STUDY PROTOCOL
Upstate Cancer Center

STUDY TITLE: Feasibility trial of high-fat low-carbohydrate (HFLC) diet with concurrent standard radiation and chemotherapy for primary (de novo) and secondary glioblastoma patients

BACKGROUND: According to the American Association of Neurological Surgeons, glioblastoma multiforme (GBM) is one of the deadliest cancers resulting in death after only 15 months of diagnosis on average. Despite decades of research, median survival time is less than 2 years from diagnosis even with the most aggressive tumor management. Furthermore, aggressive tumor management often comes with substantial side effects, such as high toxicity, that tumor management often comes with substantial side effects, such as high toxicity, that substantially lower quality of life. There is urgent need to develop additional, non-toxic, therapeutic strategies that can increase survival and patient quality of life. A recent Renaissance in oncology research has instantiated the idea that targeting tumor metabolism via dietary intervention may increase tumor cells susceptibility to chemotherapy and radiation and improving the efficacy of current therapeutic approaches in GBM. [1], [2]

Otto Warburg demonstrated that cancer cells use higher amounts of glucose in a primitive way through fermentation compared to normal cells which do not do fermentation in the presence of oxygen. Normal cells use glucose by consuming oxygen and when there is no oxygen they do fermentation by converting glucose to lactate to survive. Warburg discovered that cancer cells even in the presence of oxygen use glucose to produce lactic acid suggesting that tumor metabolism is markedly different from normal cells. [3] GBM also has a unique metabolic signature known as the Warburg Effect, which is a form of aerobic glycolysis. GBM cancer cells rely exclusively on glucose to support their rapid growth without having to generate glucose from fat or protein, a process known as gluconeogenesis, suggesting that glucose deprivation may substantially reduce cell viability. [4]

Tumor cells, like those present in GBM, rely heavily on glucose metabolism for cell growth, proliferation, and also cellular defense mechanism that allow tumor cells to survive chemotherapy and radiation. [2] For example, it is well documented that glucose metabolism and glucose flux through the pentose phosphate pathway is upregulated in tumors and allows them to make the nucleotides requiring for proliferation and for upregulating cell defense mechanism via nicotinamide adenine dinucleotide phosphate (NADPH). [2], [5] Furthermore, glucose flux through the hexosamine biosynthesis pathway is increased in cancer cells and increases cancer cell proliferation, survival. [5], [6]

Currently, there are approximately ten trials that are registered at clinicaltrials.gov using the dietary intervention of HFLC for GBM. [7] At this point, none of the studies have conclusively determined if HFLC diets can improve progression free survival (PFS) in GBM. The limited number of studies with different designs and characteristics (e.g.- sample size, lack of control group, food access, stage and therapy given, methods and technique used for assessing PFS) contribute to overall poor quality of evidence and limit the ability to draw an evidence based conclusion. Therefore, ultimately a randomized controlled trial with an adequate sample size is needed. So far there has been no randomized controlled trial including studies that are currently going on registered at clinicaltrials.gov.

There are also other clinical trials for cancer that one can draw from to determine the safety of HFLC diets in cancer. There have been 14 studies published between 1988 and 2016

that included 206 individuals (94 women, 106 men, and 6 not defined) that assessed effects of an HFLC in cancer patients. The mean sample size was 15 participants. The duration of the dietary interventions ranged from 5 days to 12 months. [8] Study designs included 2 clinical trials, [9, 10] 1 controlled clinical trial, [11] 1 randomized controlled trial, [12] 5 case reports, [13-17] 1 retrospective study, [18] 1 prospective single-arm pilot study, [19] 1 pilot clinical study, [20] 1 systematic prospective cohort study, [21] and 1 prospective observational pilot study. [22] Five of these studies assessed the safety and feasibility of a low-carbohydrate diet, it was reported that an HFLC appeared safe and well tolerated during the standard treatment of patients with various forms of cancer. [9, 11, 14, 16, 18] Amongst these studies 32 glioma patients have been treated utilizing several different low-carbohydrate diet protocols as adjunctive/complementary therapy. [15, 16, 19, 23-26] Prolonged remissions ranging from 1.5 months to more than 5 years were reported. [15, 17-19, 23-26] The HFLC diet was well tolerated with no major side effects being documented. [17]

One of the current limitations of nutritional intervention studies in GBM is dietary adherence. In order to help subjects comply with the HFLC diet, our study will provide a complete nutritional shake from Tarvalin Ltd. from Germany. [27] This shake has already been used in a feasibility study registered at clinicaltrials.gov observing HFLC diet for recurrent glioblastoma. [19] The study showed that the usage of this shake was safe and tolerable. [19] The nutritional content of the shake is shown on the last pages in the protocol before the reference section.

There is urgent need to develop therapies that improve the disease trajectory in patient's diagnosis with GBM. Utilizing dietary interventions to target cancer metabolism and directly impact tumor growth while simultaneously improving the efficacy of the current standard of care presents an exciting, non-toxic intervention that may significantly improve the survival time and quality of life of patients with GBM. Before we move on to conduct a large scale randomized controlled trial, we would like to conduct a feasibility trial to see if we can safely implement our dietary protocol for this trial.

HYPOTHESIS: A HFLC diet is tolerable and safe for people with grade IV glioblastoma multiforme when used as an adjuvant therapy to standard of care.

SPECIFIC AIM/OBJECTIVE: To test whether an HFLC diet with standard radiation/chemotherapy for GBM (WHO grade IV) can be safely and successfully implemented.

STUDY DESIGN: Interventional Study
Endpoint Classification: Feasibility Study
Primary Endpoint: Number of subjects who can tolerate and adhere to the HFLC diet before and during the radiation treatment.
Secondary Endpoint: PFS, Recurrence, overall survival
Masking: Open Label
Primary Purpose: Treatment

ELIGIBILITY CRITERIA: Patients who are diagnosed with Glioblastoma Multiforme (GBM, WHO grade IV) including both for primary (de novo) and secondary glioblastoma patients with no existing risks to an HFLC diet.

EXCLUSION CRITERIA:

1. Diagnosis of diabetes mellitus that is being treated by medication
2. Cholecystectomy within 1 year prior to study entry
3. Symptoms requiring immediate surgical intervention
4. Inability to adhere or to tolerate the dietary protocol
5. Active malignancy other than primary brain tumor requiring therapy
6. Participation in an investigational study within 6 weeks prior to study entry
7. Major co-morbidities such as liver, kidney, or heart failure that in the judgement of the investigators would disqualify the subject from the trial
8. Pregnant or lactating women
9. Inability to give informed consent.
10. Life expectancy of 3 or less months
11. Known/established glucose 6 phosphate dehydrogenase deficiency

INCLUSION CRITERIA:

1. Adults subjects over age 18 with biopsy proven GBM (WHO grade IV)
2. Life expectancy > 3 months
3. Measurable disease after standard therapies
4. Eastern Cancer Oncology Group performance status < or =2; and
5. Hgb A1C < 8%
6. AST (SGOT) < 2 X institutional upper limit or normal
7. Creatinine < 1.5 X institutional upper limit of normal

METHODS, DATA ANALYSIS AND INTERPRETATION: The study will recruit subjects diagnosed with grade IV gliomas, also known as glioblastoma. The current standard treatment for glioblastomas is surgery followed by concurrent radiation and oral chemotherapy called temolozomide over a 6-week period. Since glucose deprivation is required prior to chemotherapy and radiation to elicit the maximal effect of the intervention, dietary modification will start prior to radiation and subjects will gradually reduce the carbohydrate intake and increase fat intake. Since this is a feasibility trial, subjects will be put in a HFLC diet with the current gold standard treatment for GBM and will be monitored if they can safely and successfully adhere to the diet. The standard treatment for GBM involves maximum safe surgical resection followed by radiation combined with temozolomide (an oral chemotherapy pill). All the brain samples from the surgery will be stored by an already existing IRB protocol by Dr. Viapiano laboratory and the samples may be analyzed in the future retrospectively. After radiation, subjects receive additional cycles of temozolomide (adjuvant chemotherapy).

Total carbohydrate intake will be reduced to less than 50 grams per day one week before the treatment while maintaining appropriate total caloric intake required daily. From the first day of the radiotherapy, the total carbohydrate intake will be reduced to less than 20 grams per day. Participants will follow an HFLC diet every day during the combined radiation and chemotherapy phase and adjuvant chemotherapy phase for additional 6 weeks. Subjects will continue the diet for additional 6 weeks after the completion of radiotherapy unless there is any sign of recurrence or difficulty with dietary compliance. Therefore, subjects in the dietary intervention group will maintain a HFLC diet for a total of 12 weeks. During this period, in order to help subjects adhere to the HFLC diet, five HFLC nutritional shakes will be given to fulfill

adequate calorie intake which is about 2,000 kilocalories per day for most adults. One nutritional shake is about 413 kilocalories, thus the subject will be given five shakes every day. During this period, the subjects also can have food such as leafy green vegetables or meats as long as they do not increase readily absorbable carbohydrate intake. In order to confirm that subjects are adhering the HFLC diet, breath ketone levels will be regularly measured as the goal of the HFLC diet is to accomplish ketosis, a state that the body utilizes fat to generate ketone bodies to fuel the brain.

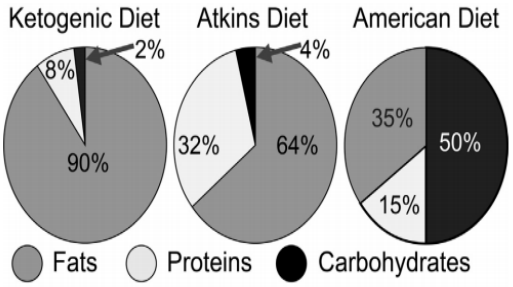


Fig. 1. Comparison of the caloric composition of the ketogenic diet, Atkins diet, and American diet. On any given day Americans consume an average of 265 g of carbohydrates (50% of total calories), 78.3 g of total fat (35% of total calories), and 78.1 g of protein (15% of total calories). Using percentage of total calories, these values are consistent with current 2010 United States Department of Agriculture recommendations that call for 45–65% of total calories from carbohydrate, 20–35% of total calories from fat, and 10–15% of total calories from protein (80).

A. Ketogenic Diet Schema

Patient Recruitment Baseline ketones, glucose, lipids and oxidative stress markers	*Patient Begins Ketogenic Diet 2 days prior to initiating radio-chemo-therapy*	*Radio-Chemotherapy With Concurrent Ketogenic Diet* Daily ketones, glucose, monitor adverse events, & weekly oxidative stress markers	*30-day follow up* Baseline ketones, glucose lipids and oxidative stress markers	*Follow up every 3 months for 1 year*

B. Typical Ketogenic Diet Plan

Course	Example	Energy, kcal	Fat, g	Protein, g	Carbohydrates, g
Breakfast	Crêpes Carb-free hot cocoa	555	54.8	11.0	2.7
Lunch	Bun-less hamburger with mustard mayo dip Chocolate frosted cupcake Low-carb milk	550	53.7	11.1	3.7
Snack	Pineapple keto-cal shake	525	52.6	11.0	2.4
Dinner	Taco salad Low-carb milk	760	75.7	15.1	3.2
Total *(% daily energy)*		2390	236.8 (90%)	48.2 (8%)	12.0 (2%)

Examples of low-carbohydrate diets from Allen et al. Ketogenic diets as an adjuvant cancer therapy: History and potential mechanism, Redox Biology 2 (2014) 963-970

STASTISTICAL ANALYSIS

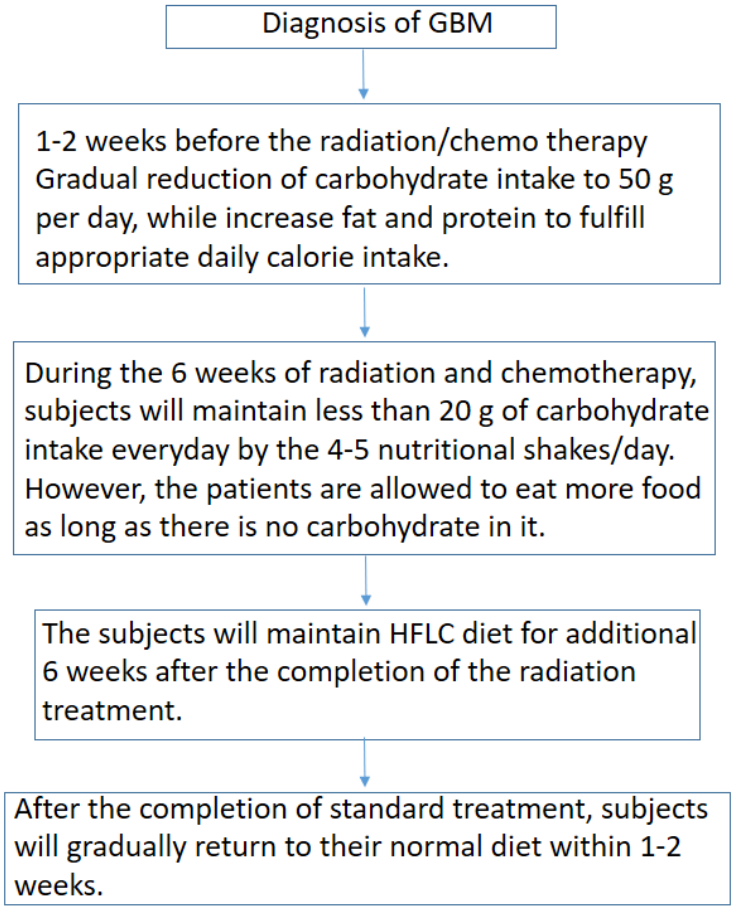
The primary outcome is the number of patients who can successfully adhere to the HFLC diet. The secondary outcome is progression free survival (PFS), which will be measured by imaging techniques such as MRI or PET periodically. The secondary outcomes will also include: time to recurrence and overall survival. All primary and secondary outcomes will be examined using general linear and mixed model analyses. Consideration for statistical adjustments will be made for age, ethnicity, enrollment date, length of treatment, stage of cancer, length of time between diagnosis and initiation of treatment and respective baseline (i.e., untreated) values. We will approach the analysis as an intent-to-treat and per protocol analyses and will further inspect missing data using standardized procedures such as data missing completely at random (MCAR), missing at random (MAR) and missing not completely at random (MNAR). Appropriate algorithms will be employed as needed. All data will be reported as mean (SD), mean change from baseline (95% confidence intervals, CI) or N (%) when describing prevalence relative to categorical analyses using SPSS (v23, Armonk, NY).

STUDY PROCEDURES: Subjects who are diagnosed with GBM and receiving standard care treatment at Upstate Cancer Center will be recruited for the study. Subjects will first be given a consent form to read and sign. Subjects will then fill out a questionnaire which includes basic demographic information, past medical history, and risks to an HFLC diet. Patient's identity will be coded. All the patients' data will be stored on a secure server at Upstate neurosurgery department. There is no direct benefit to individuals as study participants. Participants will not be

paid. However, this study will provide much needed clarity to the scientific literature that may be used to improve treatment and prognosis in several types of cancer.

EXPECTED OUTCOMES: The expected outcomes of this study are that participants are able to safely follow the HFLC diet before the radiation/chemotherapy and during the treatment. Also, we expect that they will have increased PFS when compared with the existing PFS of GBM. If successful, this intervention can be further studied to be employed adjuvant therapeutic in current GBM treatment strategies.

SCHEMATIC DIAGRAM:



Nutritional Information for the Shake

Keto-Drink BANANE: Nutritional values

Average nutritional values relative to 100 ml and per drink (250 ml).

Average nutrient content	Per 100 ml	Per drink
energy	683 kJ 165 kcal	1.708 kJ 413 kcal
fat	13.8 g	34.5 g
hereof: saturated fatty acids	2.3 g	5.8 g
Of which MCT	1.3 g	3.2 g
Of which monounsaturated fatty acids	7.9 g	19.9 g
Of which polyunsaturated fatty acids	3.6 g	9.0 g
Of which omega-3 fatty acids	0.9 g	2.4 g
Of which omega-6 fatty acids	2.6 g	6.6 g
carbohydrates	1.4 g	3.5 g
of which sugars	<0.4 g	<1.0 g
Fiber	1.8 g	4.5 g
protein	6.7 g	16.8 g
salt	0.19 g	0.48 g

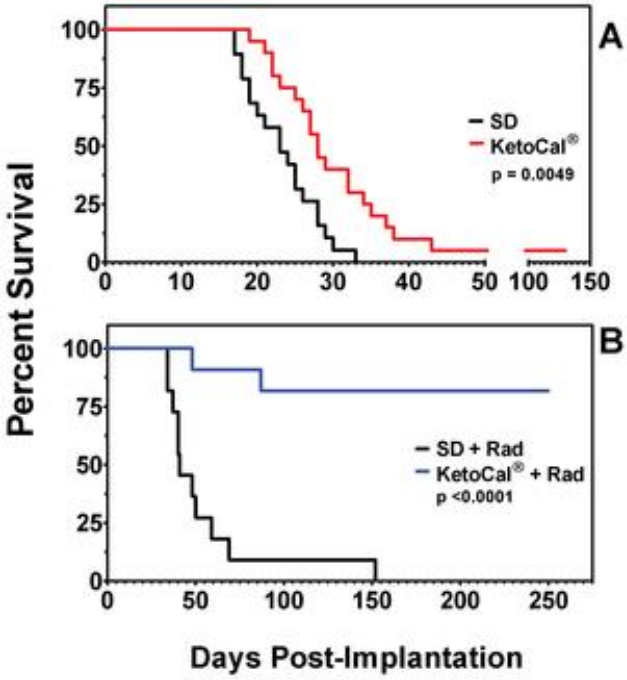
Vitamins	Per 100 ml	Per drink	NRV * / serving
Vitamin A (RE)	127 µg	317.5 µg	40%
Vitamin D	3.6 µg	9.0 µg	180%
Vitamin E (TE)	6.8 mg	17.0 mg	142%
Of which tocopherols	8.5 mg	21.25 mg	-
Of which tocotrienols	10.1 mg	25.25 mg	-
Vitamin K	5.3 µg	13.3 µg	18%
Of which vitamin K1	3.4 µg	8.5 µg	-
Of which vitamin K2	1.9 µg	4.8 µg	-
vitamin C	10.6 mg	26.4 mg	33%
Thiamine	0.2 mg	0.4 mg	35%
Riboflavin	0.1 mg	0.4 mg	26%
Niacin (NE)	3.7 mg	9.3 mg	58%
Vitamin B6	0.3 mg	0.7 mg	52%
Folic acid	50 µg	125 µg	63%
Vitamin B12	0.5 µg	1.3 µg	50%
Biotin	8.0 µg	20 µg	40%
Pantothenic acid	1.2 mg	3.1 mg	51%

Minerals	Per 100 ml	Per drink	NRV * / serving
potassium	228 mg	570 mg	29%
chloride	110 mg	275 mg	34%
Calcium	77.7 mg	194.3 mg	24%
phosphorus	77.8 mg	194.5 mg	28%
magnesium	23 mg	57.5 mg	15%
iron	2 mg	5.1 mg	36%
zinc	1.7 mg	4.4 mg	44%
copper	0.21 mg	0.53 mg	53%
manganese	0.3 mg	0.8 mg	41%
selenium	14 µg	35 µg	64%
chrome	8.2 µg	20.4 µg	51%
molybdenum	9.6 µg	24.1 µg	48%
iodine	38.4 µg	96 µg	64%

* NRV = nutrient reference values: is the reference quantity for an average adult

Other ingredients	Per 100 ml	Per drink
Choline	64.8 mg	162 mg
L-carnitine	32 mg	80 mg
Lactate	0.8 g	2 g
Osmolarity	327 mOsmol / liter	

Animal Data Using Glioblastoma Mice Model Supporting the Efficacy of HFLC Diet with Radiation Treatment.



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Treatment	Cohort Size	Median Survival (Days)
SD	19	23
KetoCal®	19	28
SD + Rad	11	41
KetoCal® + Rad	11	Undefined

SD: Standard Diet
KetoCal = HFLC diet

Abdelwahab MG, Fenton KE, Preul MC, Rho JM, Lynch A, et al. (2012) The Ketogenic Diet Is an Effective Adjuvant to Radiation Therapy for the Treatment of Malignant Glioma. PLOS ONE 7(5): e36197. https://doi.org/10.1371/journal.pone.0036197
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27. http://www.keto-drink.de/