plethora of adverse effects and undemonstrated efficacy, bevacizumab was discontinued as a therapy for brain cancer in Germany and was recently questioned by the FDA as an effective therapy for breast cancer in the US. In light of the adverse effects and potential for driving invasive cancer cells in patients, it is unclear why this drug remains in use for treating GBM. As long as brain cancer is viewed as something other than a metabolic disease, oncology will make little progress in improving progression free survival. However, once GBM becomes recognized as a metabolic disease, dependent on glucose and glutamine for progression, we anticipate major advances in treatment and with substantial improvement in outcomes.

Restricted Ketogenic Diet (RKD) as an Alternative Treatment Strategy for GBM

Emerging evidence suggests that metabolic therapies using ketogenic diets that lower blood glucose levels can help retard GBM growth in younger and older patients.[24,43] Restricted ketogenic diets are those that limit carbohydrate intake while delivering fewer total calories than are required to remain in energy balance. This hypocaloric state lowers circulating glucose levels while simultaneously raising ketone levels. Ketone bodies (β-hydroxybutyrate and acetoacetate), which are synthesized in the liver, become an alternative fuel for brain energy metabolism when glucose levels are reduced.[31,86-88] B-hydroxybutyrate (β–OHB) is the major circulating ketone body under fasting conditions[89] and certain fed states, such as the RKD, that simulate fasting. Ketone bodies have known neuroprotective and anti-inflammatory action against a number of neurological and neurodegenerative diseases and can also be toxic to some human tumor cells.[90-92] Ketone body metabolism reduces oxygen free radicals while enhancing metabolic efficiency of normal cells.[88,93] Hence, ketones are considered "good medicine" for several neurological and neurodegenerative diseases.[25,31,87]

What is the evidence supporting use of the RKD as a novel treatment strategy for malignant brain cancer? We showed that calorie restriction and the RKD are the only therapeutic approaches that simultaneously target energy metabolism, tumor cell invasion, angiogenesis, and inflammation through the IGF-1-PI3K-Akt-Hif-1a signaling pathway.[18,76,94-97] The down regulation of this pathway is considered essential for managing growth of most malignant cancers including GBM.[98-103] Unlike normal brain cells, many tumor cells cannot metabolize ketone bodies for energy due to their various mitochondrial and genetic defects.[18,21,23,104,105] Moreover, the RKD could potentially lower brain glutamine levels, thus restricting this energy metabolite for tumor growth.[106,107] The RKD could be even more therapeutic if combined with drugs that target glycolysis, e.g., 2-Deoxyglucose (2-DG) or dichloroacetate.[108,109] Although 2-DG is largely ineffective as a cancer treatment when used alone, we showed that the therapeutic efficacy of 2-DG against the malignant CT-2A astrocytoma is greatly enhanced when administered with the RKD.[108] It is also possible that therapeutic synergy might occur if any anti-glycolytic drug is administered together with the RKD.[110,111] None of these anti-glycolytic drugs would be an effective therapy if used alone, but some could be highly effective when

administered with the RKD. Although the ant-diabetic drug metformin has been suggested as an anticancer therapy,[110] metformin acts like insulin in facilitating glucose up-take into cells. Metformin could enhance tumor cell glycolysis, as lactic acidosis is an adverse effect of metformin. Drugs that might enhance glycolysis would have adverse effects in patients with brain tumors. By limiting the availability of glucose and glutamine to tumor cells, the RKD will improve progression free survival in patients with malignant brain cancer or most cancers for that matter.

Evidence from Case Reports

In 1995, Nebeling and coworkers attempted the first nutritional metabolic therapy for human malignant brain cancer using the ketogenic diet.[43] The objective of the study was to shift the prime substrate for energy metabolism from glucose to ketone bodies in order to disrupt tumor metabolism while maintaining the nutritional status of patients.[43] The patients in this landmark clinical study included two female children with nonresectable advanced stage brain tumors (anaplastic astrocytoma stage IV, and cerebellar astrocytoma stage III). Measurable tumor remained in both subjects following extensive radiation and chemotherapy. Although severe life threatening adverse effects occurred from the radiation and chemotherapy, both children responded remarkably well to the KD and experienced long-term tumor management without further chemo or radiation therapy. Indeed, one of the patients remains alive at the time of this writing (Nebeling, personal communication). Positron Emission Tomography with fluro-deoxy-glucose (FDG-PET) also showed a 21.8% reduction in glucose uptake at the tumor site in both subjects on the KD.[43] These findings indicate that a ketogenic diet, which lowers glucose and elevates ketone bodies, could reduce glycolytic energy metabolism in these brain tumors.

More recently we published a case report showing that therapeutic fasting and a modified ketogenic diet could help manage glioblastoma growth in an older female patient.[24] The GBM in this patient was managed while she remained on the RKD, but the tumor became unmanageable when the diet was no longer followed. Although the GBM was multicentric and incompletely resected (Figure 2), the RKD had a marked therapeutic benefit based on general patient health and MR imaging (Figure 3). Considered together, these case reports in children and an adult indicate that the KD, when consumed in restricted amounts, is well tolerated and can be an effective non-toxic therapy for GBM and other malignant brain cancers.

The RKD could also eliminate or reduce the need for adjuvant anticonvulsant and steroidal medications (dexamethasone) for brain tumor patients. The KD was designed initially as an antiepileptic therapy and can therefore be used to manage tumor-associated seizure activity.[112-117] It is unclear why brain cancer patients are given toxic anticonvulsant medications when the RKD should be able to achieve the same clinical endpoint while also targeting tumor growth. Indeed, the anticonvulsant action of the KD is better when administered in restricted or carefully measured amounts than when administered in unrestricted or unmeasured amounts.[28,115]

Despite the documented efficacy of the RKD as a non-toxic metabolic therapy for brain cancer in case reports, no major clinical trials of this therapy have been initiated in the US to date. A clinical trial using the unrestricted KD for recurrent glioblastoma has been initiated in Germany (ERGO Trial) under the direction of J. Rieger at the University of Tübingen. Modest improvement was reported without adverse effects, but no published information is yet available on blood glucose or blood ketone levels in the treated patients. This information will be needed to gauge the degree of energy stress on surviving tumor cells, as blood glucose levels are predictive of therapeutic efficacy. Careful documentation of blood glucose and ketone levels will be essential for predicting therapeutic efficacy.

The reason for not initiating clinical trials on the RKD for brain cancer management in the US remains unclear. Some have suggested that the North America Brain Tumor Collaborative (NABTC) prefers "hand-me-down" drug therapies from other cancer studies rather than exploring potentially more effective alternative approaches.[18,21,49] It is unknown if the reluctance to consider alternative approaches to GBM management

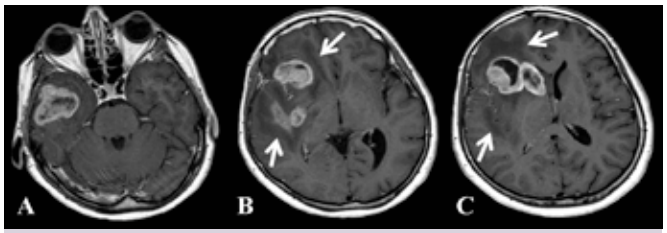

Figure 2. MR contrast enhanced images of a large multi-centric mass involving the right temporal pole. A) Frontal operculum, B) insular lobe and basal ganglia, C) head of caudate nucleus. Note the presence of peripheral edema (arrows). The image was taken prior to surgical resection and implementation of diet therapy. Reprinted from *Nutrition & Metabolism*.[24]

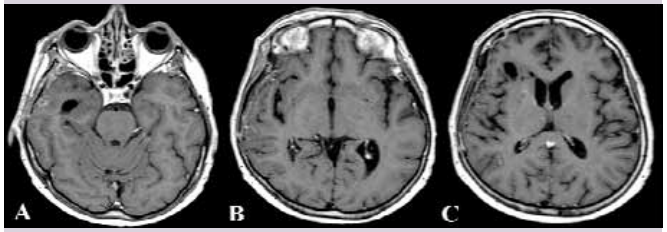

Figure 3. Brain MR imaging taken approximately 2.5 months after the first MR imaging shown in Figure 2. The patient had completed the standard radiotherapy plus concomitant temozolomide therapy together with RKD protocol. No clear evidence of tumor tissue or associated edema was seen. Arrow indicates porencephaly. Further details are described in.[24] *Reprinted from Nutrition & Metabolism*.[24]

rests with the NABTC or with those who advise the Collaborative. Based on pre-clinical studies in mice and case studies in patients,[17,20,23] it is obvious that the RKD should be investigated for its therapeutic potential for managing GBM and other malignant brain cancers. The failure to initiate clinical trials is unfortunate especially for those GBM patients who might benefit from using the RKD for managing their disease.

Guidelines for Implementing the RKD for GBM

We suggest a sequential series of therapeutic phases for treating GBM patients with the RKD. The protocol for using the RKD could differ from one patient to the next depending on the age and health status of the patient. Consequently, the information presented in the use protocol below can be modified for individual cases.

Phase 1: Initiation

Phase one of the treatment strategy would require GBM patients to gradually lower their circulating glucose levels while concurrently elevating circulating ketone (β–OHB) levels. The procedures used for measuring blood glucose and ketone levels in GBM patients are essentially the same as those that would be used by individuals with diabetes. The Medisense Precision Xtra blood glucose and ketone monitor (Abbott Laboratories) is suggested for measuring blood ketones and glucose, but any meter that can measure glucose and ketones in the blood will be adequate. Patients can measure their blood glucose three times a day preferably before breakfast, and about two hours after lunch and dinner. It is essential to keep accurate food records that specify the type and quantity of all foods and beverages along with other potential sources of carbohydrates (e.g. medications and supplements). Precise blood glucose and ketone measurements should also be documented. This information is essential in identifying factors that have an impact on compliance and efficacy. Although blood ketone measures are a more precise measure than urine ketones[24,118,119] it may aid compliance to track urine ketones at frequent intervals during the early stages of implementation or until patients become familiar with the procedures for using the blood ketone meter. Thereafter, urine testing may be used in addition to blood testing as an additional measure of dietary compliance. As finger blood withdrawal is more easily tolerated in adults than in children, the protocol can be modified for children.

Blood glucose ranges between 3.0-3.5 mM (55-65 mg/dl) and β–OHB ranges between 4-7 mM should be effective for managing GBM growth in most patients. These values are within normal physiological ranges of glucose and ketones in humans and, based on our findings in mice, should have anti-angiogenic, ant-inflammatory, and pro-apoptotic effects. This treatment will induce metabolic isolation and significant growth arrest of tumor cells. We refer to these glucose and ketone levels as the zone of metabolic management (Figure 4).[17]

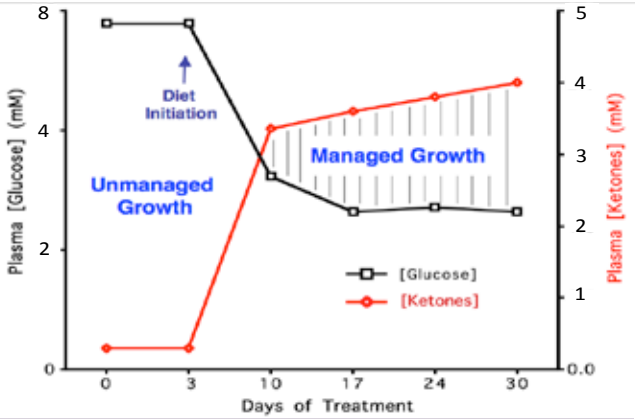

Figure 4. Relationship of circulating levels of glucose and ketones (β-hydroxybutyrate, β-OHB) to brain tumor management. The glucose and ketone values are within normal physiological ranges under fasting conditions in humans and will produce anti-angiogenic, anti-inflammatory, and pro-apoptotic effects. We refer to this state as the zone of metabolic management. Metabolic stress will be greater in tumor cells than in normal cells when humans with brain cancer are in the metabolic zone. The values for blood glucose in mg/dl can be estimated by multiplying the mM values by 18. The glucose and ketone levels predicted for brain tumor management in human brain cancer patients are 3.1-3.8 mM (55-65 mg/dl) and 2.5-7.0 mM, respectively.[18,21] Reprinted with permission from *Epilepsia*.[17]

Blood ketone levels are higher when the KD is administered in restricted amounts than when administered in unrestricted amounts,[28] and when ketone synthesis is supported by the addition of medium-chain triglycerides to the diet. It is important to recognize, however, that circulating ketone levels will rarely exceed 7-9 mmol in most non-diabetic patients since excess ketones will be excreted in the urine.[34,87,88] Hence, it is unlikely that the RKD will elevate blood ketones to pathological levels (greater than 15 mmol) in most patients with normal physiology.

Patients in good health should start the therapy with a water-only fast. Therapeutic fasting will lower blood glucose and will elevate blood ketones to the therapeutic ranges within 48-72 hrs. While this degree of food abstinence might sound draconian, fasting for 2-3 days should not be difficult for those individuals in good physical health. Fasting is often used to initiate the KD as a therapy for managing refractory seizures in children with epilepsy.[120] Diet initiation can be done in hospital or under the guidance of a physician in the home environment. It would be advisable for patients to read the book "Fasting for Renewal of Life," by Herbert Shelton.[121] The information in this book will allay concerns regarding adverse effects of fasting and will highlight the multiple health benefits associated with reduced food intake. The book by John Freeman and colleagues, "The Ketogenic Diet: A Treatment for Children and Others with Epilepsy," also provides excellent information on how the ketogenic diet can be implemented and the role of fasting in jump-starting the necessary metabolic adjustments for initiating the diet.

A gradual introduction of the diet without fasting is recommended for

those patients who are fragile or in poor health. For those patients not conducting a water-only fast, a restriction of carbohydrates to <12 g/day and a limitation of protein to about 0.8-1.2 g/kg of body weight/day is one way to enter the therapeutic ranges for blood glucose and ketones. Consumption of fats and oils can be used to make up the balance of energy needs. This approach will, however, require longer periods of time (perhaps several weeks) to reach the therapeutic ranges. Attending physicians should determine which course of action would be best suited for each patient, i.e., a full therapeutic fast or a more gradual introduction of the RKD. However, it is imperative that implementation reduce blood glucose levels to the therapeutic range as quickly as possible in order to limit tumor progression. Once patients get their blood glucose levels to 55-65 mg/dl range and get their blood ketones to the 3-5 mmol range, they can maintain this metabolic state using various ketogenic diets and caloric adjustments. As the KD can have a diuretic effect, it is best to avoid diuretic drugs such as Lasix (the prescribing physician should be consulted here). Indeed it is best to monitor the effects of all medications and to keep dosages at minimum levels while on the RKD. Electrolyte levels should also be monitored or replenished if needed while on the diet for extended periods.

Many recipes for ketogenic meals are available on the Charlie Foundation website (http://www.charliefoundation.org). The Charlie Foundation was established to provide information on how the KD is used to manage refractory seizures in children. It is our opinion that any ketogenic diet, consumed in restricted amounts, will be effective in maintaining reduced glucose and elevated ketones. The composition of fats in the diet can be flexible as long as blood glucose and ketones are maintained in the therapeutic ranges. Like the ketogenic diet, low glycemic diets have also been used to manage seizures in children.[122] Low glycemic diets might also be effective in helping to maintain low blood glucose levels in some GBM patients, as glucose is released slowly from low glycemic foods. It remains to be determined if low glycemic diets are as effective as the RKD for maintaining glucose levels low enough to retard tumor progression.

Most GBM patients will require some degree of professional nutritional guidance, particularly in the first few weeks of diet implementation. The key is to maintain a KD that is nutritious, but is consumed in limited amounts. The definition of "ketogenic diet" allows for considerable leeway in food choices as long as the individual has reduced blood glucose and is producing ketones. Ghee, a clarified butter, can be combined with egg yolk as a ketogenic option. Coconut oil, safflower oil, and sunflower oil can also be included as part of the KD. Medium-chain triglyceride (MCT) oil is another choice, as MCTs are transported directly from the small intestine to the liver, where they are metabolized to ketones.[123] However, some gastrointestinal problems might arise from too rapid introduction of MCT to the diet or from prolonged use. Consumption of the KD in unrestricted amounts will prevent blood glucose from reaching the reduced levels needed to target tumor progression and can have adverse effects for patients. Excessive or unrestricted KD consumption can cause insulin resistance and hyperglycemia.[23] All brain cancer patients and their physicians should know that "less is better" when it comes to using the KD for managing brain cancer growth.

It is helpful for patients to keep accurate food records during diet implementation, and to share this information with healthcare professionals who are experienced in implementing very-low-carbohydrate therapies. In order to maintain compliance, patients can use the "KetoCalculator" (see Charlie Foundation web site for information on the KetoCalculator, http://www.ketocalculator.com/ketocalc/diet.asp) to facilitate menu planning and to identify foods that are non-compliant with a therapeutic KD. After the initial acclimation period, heavier individuals can safely lose up to 2 lbs/week until they are in the lower end of the normal range. Slight or severely compromised individuals should be monitored carefully to avoid rapid weight loss. As ketogenic diets are deficient in select vitamins and minerals,[115] daily vitamin/minerals supplementation will be needed during a sustained RKD. Sugar-free multivitamins and calcium are the standard supplements when on the KD.[115]

Variability in Caloric Adjustments

As calories can be metabolized differently among individuals, the dietary adjustments needed to achieve the glucose/ketone metabolic state will differ among patients. In mice we used body weight as the independent variable for adjusting the degree of calorie restriction.[23,28] This practice, however, will not be effective in humans. Some patients might achieve the therapeutic glucose/ketone range without significant weight reduction, while other patients might require weight reduction to achieve the metabolic state. Importantly, the weight loss associated with the RKD is part of a metabolically appropriate response to calorie restriction. In contrast, the weight loss sometimes seen following chemotherapy is due to toxicity and to the effects of the therapy on appetite. We know from the results of the case studies with two children and an adult GBM patient that malignant brain tumor growth can be reduced if blood glucose is lowered and ketones are elevated.[24,43] We know from our work in mice that tumor growth is not slowed if blood glucose is not lowered despite elevations in ketones and the persistence of normal body weight.[19,23] Given the wide variations in age, body type, weight, and metabolic status that we are likely to encounter in humans as compared to our mouse models, we anticipate the need to individualize the degree of caloric restriction to lower glucose and elevate ketones to ranges that will retard GBM progression. Frequent blood glucose measurements, as described previously, will help refine that process. Considering that personalized therapy is the new mantra for cancer management[124,125] we suggest that personalized caloric adjustment will result in maximum therapeutic benefit.

Some patients might experience light-headedness, nausea, headache, etc. in the first few days of the RKD, especially if they initiate the therapy with a multi-day fast. These symptoms are transient and associated more with glucose withdrawal than with adverse effects of the diet. Evidence suggests that the human brain can become addicted to glucose from a life-long consumption of energy-dense foods of low nutritional value.[126] Consequently, the abrupt cessation of food intake may produce temporary withdrawal symptoms similar to those experienced from cessation of any addictive substance. Glucose withdrawal symptoms can be greater in those individuals who have never fasted than in individuals who have experience with fasting. As most people in modern industrial societies do not practice therapeutic fasting as a life style, glucose withdrawal symptoms will likely be encountered in most patients who attempt the RKD as a brain cancer therapy. When compared to the debilitating effects of conventional chemotherapies and radiation, the symptoms associated with the RKD are minor and will pass after two to three days for most people.

The high fat content of the RKD can reduce the feeling of hunger while maintaining low glucose and elevated ketone body levels. A recent study in rats suggests that diets supplemented with ketone esters might produce physiological effects similar to those for the RKD, but without significant food restriction.[106] However, administration of ketone esters has not yet been tested in GBM patients. It is also important for physicians to recognize that some patients are unable or are unwilling to implement the RKD for various reasons. It is not good to force the RKD on any patient who does not want this therapy. The RKD should be used only for those patients who are motivated, disciplined, and healthy enough to make the necessary changes to diet. Patient education and engagement in the process is key to the success of the therapy.

Dosages of any medications (i.e., anti-epileptic drugs) will need to be monitored carefully under the RKD. We showed that therapeutic action as well as toxic effects for 2-DG was greater when administered with the RKD than when administered with an unrestricted KD.[108] The RKD will not be effective in the presence of dexamethasone (Decadron) or other steroid medications. Steroid medications prevent glucose levels from reaching the therapeutic zone and therefore antagonize the therapeutic effects of RKD. While steroids can rapidly mitigate some aspects of the brain tumor-related neurological symptoms traits over the short term (paralysis, edema, etc.), chronic steroidal use will ultimately accelerate growth of surviving tumor cells and thus the demise of patients. Hence RKD therapy with its neuroprotective and neuro-therapeutic effects will not harm patients as can high-dose dexamethasone.

Phase 2: Surgery

Phase two of the treatment strategy would involve surgical resection. We suggest surgical resection as an option after first implementing the RKD therapy.[18] This option will be possible only if there is an opportunity for a "watchful waiting" period prior to scheduled surgery.[48] This option will not be possible for those patients in a critical condition at the time of presentation. Calorie restriction and RKD will reduce tumor vascularization and inflammation and will more clearly delineate tumor tissue from surrounding normal brain tissue, as we showed in mice.[94,95] It remains to be determined whether the RKD will produce the same effects in the human brain, but we believe it will. This could be assessed through histological and MR imaging analyses.

Neuro-oncology teams should recognize that smaller brain tumors with reduced vascularity and clearly circumscribed boundaries should be easier to resect than larger brain tumors with poorly circumscribed boundaries and extensive vascularization. Tumors in this state should also ensure greater debulking thereby increasing the likelihood of longer-term survival. The urge to debulk malignant brain tumors as soon as possible after diagnosis may not be in the best interests of all patients and could actually exacerbate disease progression by inducing inflammation in the microenvironment.[18,57] The RKD could confer an additional advantage for some GBM patients, as surgical resection alone can alter the microenvironment thus enhancing the invasive behavior of tumor cells. This practice of surgical resection as soon as possible after tumor diagnosis could also be counter productive for a sub-set of patients, especially those with lower-grade gliomas. The metabolic diet therapy will target angiogenesis and inflammation thus slowing tumor progression naturally. This will provide more time before moving to the surgical option. It is therefore possible that progression free survival could be extended in some GBM patients if an aggressive metabolic therapy were implemented prior to surgery.

Phase 3: Maintenance

Finally, phase three of the treatment strategy is to maintain metabolic pressure on surviving tumor cells. The GBM patient should initiate a fasting regime and/or the RKD within days following debulking surgery.[24] Metabolic pressure could also involve carefully executed diet cycling strategies.[21,24,127] Diet cycling for GBM patients could involve weekly transitions from calorically restricted ketogenic diets to nutritious low calorie, low glycemic diets. Patients should continue monitoring their blood glucose and ketones levels for as long as possible or until disease resolution is achieved. Periodic MR imaging analysis including MR spectroscopy (once every three to four months) can help in assessing therapeutic progress.[24,128]

While the RKD will target energy metabolism and improve progression free survival in GBM patients, we do not believe that the RKD, used as a singular therapy, will provide complete disease resolution. Instead, the goal of the

maintenance strategy is to increase the probability of patient survival for at least 36 months. Recent studies suggest that many patients with advanced cancer should be given the details of their condition.[121] GBM patients should know that they can be considered long-term survivors if they can live 36 months beyond diagnosis. Patients who understand that 36 months marks them as long-term survivors might be more motivated to comply with the dietary restrictions and adhere to the protocol.

In order to significantly extend patient survival, we recommend combining the RKD with drugs that also target energy metabolites. The RKD can be administered together with 2-deoxyglucose (30-40 mg/Kg) and with phenylbutyrate (3-5 g/day) as a diet drug cocktail for targeting both glucose and glutamine in GBM patients. The 2-deoxyglucose will target glucose metabolism and glycolysis, while phenylbutyrate will target glutamine. Phenylbutyrate is metabolized to phenylacetate, which binds to glutamine for elimination in the urine.[52] The drug AN-113 appears to access the brain better than phenylbutyrate and could therefore be more effective than phenylbutyrate in reducing brain levels of glutamine.[129] As we have not yet tested the therapeutic effects of AN-113 in our VM-M3 model of GBM, our recommendation for using this drug as a GBM therapy is speculative at this time.

In light of the non-toxic therapeutic efficacy of the RKD demonstrated in pre-clinical studies, we believe that this metabolic therapy could be used together with a broad range of drugs that also target GBM energy metabolism such as dichloroacetate, 3-bromopyruvate, glufosfamide, omega-3 fatty acids, and resveratrol.[110,130-136] While many of these drugs and strategies might have little therapeutic efficacy or express unacceptable toxicity when used alone, their therapeutic benefit might be significantly enhanced and their toxicity reduced when combined with the RKD, especially since the RKD would allow for the use of lower dosage levels. Similarly, we believe that ketone bodies can protect normal cells from the adverse effects of low glucose and reactive oxygen species (ROS) while effectively targeting the energy metabolism of the tumor cells.

Despite the adverse effects and tumor provocative properties of radiation and temolozomide, it is unlikely that the neuro-oncology field will abandon these therapies anytime soon. It is more probable that oncologists will opt to use these therapies in combination with the RKD. Moreover, radiation and toxic drug therapy will remain as a mainstay for those patients who are incapable or unwilling to use the RKD as a treatment strategy. Radiation therapy can be delayed for four to six weeks following surgery without affecting tumor growth.[137] This could give patients an opportunity to consider whether radiation therapy or the RKD might be best for their situation. In light of the vast data showing that cancer is primarily a metabolic disease and that current treatment strategies result in consistently poor outcomes, it is only a matter of time before the standard therapeutic practices are revised.

Complicating Issues for Implementing the RKD as a Treatment Strategy for GBM

Several issues can complicate attempts to implement RKD as a GBM treatment strategy. One issue is the non-conventional and non-pharmacological nature of the metabolic therapy. Modern medicine has not looked favorably on metabolic diet therapies for managing complex diseases especially when well-established procedures for acceptable clinical practice are available, regardless of how ineffective these procedures might be in managing the disease.[18] Availability of a drug that would mimic the global therapeutic effects of the RKD would certainly be the easiest way to implement the therapy. However, no drugs are known that can lower glucose levels, while simultaneously elevating ketones in the absence of some form of restricted food intake. The recently described ketone ester diets, however, could be an exception and warrant further investigation.[106] Difficulty with implementation and a paucity of experienced practitioners remain as complicating issues in adapting the RKD as a standard therapy for GBM.

Radiation and temozolomide are the standard treatments for GBM. Although radiation therapy was used with the RKD in the human case studies,[24,43] we believe radiation therapy impedes long-term patient survival. Radiation will damage mitochondria in normal cells while creating an inflamed microenvironment. Inflammation enhances glucose energy metabolism and further damages mitochondria. Mitochondrial damage is the origin of most cancers.[22] It makes little sense to treat delicate brain tissue with a therapy that is toxic to normal cells, provides a growth advantage to the surviving tumor cells, and increases risk for new cancers. Although temolozomide has increased GBM patient survival, the benefit has been marginal at best.[1] Like radiation therapy, temolozomide also increases necrotic brain inflammation.[128,138,139] Considering that healthy long-term survivors of the conventional GBM treatment strategies are more the exception than the rule, the RKD administered with energy targeting drugs could represent a novel alternative treatment strategy to the conventional standard treatments.

Compliance

Strict compliance with the requirements of the RKD poses the most significant challenge for implementation. The consequences of non-compliance are not as obvious to patients with GBM as they are to patients with epilepsy. Non-compliance for the epilepsy patient is associated with breakthrough seizures, which are immediate and disturbing for both the patient and the family. In other words, the epilepsy patient experiences an immediate and unambiguous consequence following a failure to comply with the dietary requirements. The consequence of non-compliance for GBM patients would be a subtle

increase in tumor progression that would not be immediately obvious to either the patient or the family. In contrast to breakthrough seizures, shortened survival would be the likely consequence of non-compliance for the GBM patient.

Cancer as a Genetic Disease

Another complicating issue for implementing the RKD as a brain cancer therapy is the persistent view that all cancer including GBM is a genetic disease.[125,140] Why should the neuro-oncology field switch to metabolic therapies if the disease is primarily of genetic origin? The view of cancer as a genetic disease is the driving force for investment in targeted molecular therapies and for the idea that cancer treatments should be personalized in order to target defective signaling pathways within tumors. However, emerging evidence indicates that cancer is primarily a metabolic disease and that the vast numbers of mutations found in tumor cells arise as downstream epiphenomenon of mitochondrial damage.[18,22] Recent studies indicate that over one million mutations can be found in the cells of most tumors.[141,142] How will it be possible to target all these mutations to achieve a cure? We find it remarkable that many brain cancer patients are recruited for molecular therapies that are toxic, potentially lethal, and offer little hope for improved clinical outcome.[143-145] Once cancer becomes recognized as a metabolic disease, more effective and less toxic therapeutic strategies will hopefully emerge.

Mechanism of Action?

Another concern in implementing the RKD for brain cancer management involves the mechanism of action. How can the process of targeting glucose and glutamine, while elevating ketone bodies through dietary energy restriction, be so effective in managing malignant brain cancer? The mechanism of action is rooted in the well-established scientific principle that tumor cells largely use fermentation energy for their growth and survival.[22,23,43,64,76,146,147] Glucose and glutamine drive cancer cell fermentation through substrate level phosphorylation.[18,21,61,62] Because tumor cells are less flexible than normal cells in using alternative energy substrates (ketones), tumor cells will experience more energy stress when access to glucose and glutamine become restricted. While the concept might appear simple, the underlying mechanisms are the subject of considerable investigation and debate. This should not, however, delay the use of the RKD as a systemic metabolic therapy for GBM management.

Cachexia

Another concern is how a metabolic therapy that reduces food intake and body weight can be recommended to patients who might be loosing body weight because of cancer cachexia.[22] Although cachexia is not common in GBM, prognosis is worse in GBM patients that express higher than normal levels of IL-6, a biomarker of cachexia.[148] Other pro-cachexia molecules such as proteolysis-inducing factor are released from the tumor cells into the circulation and contribute to the cachexia phenotype.[149-151] The RKD will reduce inflammation and expression of IL-6.[152] By eliminating the fermenting tumor cells that produce pro-cachexia molecules, the RKD can potentially reduce tumor cachexia.[19,22,151] Once the tumor becomes managed, patients can increase caloric consumption, which will accelerate weight gain and improved health. Nebeling used the KD to improve the nutritional status of her brain cancer patients.[43] Hence, restricted consumption of ketogenic diets could be effective, in principle, for managing tumor growth in brain cancer patients that express biomarkers of cachexia.[43,149]

In contrast to most conventional brain tumor therapies, which expose both normal cells and tumor cells to toxic assaults, dietary restriction and particularly the RKD, are the only known therapies that can target brain tumor cells while enhancing the health and vitality of normal brain cells.[21,23,24,43] In this regard, restricted calorie intake is conceptually superior to most current conventional brain cancer therapies. Support for our position on this issue can be established through clinical trials for brain cancer patients similar to those trials conducted previously for epilepsy patients.[153]

Patient Information

How can effective non-toxic metabolic therapies be introduced as part of the standard clinical practice in the field? It is incumbent upon neuro-oncologists to notify patients that effective alternatives to the current standards of care exist for managing brain tumors. As GBM can be considered an advanced cancer, patients should be aware of all therapeutic options, not just the conventional treatment strategies.[154] Patients should also know that the RKD would retard tumor growth without producing toxic adverse effects. It should be up to the patient and their family to decide whether or not the RKD is a viable therapeutic option for their situation. Patients with malignant brain tumors, especially those with GBM, should have the opportunity to compare and contrast the results from recent drug studies,[144,155] with those of metabolic therapy using restricted diets.[24,43] While standard practices within the field and a paucity of education regarding dietary therapies might make it difficult for some physicians to suggest the RKD as a therapeutic option for brain cancer management, it is hoped that all neuro-oncologists will come to recognize the potential value of the RKD as an effective treatment strategy for GBM.

Conclusions

We provide information on a new, alternative treatment strategy for GBM management that targets tumor energy metabolism. The objective of this new therapeutic strategy is to change the metabolic environment of the tumor and the host. Access to glucose and glutamine within the tumor microenvironment provides neoplastic GBM cells and associated

host stromal cells (macrophages) with fermentable fuels necessary for maintaining the growth and survival of malignant brain tumor cells in the hypoxic microenvironment. The low-carbohydrate, high-fat ketogenic diet, will reduce circulating glucose levels and will elevate circulating levels of ketone bodies especially when consumed in restricted amounts (RKD). A transition from glucose to ketone bodies will restrict glucose availability to the malignant tumor cells while protecting and enhancing the health and vitality of normal brain cells. The therapeutic efficacy of the RKD against GBM can be enhanced when combined with drugs that also target or reduce access to glucose and glutamine. A use protocol is presented for assisting physicians and GBM patients in implementing the RKD as a treatment strategy. Although the RKD is less toxic and potentially more effective in managing GBM than the conventional standard of care, considerable patient education, motivation, and discipline will be necessary for implementing this therapy. Considering the unfavorable outcome of most GBM patients treated with the current standard of care, the RKD treatment strategy could be an attractive treatment option for some GBM patients.

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