THE KETOGENIC DIET AS AN ADJUVANT THERAPY FOR TREATMENT OF ADVANCED GLIOMAS AND GLIOBLASTOMAS

By Erin Einarson

Submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Dietetics

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August 2018

Abstract

Objective: The purpose of this study is to assess the effect of the ketogenic diet on treatment sensitivity and progression-free survival in the patients with glioblastoma. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing evidence on the ketogenic diet therapy as a medical nutrition therapy for the oncologic population. It is hypothesized that the ketogenic diet as an adjuvant therapy will improve sensitivity to traditional cancer treatment of chemotherapy in human subjects with advanced gliomas or glioblastomas and will increase progression-free survival.

Methods: The proposed study will be the first randomized controlled trial with human subjects to assess the ketogenic diet as a medical nutrition therapy along with traditional cancer treatment. Eligible participants with advanced gliomas or glioblastomas will be recruited by convenience sampling throughout the Essentia Health system. Patients will be randomized into either the experimental group with a ketogenic 60-gram carbohydrate restricted diet for the 8-week study period or the control group (standard diet). Participants will receive education regarding their respective diets. A registered dietitian will check in with participants bi-weekly to answer any questions and provide support and resource information. Participants in both diet groups will receive the chemotherapy Bevacizumab (Avastin) dosed per the oncologist recommendation. Statistical analysis will include comparisons between the control and experimental group using appropriate statistical methods for continuous and categorical variables. Survival analysis will be measured using the Kaplan-Meier test. Comparisons of mean lab values and weight changes between baseline and the end of the study within groups will be analyzed by a paired t-test. One-way repeated-measures analysis of variance (ANOVA) will be used to evaluate the between group changes in variables during the study.

Anticipated Results: It is expected that the ketogenic diet will be well tolerated by the experimental group and that the ketogenic diet will improve sensitivity to chemotherapy, resulting in a significant difference in progression-free survival compared to the control group. It is also expected that any resulting data from the proposed study will provide more information regarding the ketogenic diet as an adjuvant therapy for cancer and may spur on further research on this topic.

Conclusion: More research is needed in the form of randomized controlled human trials to identify whether the ketogenic diet should be recommended as a complimentary therapy in human subjects with cancer. The proposed study will help to contribute to the knowledge of medical nutrition therapy for the field of oncology.

Acknowledgments

The completion of this research proposal would not have been possible without the guidance and support from many people. I am extremely fortunate to have received their help and encouragement throughout the development of my thesis project.

First, I would like to thank Dr. Megan Baumler for providing me with advice and support over the last year. I am extremely thankful to her for taking the time to review each draft of this paper and providing such good feedback. I would also like to thank Dr. Tara LaRowe, for her feedback as well as all of my teachers at Mount Mary University who have taught me so much.

Lastly, I would like to thank my family. Thank you to my parents for their loving support throughout my academic career and my soon-to-be husband Logan for all your help and reassurance during this process. I would not have been able to do this without you.

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Chapter 1: Introduction

Cancer is a growing burden worldwide. Globally, cancer is responsible for an estimated 1 in 7 deaths, and it is expected that by 2030, cancer-related deaths will be 13 million, according to the American Cancer Society (ACS). The ACS also predicts that the yearly diagnosis of cancer will reach 21.6 million new cases. Roughly 41% of US men and 38% of US women will develop some form of cancer over their life time. Advanced gliomas and glioblastomas are particularly devastating brain cancers that are the focus of this proposed study. It is a difficult form of cancer to treat and typically requires multiple treatment therapies. Treatment of cancer is very expensive. In 2014, the estimated direct cost of cancer in the US was $87.8 billion, according to the Agency for Healthcare Research and Quality (American Cancer Society, 2017a).

The most common treatment options for cancer include surgery, immunotherapy, chemotherapy and radiation. Despite advances in cancer treatment, many patients still have poor prognoses. Many primary tumors can be controlled with conventional therapies; however, these treatments are often unsuccessful when treating advanced gliomas and glioblastoma as most of these patients fail traditional treatments within a year. Most of the current treatment options come with harsh side effects that can be detrimental to the health outcome of the patients. Thus, there is a substantial need for non-toxic therapy options to improve cancer treatment outcomes.

One such option is the ketogenic diet, which is extremely low in carbohydrate and very rich in fats. This diet typically consists of 90% calorie from fat and 2% form carbohydrate, compared to the 2015-2020 USDA guidelines of 20-35% calories from fat and 45-65% calorie from carbohydrate (USDA, 2015). The ketogenic diet causes the production of ketones as a byproduct of the metabolism of fatty acids. Ketones can be used as an alternate source of energy by healthy cells, but interestingly, not tumor cells. While research on the ketogenic diet as a therapy

for cancer is limited, three studies from Allen, et al., Poff et al., and Rieger, et al. have shown it to significantly reduce tumor size and progression in combination with a variety of therapies in animal models. Prospective observational studies on human subjects have been small, and without a control group, are not able to conclude causality. However, the available studies found that while the diet may be challenging to follow, it has no adverse effects and was relatively well tolerated, indicating that it is a low risk option. The current study proposes combining the ketogenic diet with conventional chemotherapy to assess progression-free survival in human subjects with advanced gliomas or glioblastoma.

Rationale

The rationale for conducting the proposed study is that this would be the first randomized controlled trial of the effect of the ketogenic diet on treatment sensitivity and progression-free survival in the human oncology population.

Potential significance

The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing evidence on the ketogenic diet therapy as an adjuvant therapy for cancer research. In addition, the ketogenic diet as an adjuvant therapy may benefit the oncology population by increasing sensitivity to chemotherapy, improving survival, and reduce the cost of treatment.

Hypotheses

The ketogenic diet as an adjuvant therapy will improve sensitivity to traditional cancer treatment of chemotherapy in human subjects with advanced gliomas or glioblastomas and will increase progression free survival.

Sub-Problems

1. The ketogenic diet will be well tolerated by the experimental group.

2. Participant surveys will show increased progression free survival resulting from diet intervention will not decrease quality of life.
3. Participants on the ketogenic diet will not have adverse changes in laboratory parameters.

Limitations

1. Because advance gliomas and glioblastomas have a much lower incidence compared to other cancers, there may be a limited number of people who meet the eligibility criteria.

Delimitations

1. This study will only include patients with advanced gliomas and glioblastoma.
2. The study will only examine the effect of the ketogenic diet in relation to sensitivity to chemotherapy using Bevacizumab, no other cancer treatments or chemotherapy regimens will be studied.

Assumptions

1. Patients in the study will be able to tolerate and comply to the diet assigned.
2. Participants in the ketogenic diet group will be able to achieve ketosis.

Definition of Terms

Acetoacetate: A ketone body

Acetone: A ketone body

Alkylating Agents: A group of synthetic compounds containing alkyl groups that combine readily with other molecules. They act by cross-linking the strands of DNA, preventing its replication and the transcription of RNA. They are primarily used in chemotherapy of cancer

Anaplerosis: The process of replenishment of depleted metabolic cycle or pathway intermediates; most commonly referring to the tricarboxylic acid cycle

Anthracyclines: A class of antineoplastic antibiotics

Astrocytes: Star-shaped cells that make up the supportive tissue of the brain

Astrocytoma: A tumor composed of astrocytes

Bevacizumab: Recombinant humanized monoclonal antineoplastic antibody

Cachexia: A state of severe weight loss and muscle wasting secondary to underlying disease

Chemotherapy: The treatment of disease using chemical agents or drugs that are selectively toxic and destructive to the causative agent of the disease or malignant cells and tissue

Glial Cells: Any of the cells making up the neuroglia, especially the astrocytes, oligodendroglia, and microglia

Glioblastoma: The most malignant type of astrocytoma; it usually occurs in the brain but may occur in the brain stem or spinal cord. Also called Glioblastoma Multiforme

Glioma: A tumor composed of neuroglia in any of its stages of development; sometimes extended to include all intrinsic neoplasms of the brain and spinal cord, such as astrocytomas, ependymomas, and so on. Also called neuroglioma

Hyperbaric Oxygen Therapy: Treatment in which oxygen is provided in a sealed chamber at an ambient pressure greater than one atmosphere

Hypofractionated Radiation: Larger fractions of a dose of radiation given less frequently than daily

Karnofsy Performance Score: A tool to estimate clinically a patient's physical state, performance, and prognosis. The scale is from 100%, perfectly well and active, to 0%, completely inactive, or dead. It has been used in studying cancer and chronic illness. Lower Karnofsky scores are generally associated with poorer treatment response and prognosis

Ketoacidosis: The accumulation of ketone bodies in the blood, which results in metabolic acidosis; it is often associated with uncontrolled diabetes mellitus

Ketogenic Diet: A diet that produces ketones or acetones, or mild acidosis, such as one that is insufficient in carbohydrate and protein

Ketone: Any of a class of organic compounds containing the carbonyl group whose carbon atom is joined to two other carbon atoms; metabolic products usually derived from excess acetyl CoA from fatty acids within the liver, and are oxidized by the extrahepatic tissues

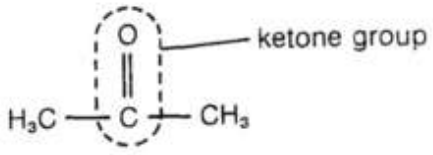


Fig. 199 **Ketone.**
The molecular structure of acetone.

(Collins Dictionary of Biology, 3rd ed. 2005)

Ketonuria: The presence of ketone bodies in the urine

Ketosis: Accumulation of excessive amounts of ketone bodies in body tissues and fluids, occurring when fatty acids are incompletely metabolized

Malignant: Dangerous to health; characterized by progressive and uncontrolled growth (especially of a tumor)

Metastasis: The spread of a disease process from one part of the body to another, as in the appearance of neoplasms in parts of the body remote from the site of the primary tumor; results from dissemination of tumor cells by the lymphatics or blood vessels or by direct extension through serous cavities

Neoplasm: A new, often uncontrolled growth of abnormal tissue; tumor

Oligodendrocytes: A type of neuroglial cell with dendritic projections that coil around axons of neural cells. The projections continue as myelin sheaths over the axons

Progression-Free Survival (PFS): In cancer care, the time during which a patient shows no signs or symptoms of the growth or the spreading of a tumor

Radiation Therapy: The treatment of disease, especially cancer, by means of alpha or beta particles emitted from an implanted or ingested radioisotope, or by means of a beam of high-energy radiation

Topoisomerase II inhibitors: A class of antineoplastic agents that interfere with the arrangement of DNA in cells

β-hydroxybutyrate: The major circulating ketone body

Chapter 2: Literature Review

Introduction

Cancer is a growing burden worldwide. Globally, cancer is responsible for an estimated one in seven deaths. An estimated 1,688,780 new cancer cases have been diagnosed in the US during 2017 alone and is responsible for an average of 1,650 deaths per day for an estimated total of 600,920 this year (American Cancer Society, 2017a). It is expected that by 2030, yearly cancer-related deaths will be 13 million, according to the American Cancer Society and the yearly diagnosis of cancer will reach 21.6 million new cases. Roughly 41 percent of US men and 38 percent of US women will develop some form of cancer over their life time. In 2014, the estimated direct cost of cancer in the US was $87.8 billion, according to the Agency for Healthcare Research and Quality (American Cancer Society, 2017a). Improvements in treatments and cancer screenings has resulted in earlier diagnosis and has increased five-year relative survival rates over the last three decades, now ranging from 61-68% (American Cancer Society, 2017a).

The most common treatment options for cancer include surgery, immunotherapy, chemotherapy, and radiation. These treatments can be used alone but are often used in combination. Chemotherapy is a common option with over 100 chemotherapy drugs that can be used for cancer treatment. These drugs target cells in different phases of the cell cycle but unfortunately, do not differentiate between cancer cells and healthy cells. Chemotherapy is harsh on the body, and some drugs can cause serious side effects such as nerve damage from mitotic inhibitors, heart damage from anthracyclines, leukemia from high doses of alkylating agents, or topoisomerase II inhibitors. Others may cause damage to the cells of the heart, kidneys, bladder, lungs, and nervous system. Typical side effects of chemotherapy include fatigue, hair loss,

infection, anemia, nausea and vomiting, loss of appetite, constipation or diarrhea, mouth/throat sores, and weight loss (American Cancer Society, 2017b).

Radiation therapy is one of the most common cancer treatment options with more than half of patients with cancer undergoing radiation therapy (American Cancer Society, 2017b). Unlike chemotherapy, radiation is localized to the part of the body being treated; however nearby healthy cells may be affected. Radiation can be delivered in three ways: External radiation made of high-energy particles or waves aimed at the affected area from outside the body, internal radiation – the placement of a radioactive source near the tumor area inside the body, or systemic radiation which is the use of radioactive drugs. There are differing side effects depending on the area that radiation is targeting but common side effects can include weakness, sore throat, dry mouth, nausea, vomiting and diarrhea. Radiation can be effective in reducing solid tumor sizes, but it does slightly raise the risk of developing a second cancer (American Cancer Society, 2017b).

Despite advances in cancer treatment, many patients still have poor prognoses. Many primary tumors can be controlled with conventional therapies however these treatments are often unsuccessful against metastatic disease and in some cases, may promote cancer progression and metastasis. Most of the current treatment options come with harsh side effects that can be detrimental to the health outcome of the patients. There is a substantial need for non-toxic therapy options to improve cancer treatment outcomes. One such option is the ketogenic diet, which is a diet low in carbohydrate and rich in fats. This diet results in the production of and subsequent increase in serum concentration of ketones, as a metabolic byproduct of oxidation of fatty acids, that can be used as an alternate source of energy by healthy cells but not tumor cells. The ketogenic diet may be a promising complimentary therapy that makes cancer more sensitive to treatment. Due to the increased requirement of glucose for energy production, tumors of the

brain and central nervous system may be particularly affected by the ketogenic diet. The purpose of this literature review is to critically analyze the evidence on the ketogenic diet as a potential non-toxic adjuvant therapy to cancer treatment particularly for patients with brain cancer.

Background

Glioblastoma

Glial cell tumors, or gliomas, make up more than two thirds of primary brain tumors. Gliomas may be made up of astrocytes, oligodendrocytes, or both making it a mixed glioma. Gliomas are not staged like other cancers because they rarely metastasize outside of the central nervous system. Instead, the World Health Organization has come up with a three-tiered grading system. Grade one and two are lumped together and are low-grade gliomas, grade three is anaplastic glioma, and grade four is the most devastating of all central nervous system malignancies: Glioblastoma also known as Glioblastoma Multiforme (Jones, et al., 2012).

Glioblastoma Multiforme is an extremely malignant brain tumor characterized by high growth rates and necrosis. Glioblastomas arise from astrocytes, the star-shaped cells that make up the supportive tissue of the brain. It represents about 15.4% of all primary brain tumors and about 60-75% of all astrocytomas, the highest number of cases of all malignant brain tumors, with an estimated 12,390 new cases in 2017 (ABTA, 2014). The incidence is estimated to be two to three new cases per 100,000 people (AANS, 2018). The etiology of this cancer is unknown however risk increases with age and more men are affected than women (ABTA, 2014). This category of brain tumors frequently presents with headache, weakness on one side of the body, visual changes, seizures, aphasia, or other cognitive or personality changes indicating the area of pathologic origin. MRI is the most common diagnostic tool. On most occasions, a focal heterogenous irregular-

margined cystic mass lesion with edema, often enough mass to produce herniation, can be seen on the MRI (Jones, et al., 2012).

Glioblastoma is the most aggressive and least likely form of brain cancer to respond to treatment. Even with early diagnosis of Glioblastoma the prognosis is quite poor. Most patients fail therapy within a year of diagnosis. The first step in treatment is usually to perform surgical resection, removing as much of the tumor as possible. Combining radiation therapy with adjuvant chemotherapy postoperatively is now considered the standard treatment regimen for glioblastoma (Jones et al., 2012). However, even with the combination of therapies the outcome remains poor. The average survival with treatment is 14.6 months and two-year survival is only 30% (ABTA, 2014).

Bevacizumab is a chemotherapy agent that has recently received FDA approval for treatment as it has proven safe and effective in patients with recurrent glioblastoma (Jones et al., 2012). Bevacizumab is a recombinant humanized monoclonal antibody that acts as an agonist of the vascular endothelial growth factor (VEGF), a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits receptor binding, preventing the formation of blood vessels that bring oxygen and nutrients to tumors, potentially slowing the tumor's growth (NCI, 2017).

Bevacizumab, or brand name Avastin, comes as a solution to be administered intravenously. It is usually given once every 14 days for treatment of glioblastoma, as well as other cancers such as colon, rectal or renal cell cancer. It takes 90 minutes for the first dose to be administered. If the first dose is tolerated consecutive doses typically take 30 to 60 minutes for administration. Common side effects are similar to other chemotherapy agents including loss of appetite, diarrhea, weight loss. More serious side effects include increased risk of gastrointestinal bleed, infertility, and delayed wound healing. Due to the risk of increased bleeding and delayed

wound healing, patients should wait 28 days after surgical wounds have healing before starting Bevacizumab (US National Library of Medicine, 2012). Reports show a six-month progression free survival of up to 46% with the use of this chemotherapy (Jones et al., 2012). Perhaps combining traditional treatment for Glioblastoma with the adjuvant therapy of the ketogenic diet would improve progression-free survival for this population.

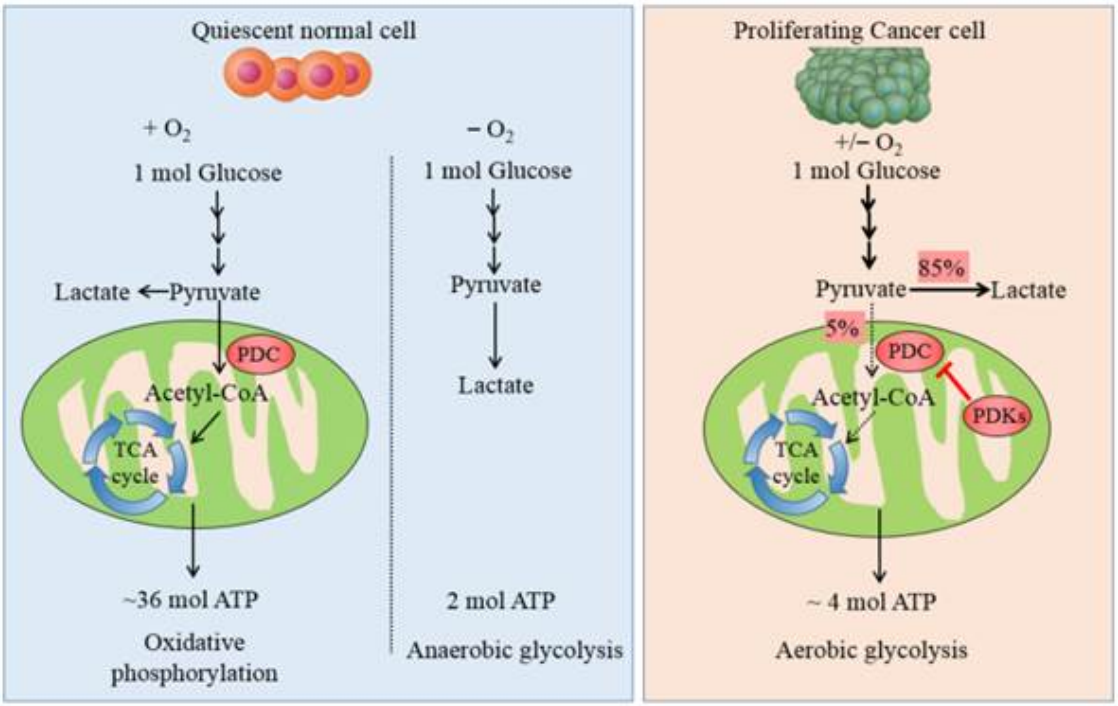
Tumor Metabolism

The mitochondrion is the powerhouse of the cell, most of the cell's energy production occurs here. In normal cells, glycolysis is the first step in the production of energy for the cell. In the process of glycolysis, glucose is broken down into pyruvate. In the presence of oxygen pyruvate is completely oxidized in the mitochondria and produces a total of 36 ATP through oxidative phosphorylation to be used for energy (Tennant, 2010). During this step oxygen is reduced and creates reactive oxygen species (ROS) commonly known as free radicals. These ROS can damage nucleic acids in DNA. Nuclear DNA is susceptible to ROS mediated damage, however, given the lack of protective histones, decreased ability for repair, and proximity to the production of ROS, the mitochondrial DNA is especially at risk for damage. Mitochondrial DNA mutations have been detected in most cancers (Fogg, et al., 2011). The mitochondrial DNA code for the process of oxidative phosphorylation and without a properly functioning electron transport chain the cell is not able to produce energy efficiently.

Cancer has developed an alternative way to produce energy despite a dysfunctional electron transport chain that inhibits normal energy production. In the absence of oxygen, pyruvate is normally reduced to lactate and produces a total of only two ATP, but cancer cells reduce pyruvate to lactate, even in the presence of oxygen (Tennant, 2010). This alternate method of energy metabolism, known as the Warburg effect, is likely due to the mitochondrial damage within

cancer cells. Because of the incomplete oxidation and failure to produce higher amounts of ATP through mitochondrial respiration, cancer cells rely more heavily on glucose for energy (Poff, 2013). Tumors consume considerable amounts of glucose and can take in more than some of the most metabolically-active organs such as the brain or heart (Tennant, 2010). Glucose uptake by tumor cells has been shown to exceed that of normal tissue 30-fold while lactate release is 43 times more than normal tissue in studies on colon cancer (Allen, et al., 2014).

Figure 1



(Figure from, Zhang, et al., 2015)

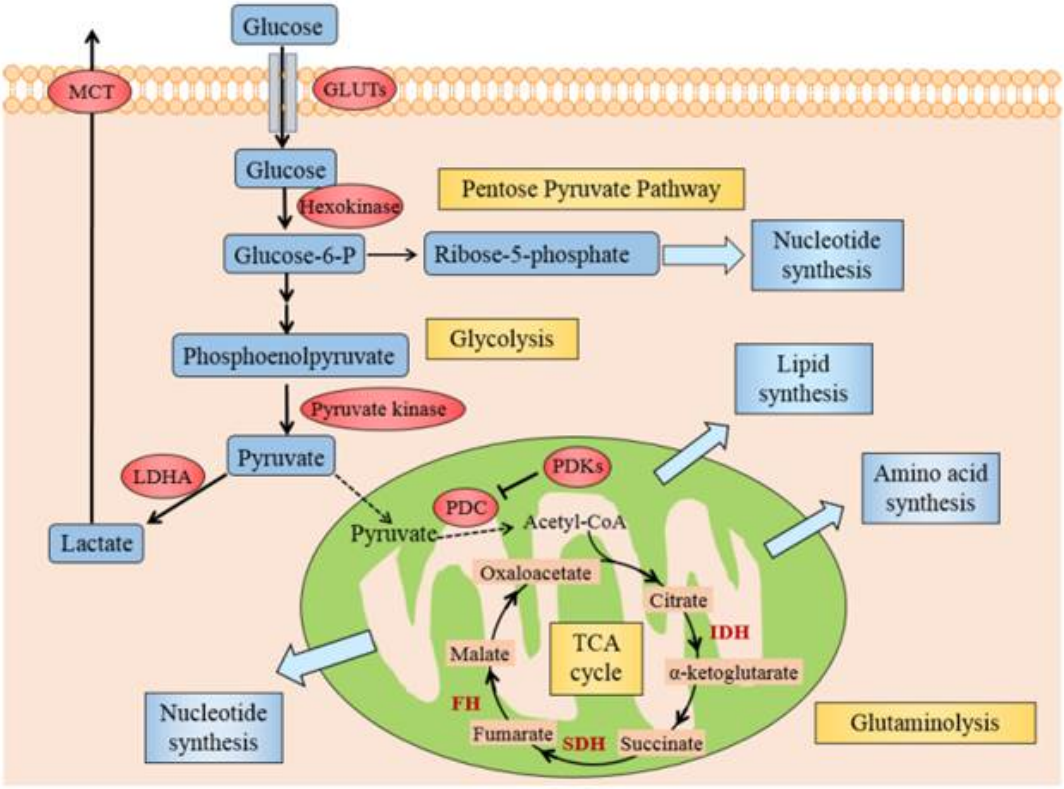
Figure 1 shows the metabolic pathway of glucose in normal cells and cancer cells. In normal cells, with sufficient oxygen, glucose is metabolized to pyruvate by which is transformed to acetyl-CoA for entering the mitochondria to be used in oxidative phosphorylation. In an anaerobic environment, pyruvate is reduced to lactate in in the cytoplasm. In cancer cells, pyruvate is deterred from entering the mitochondria and the majority is fermented into lactate in the cytoplasm even in the presence of oxygen.

The Warburg Effect

Increased dependence on glucose rather than other substrates would appear to make for an inefficient production of energy for cell growth. The cause and function of the Warburg Effect

remain debated. However, there is a growing consensus that it is not a metabolic mishap but a vital process for cancer cells to maintain rapid proliferation. If glucose were completely oxidized this would inhibit the use of the glucose's additional carbons for synthesis of other biological molecules such as lipids, amino acids and nucleotides required for rapidly multiplying cells (Fogg. et al., 2011). Eighty-five percent of pyruvate in malignant cells is fermented into lactate and only five percent of pyruvate is used for anaplerosis of the intact TCA cycle for conversion into intermediates that can be used for the synthesis of those biological molecules necessary for tumor growth (Zhang, et al., 2015).

Figure 2



(Figure from, Zhang, et al., 2015)

Figure 2 displays the aspects of Warburg effect in cancer cell, including glycolysis, lactate fermentation, and the use of TCA cycle intermediates to synthesize lipids, amino acid and nucleotides.

Additionally, the biosynthetic pathways of fatty acid synthesis and the pentose phosphate pathway parallel to glucose metabolism generates the byproduct nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) which is a regulator or cellular redox and can rid cells of ROS. Cancer cells exist in a condition of chronic metabolic oxidative stress with increased levels of ROS, primarily O_2 and H_2O_2, because of a dysfunctional mitochondrial electron transport chain. Increases in glucose metabolism may be compensating for the increased level of ROS by generating NADPH to be used as a cofactor in hydroperoxide metabolism (Allen et al., 2013).

It is also possible that the Warburg Effect develops as a preemptive strategy to maintain energy production in a hypoxic state that is common in solid tumors due to increased mass and limited vasculature. Increased lactate production is a consequence that may also favor tumor invasion as it creates an acidic environment that is toxic to normal cells (Fogg. et al., 2011). The inhibition of pathways linked to glycolysis, such as ATP citrate lyase in lipid synthesis, results in reduced tumor growth, supporting the hypothesis that outcomes of the Warburg effect contribute to tumor proliferation. (Fogg. et al., 2011).

Ketones

Ketones, a metabolic by-product of fatty acid metabolism, can serve as a potential energy source for cells with normal mitochondrial function in the absence of glucose, however would not be a source of energy for cancer cells. Fat metabolism produces ketones bodies, primarily β-hydroxybutyrate and acetoacetate, as well as acetone which is less abundant, through oxidation of fatty acids in the liver. Ketones are then converted to acetyl-CoA to be used as a substrate for energy production through mitochondrial respiration. Cancer cells with damaged mitochondria would not be able to adequately use ketones for energy, substrates would not be able to progress on to the oxidative phosphorylation step in energy production, due to the improperly functioning

electron transport chain. In addition, many cancers do not express the succinyl-CoA: 3-ketoacid Co-A transferase (SCOT) enzyme which is required for ketone body metabolism (Poff et al., 2013).

Ketogenic Diet

The ketogenic diet originated in 1921 by Dr. Wilder at the Mayo Clinic to mimic fasting as a treatment for epilepsy and has been a successful therapy in cases of refractory epilepsy (Allen, et al., 2014). The diet is designed to maximize production of ketones by providing fat as the main dietary substrate. As the fatty acid oxidation process becomes overloaded with fatty acids and limited by lack of oxaloacetate, ketones will be produced. The ketogenic diet consists of high fat, very low carbohydrate, and moderate to low protein with the general macronutrient distribution being around 90% fat, 2% carb, and 8% protein (Allen, et al., 2014). Ketosis, an accumulation of excessive amounts of ketone bodies in the blood or tissue, can be monitored by urinary and serum ketones. Urinary ketone tests range from negative, small: <20 mg/dL, moderate: 30 to 40 mg/dL, large: >80 mg/dL. Serum ketones are in normal range when less than 0.6 mmol/L and reaching ketosis when serum ketones are above 0.6 mmol/L. Ketone production is a normal metabolic process but can become dangerous and result in ketoacidosis at levels >3 mmol/L (Blahd, 2016). People with uncontrolled diabetes are at an increased risk of this potentially life-threatening condition. However, the level of serum ketones in most adults on the ketogenic diet is modest and not accompanied by elevated blood glucose or lack of insulin, so the risk of ketoacidosis is much lower (Allen, et al., 2014).

Because ketogenic diets force cells to rely on mitochondrial oxidative metabolism for energy production while limiting glucose availability, it would be expected that a state of ketosis

would exacerbate metabolic oxidative stress in cancer cells relative to normal cells (Allen et al., 2013). This may improve cancer cell sensitivity to chemo and radiation therapy.

Current Research

Ketogenic diet for treatment of Glioblastoma

Metabolic activity between healthy brain cells and glioblastoma cells may differ, resulting in a vulnerability in tumor growth that could potentially provide more specific targets for treatment. A study conducted by Marin-Valencia et al. published in the journal of *Cell Metabolism* aimed to determine the fate of glucose in glioblastoma cells in vivo and to determine metabolic differences between the tumor and surrounding brain. To study glucose metabolism in glioblastoma the researchers implanted human glioblastoma tumor cells directly into the brains of immunocompromised mice. After two to three months the mice displayed symptoms of expanding intracranial mass. Mice were infused with C-glucose for up to 300 minutes. At the end of the infusion the tumor was rapidly dissected. Spectroscopy of the tissue revealed C-signaling in several metabolites including extensive labeling in lactate, glutamate and glutamine. The tumor cells had much higher labeling in glutamine relative to glutamate compared to normal brain cells. In the normal brain, glutamine can be synthesized and rapidly exchanges with glutamate between neurons and astrocytes, but this was markedly disrupted in brains with glioblastoma (Marin-Valencia et al., 2012).

The presence of glutamine in the tumor cells is significant as glutamine can be used as a major anaplerotic nutrient for many cancer cells. Glutamine functions as a source of nitrogen and carbon which makes it a versatile nutrient for amino acid and lipid synthesis during cell proliferation (Rajagopalan, 2011). However, the accumulation of glutamine and decreased amount of glutaminase in tumor cells compared to surrounding brain cells indicated that it was not being

metabolized for anaplerosis. Cancer cells are also able to use a glucose-dependent anaplerotic pathway that requires pyruvate carboxylase which would not rely on glutamine for anaplerosis (Marin-Valencia et al., 2012). The researchers found several features of carbon labeling consistent with pyruvate carboxylase activity in the tumor cells, reinforcing the observation that glucose is the preferred nutrient for metabolism in tumor cells. The researchers concluded that glucose was allocated into both oxidative and non-oxidative pathways, and the use of glucose was not confined to aerobic glycolysis for energy production but also alternate pathways for production of macromolecules to promote cell proliferation (Marin-Valencia et al., 2012). The comparison of metabolism in the tumors and surround brain and the fate of glucose in glioblastoma tumor metabolism demonstrates that there are distinct differences in metabolic profiles and could be an area to target for treatment.

Researchers Stafford et al. investigated whether decreasing the availability of glucose as a preferred nutrient for tumor metabolism through the provision of a ketogenic diet would have an effect in mice gliomas. To observe the tumor response to the ketogenic diet the researchers implanted glioma cells into female mice. Following implantation, the mice were fed regular chow for three days until randomization into groups. Twenty mice remained on rodent chow while 20 mice switched to a ketogenic diet using the animal formula Bio-Serv (8.36% protein, 0.76% carbohydrate, and 78.8% fat). To ensure stable ketosis, serum β-hydroxybutyrate and blood glucose levels were measured weekly. Animals were also weighed daily. The mice were euthanized after observation of symptoms of decline such as reduced mobility or hunched posture. Kaplan-Meier analysis of survival data using a Gerhan-Breslow Wilcoxon test revealed a significant increase in survival among animals fed the ketogenic diet; mean survival of 19 days

post implantation for mice on the standard diet vs a mean survival of 25 days for mice on the ketogenic diet (P=0.0196) (Stafford, et al., 2010).

The increased survival time in animals on the ketogenic diet was further analyzed by the researchers. They wanted to see if the ketogenic diet influenced reactive oxygen species as well as genetic expression. To evaluate reactive oxygen species measurements were taken from cultured glioma cells in vitro and on tumor slices ex vivo from both groups. There was a significant reduction ($p<0.05$) in reactive oxygen species in tumors from the mice fed the ketogenic diet compared the mice fed the standard diet in both core and invading tumor cells. Interestingly while there was a reduction of reactive oxygen species over all in mice in the ketogenic group, the researchers found areas of higher levels in some tumor cells than surrounding brain. This may be due to the heterogeneity of brain tumors (Stafford, et al., 2010).

Because neuroprotective effects of the ketogenic diet are thought to be related to reduction in reactive oxygen species, which was evident during the study, the researchers analyzed gene expression for those involved in oxidative stress and antioxidant defense pathways. RNA from both normal brain and tumor cells of two animals in each group were analyzed. For comparison a two-fold difference in expression was determined to be the cut off. Genes that fit the inclusion criteria were analyzed and comparisons were made between and within diet groups, comparing normal cells to tumor cells. The researchers found genes that were expressed more in tumor brain cells compared to normal brain cells in standard diet mice, were expressed less in tumor brain cells compared to normal brain cells in ketogenic diet mice. A specific gene of interest is the ptgs2 (COX2) gene. The inhibition of COX2 is associated with increased apoptosis. Expression of COX2 was found to be reduced in the tumor cells of mice fed the ketogenic diet to levels comparable to normal cells (Stafford, et al., 2010). The researchers concluded that the ketogenic

diet may be beneficial in not only treatment of cancer, but it also may have a protective effect for normal cells due to the changes in gene expression and subsequent reduction of reactive oxygen species.

It is evident in mice studies that glucose is the preferred nutrient for brain tumors. When availability of glucose is reduced and replaced with ketones not only does it disrupt tumor proliferation, but may also offer benefits to normal brain cells indicating that cellular metabolism may be an area of weakness for tumor growth. The differences in metabolism between tumor cell and normal cells could pose as an area for treatment of brain cancer if the ketogenic diet is well tolerated and feasible for humans to comply with.

The *Pilot study of Ketogenic diet in Recurrent Glioblastoma* published in the *International Journal of Oncology* is a study that aimed to test the feasibility of the ketogenic diet in human subjects. In addition to assessing feasibility of maintaining the diet, the purpose of the study was also to determine the percentage of patients that were able to reach urinary ketosis, and to study the progression-free survival in patients with recurrent glioblastoma. The human trial was an open-label prospective pilot study that took place at the Frankfurt and Tubingen University Hospitals in Germany. Twenty participants were enrolled from December 2007 through March 2010. Inclusion criteria consisted of diagnosis of glioblastoma with relapse at $\geq$ 6 months after surgery or $\geq$ 3 months after completion of radiation therapy and had exhausted their options for chemotherapy. Exclusion criteria was patient requiring insulin for management of diabetes mellitus (Rieger, et al. 2014).

The participants were put on a ketogenic 60-gram carbohydrate restricted diet. The participants were to individually prepare their own meals and no calorie restriction was implemented. Prior to the start of the diet, participants received education on the principles of the

ketogenic diet as well as written information and recipes. Feasibility was assessed by patient reported tolerance to the diet. Three of the initial 20 patients dropped out the study as they felt the diet impacted their quality of life, and the remaining 17 participants reported they were able to adhere to the diet an average of 6.8 days per week. The attrition rate of this study is 15%.

Blood glucose was drawn to assess the safety of the diet. It was found that none of the patients had experienced hypoglycemia. However, it is important to note that the labs were drawn at baseline and a follow up visit after 36 days, leaving room for variability in the time between lab draws. Ketosis was measured by self-monitoring urine ketones 2 to 3 times per week using urine test sticks. The study found that ketosis was achieved at least one time by 12 of the 13 participants that had a consistent urine ketone analysis during the treatment. An average of 73% of participants were able to achieve stable ketosis (Rieger, et al. 2014).

The last variable assessed was the progression-free survival and overall survival. The median time to disease progression while on the diet was 5 weeks. All participants experienced disease progression, which ranged from 3-13 weeks. There was a trend of increased progression-free survival of participants that were able to reach stable ketosis compared to those that did not, with a median of 6 weeks compared to 3 weeks respectively, ($p=0.069$). The study protocol allowed for the addition of salvage treatment while continuing the diet once the disease progressed. Seven participants continued the diet while receiving the salvage treatment of Bevacizumab. The overall chemotherapy response rate was 85% including full or partial response. A similar cohort of 28 patients receiving Bevacizumab chemotherapy alone had a response rate of 65% (Rieger, et al. 2014). This led the researchers to test the efficacy of Bevacizumab in combination with the ketogenic diet via research on mice.

The animal trial briefly explored the effect of the ketogenic diet alone vs in combination with the chemotherapy treatment, bevacizumab (Rieger, et al. 2014). This randomized control trail consisted of 44 female mice that were inoculated with human glioma cells at the start of the study. On day 7 the animals were randomly assigned to a standard diet group or a ketogenic diet group. Bevacizumab chemotherapy or a control of phosphate-buffered saline was administered on day 12. On the 28th day, 12 mice (3 from each group) underwent an MRI to analyze tumor volumes. Mice that consumed the ketogenic diet in addition to chemotherapy had significantly smaller tumors than the mice that received chemo alone (13.8 mm^3 vs 23.9 mm^3, $p < 0.05$) (Rieger, et al. 2014). The results of the study again show the potential benefit of the ketogenic diet in combination with traditional cancer therapies and should be further researched in human subjects.

The researchers found the diet to have no adverse effects for human subjects and was relatively well tolerated. Participants did not complain of bowel distress. Although there was no calorie restriction, hunger was prominent within the first week of starting the diet but decreased as the trial went on. There was a statistically significant decrease in weight on average of 2.2% during the study; this may be attributed to the appetite suppressing effect of the diet but may also be a result of disease progression or a side effect of chemotherapy. It should also be noted that the use of steroids is typical in this population and may have produced a challenge in effectively lowering blood glucose to allow for ketosis. The human portion of the study did not have a control group to allow for a definitive conclusion on the efficacy of the ketogenic diet on progression free survival, so conclusions are limited, but it was interesting that the animal trail resulted in a significant reduction in tumor size when combining chemo therapy with the ketogenic diet. Further research is needed in the form of randomized control trials to clarify the potential benefit of the ketogenic diet on human subjects alone or in combination with conventional therapies.

Additional Research on the Ketogenic Diet as an Adjuvant Therapy

There are limited studies on brain cancers however there are several other studies on various cancer types that have investigated that potential benefit of a ketogenic diet as a possible cancer treatment. In the study published in the journal *Clinical Cancer Research*, researchers hypothesized that consuming a ketogenic diet would induce oxidative stress in cancer cells and improve the efficacy of radio-chemotherapies for lung cancer in mice. They believe the ketogenic diet may be of benefit because the combination of reduction in blood glucose and rise in ketones resulting from the high fat, low carbohydrate diet is thought to force cells to rely on mitochondrial respiration more than glycolysis for energy metabolism. This switch in metabolic focus may increase oxidative stress in cancer cells and result in enhanced responses to chemo and radiation therapies. They performed a randomized control trial on mice to test their hypothesis (Allen, et al., 2013).

Lung cancer cell xenografts H292 (non-small-cell lung carcinoma) were subcutaneously injected into the right flanks of four to six-week-old female mice. When tumor cells grew to 4 mm in diameter mice were treated with radiation alone, the ketogenic diet, a combination of radiation and chemotherapy, or a combination of radio-chemotherapy and ketogenic diet. Ketocal formula, which has a macronutrient composition of 90% fat, 1.6% carbohydrate and 8.4% protein, was used. Mice on the ketogenic diet had serum ketone levels >0.3 mEq/L and were considered to be in stable ketosis. The standard chow diet provided to the control groups consisted of 25% protein, 21% fat and 54% carbohydrate macronutrient distribution. All mice were allowed to eat as desired (Allen, et al., 2013).

A total of 91 mice were involved in assessing the effect of ketogenic diet in combination with chemo and radiation therapy. The mice were divided into the following treatment groups:

control group (N = 10), Ketogenic diet group (N=12), radiation group (N = 16), radiation + Ketogenic diet group (N = 16), chemotherapy group (N = 6), chemo + ketogenic diet (N = 5), radiation + chemo (N = 12) radiation + chemo + ketogenic diet (N= 14). The results of the study showed mice treated with radiation along with the ketogenic diet or radiation, ketogenic diet and chemotherapy had a significant decrease in tumor size compared to identical therapies with standard diet (P = 0.047 and P = 0.0046 respectively). Weight maintenance, activity and general condition showed that all treatments were well tolerated amongst these mice (Allen, et al., 2013).

The researchers also assessed the effect of the ketogenic diet on mice treated with a higher dose of radiation over a longer duration to replicate the duration and dose of common radiation protocols for humans with lung cancer. Twenty-eight mice were involved in this portion of the study and were divided into groups: control (N= 6), Ketogenic diet (N=7), radiation alone (N=8) and Ketogenic diet + radiation (N=7). The researchers found that mice receiving the more protracted radiation lost a significant amount of weight (15% of initial weight) by the end of the third to fourth week regardless of diet type. All mice receiving the ketogenic diet alone were able to complete the course of radiation therapy and had a slower tumor growth (p= 0.021) and longer survival time (p= 0.0041) compared to mice receiving radiation alone, based on Kaplan-Meier survival curve (mean survival not provided) (Allen, et al., 2013).

The researchers also assessed the effects of ketogenic diet in combination with hypofractionated radiation, maintaining the total treatment dose while shortening treatment duration by delivering higher doses of radiation with few fractions. The mice in this portion of the study received the ketogenic diet for a total of seven days starting two days prior to radiation and continuing for two days after. The mice were split into four groups: control, ketogenic diet, radiation, and ketogenic + radiation with each group containing nine mice. Again, mice receiving

both ketogenic diet and radiation had significantly lower tumor growth rates ($p=0.029$) and longer survival time ($P=0.0006$) in comparison with radiation alone. To assess oxidative stress, tumor samples were harvested at the end of the experiment. Dot blot analysis showed significantly increased 4HNE-modified protein, in mice treated with ketogenic diet ($p<0.05$) compared to radiation alone. 4HNE-modified protein is generated during conditions of oxidative stress and peroxidation of polyunsaturated fatty acids. This supports the hypothesis that ketogenic diet along with radiation therapy causes lipid peroxidation-derived aldehydes that result in oxidative damage to tumor tissue. The authors concluded that the diet was well tolerated by the mice and that the ketogenic diet could be easily implemented as an adjuvant therapy for improving response to radio-chemotherapy in the treatment of lung cancer (Allen, et al., 2013).

This study shows that the ketogenic diet can be an effective complimentary therapy capable of increasing response to chemo and radiation in mice with xenograft models of human lung cancer. The researchers were able to show a benefit of the ketogenic diet in two different radiation dosing regimens as well as the hypofractionated radiation therapy. This could potentially increase compliance to the ketogenic diet during radiation treatment in future human trials, as the regimen runs over a shorter amount of time. A weakness of the study is that xenograft models of human cancers in immune compromised mice are not an accurate representation of human cancer because the immune system is highly involved in disease progression, and most often fail to metastasize. More research is needed in the form of human trials to prove a potential benefit of the ketogenic diet as a complimentary therapy in human subjects.

Ketogenic Diet and Complimentary Therapy

Not only has the ketogenic diet been shown to increase the efficacy of traditional therapies for lung and glioma cancers, there is also potential for it to increase the effect of complimentary

therapies as well. The researchers Poff et al. hypothesized that the ketogenic diet along with hyperbaric oxygen therapy would work together to inhibit systemic metastatic tumor progression. Tumors contain abnormal vasculature that prevents appropriate tissue profusion creating a hypoxic region that diminishes the efficacy of radiation and chemotherapy. Additionally, tumor hypoxia activates oncogenic pathways such as Hypoxia Inducing Factor-1 (HIF-1) transcription factor that can cause tumor proliferation, metastasis, angiogenesis and hinders apoptosis. The researchers of this study suggested that a ketogenic diet and hyperbaric oxygen therapy could target overlapping pathways of metabolism and reactive oxygen species production and that these therapies in combination may improve progression free survival in patients with advanced metastatic cancer (Poff et al., 2013).

To test their hypothesis, a randomized controlled trial was initiated to apply these complementary therapies on mice. Forty adult male mice were used in this study which ran over three weeks. Researchers used VM-M3 cancer cells as they closely mimic the natural progression and invasion of metastasis. VM-M3 cells were implanted into the abdomen of the mice and quickly spread to major organ systems including liver, lung, kidney, spleen, brain and bone marrow over the first day. The mice were then randomly assigned to four groups: a control group, a hyperbaric oxygen therapy group, a ketogenic group, and a combination group. The ketogenic diet group received Keto-Solace formula while the standard diet groups received typical rodent food. Mice were allowed to eat as much as desired. Hyperbaric oxygen groups received 100% oxygen for 90 minutes three times weekly (Poff et al., 2013).

Blood glucose and β-hydroxybutyrate were measured weekly. Weight was taken at the same time twice weekly. Tumor growth was monitored by bioluminescent signaling and was measured over time through the body of the animal as an indicator of tumor size and metastasis.

Mice were euthanized after presentation of tumor-associated ascites, diminished response to stimuli, lethargy, or failure to thrive. Survival time was recorded (Poff et al., 2013).

The results of the study showed that the keto diet group and the keto-hyperbaric group had significantly increased survival times compared to standard diet groups ($p = 0.0194$ and $p = 0.0035$ respectively). There was no significant difference in survival time with hyperbaric oxygen therapy alone compared to the control group whose mean survival was 312 days. The Ketogenic diet group had an increased mean survival of 17 days (56.7%), while the combination therapy group had an increased mean survival of 24 days (77.9%). At the conclusion of the study, tumor growth rate was noticeably reduced among mice fed the ketogenic diet and was the most prominent in the combined therapies group with mean tumor size significantly smaller than the control group ($p = 0.0266$). Both reduction in blood glucose and percent body weight change were significantly associated with increased survival time ($p= 0.0189$ and $p = 0.0001$ respectively) (Poff et al., 2013).

The ketogenic diet has appetite suppressing effects that may lead to weight loss. Another contributing factor is the possibility that the mice found the diet to be less appealing and were therefore restricting intake. The ketogenic diet may reduce cell proliferation in part by caloric restriction. The researchers suggest that the targeting of tumor metabolism may make the cancer cells more sensitive to oxidative damage from the hyperbaric oxygen therapy. Additionally, ketone metabolism can protect cells from oxidative damage by enhancing antioxidant capabilities and decreasing production of free radicals. This study concluded that combining the ketogenic diet with the hyperbaric oxygen therapy may be an effective treatment for metastatic cancer however more research should be performed to test the potential of these alternative therapies in clinical use as an addition to the current standard of care.

Ketogenic Diet Implementation for Cancer Patients

The research on mice show there is potential for the ketogenic diet as an adjuvant therapy. Is there potential for this diet as a cancer therapy in human subjects? As discussed above, research conducted by Rieger et al. presented that the diet was relatively safe and tolerable for patients with glioblastoma, although all the participants experiences progression when consuming the ketogenic diet without traditional methods of treatment. More research conducted by Schmidt et al. presents a 12-week prospective observational study was published in the journal *Nutrition and Metabolism* that assessed not only the tolerability of the ketogenic diet in patients with advanced tumors, but also the effects it may have on quality of life. In addition, the study analyzed the effects of the diet on blood parameters as well as the course of disease. The researchers hypothesized that a high fat-low carbohydrate diet could be a feasible option for patients with advanced cancer that would not result in adverse side effects and may improve their quality of life.

Sixteen patients with advanced or metastatic cancers of different origins were included in the study that took place in Wuerzberg, Germany. Inclusion criteria included patients that were not receiving chemotherapy or radiation therapy, had measurable tumor-markers in serum or visible tumor in CT/PET/MRI, were in acceptable condition as measured by the Karnofsky Performance Score, had lab values in normal limits, and felt capable of following the dietary guidelines of the study. The mean age of the patients was 50.4 years. Of the 16 participants in the study, 12 were women. All 16 patients had received traditional therapies with no further conventional options available to them (Schmidt et al., 2011).

Prior to the start of the diet, participants were thoroughly instructed on the principles of the diet. They were provided a diet manual, list of acceptable food items, exchange list and recipes. Carbohydrates were to be restricted to 70 grams per day and 20 grams per meal. Patients were

encouraged to reduce carbohydrate intake even further if possible, however calories were not restricted. The diet plan contained two liquid meals to be consumed as a morning and afternoon snack, these were provided by the researchers and were made up of fermented yogurt, vegetable oil and 10 grams of protein preparation. Participants were to follow the diet for 12 weeks. (Schmidt et al., 2011).

The stage of cancer was determined by the oncologist prior to the start of the study in order to assess progression. Ketones were measured by assessment of ketonuria through patient self-collection in the morning. Stable ketonuria was determined to be at least 0.5 mmol/L of ketone bodies more than half the time. The researchers also assessed lab parameters by collecting labs at baseline and every two weeks. To assess the effect of the diet and disease on quality of life, patients took the EORTC QLQ-C30 questionnaire at the beginning of the study, every two weeks during the study and at the completion of the study. This validated questionnaire evaluates global health status, physical function, emotional health, cognitive function, social health, and symptoms such as loss of appetite, diarrhea, pain and fatigue (Schmidt et al., 2011).

Of the 16 patients that started the study only five were able to finish the 12-week trial, for an attrition rate of 69%. The participants dropped out for various reasons including inability to comply with diet (N=3), excessive weight loss (N=1), resuming treatment (N=1), disease progression (N=4) and death (N=2). Of the five who reached the end of the study only three were able to reach stable ketonuria; three others were also noted to have reached stable ketonuria but dropped out before completion of the study. Patients were asked to rate the feasibility of the diet. Acceptance was variable: one stated it was not feasible at all, seven rated it to be good, three rated it moderate, one poor, and two did not rate the feasibility of the diet (Schmidt et al., 2011).

The results of the quality of life questionnaire showed low rating at baseline due to advanced tumor stage. Global health scores remained stable through the duration of the study. It was not surprising that scores on physical function, fatigue or pain worsened over time as five of the 16 participant had progression in their disease and two died during the study. Despite disease progression, emotional health and insomnia ratings improved on overall scores (Schmidt et al., 2011). Participant also had improvements in blood parameters including a significant reduction in total cholesterol ($p < 0.001$), significant improvements in liver function tests ($p < 0.01$), and total leukocytes count increased significantly ($P < 0.001$). All of the patients in the study also had a reduction in blood glucose level, although statistical significance was not reported. All patients also lost a significant amount of weight during the trial (2 kg from 68.5 kg ±6.8 SD to 66.5 ± 6.8 SD; $p < 0.05$) (Schmidt et al., 2011).

The pilot study was to assess the safety, feasibility and impact of a low carbohydrate high fat diet on patients with advanced cancer. Unfortunately, because the low number of participant and the variety of cancer types in the study, statistical evaluation on the effect of tumor growth was not feasible. The ketogenic diet may not be an acceptable diet for all patients as evident by the dropout rate in this study however it may be an option for some. The results of the study found improvements in blood parameters and well as quality of life in the respects of emotional health and insomnia ratings. It cannot be ruled out that the placebo effect played a part in the study. Emotional well-being may have been improved by the opportunity for patients to control some aspect of their treatment or by the fact that the ketone body β-hydroxybutyrate can lead to mild euphoria (Schmidt et al., 2011).

This was a small study and only a few participants were able to complete the trial. There was no control group in this trial and the patients had varying types of cancer making cause and

effect relationships impossible to determine. Further research is needed to assess the influence of the diet on disease progression throughout the duration of the disease rather than just end stages. More research is also needed on the impact of the ketogenic diet on tumor metabolism in specific tumor entities.

Summary and Conclusion

It is quite evident that more research is necessary on the effect of the ketogenic diet as an adjuvant therapy for treatment of cancer. The study conducted by Marin-Valencia et al. showed that glucose is the preferred nutrient for tumor energy production and anaplerosis of substrates that led to synthesis of amino acids, lipids, and nucleic acid, even when glutamine is abundant. Energy production and synthesis of these molecules are necessary for tumor cell proliferation. This study indicated a weakness in tumor metabolism that could be targeted for treatment but reducing the availability of glucose.

Many of the studies discussed above included different facets of ketone metabolism as potential reasons for improving treatment. Of course, supplying an alternate source of energy for healthy cells while inhibiting energy production for tumor cells is a primary explanation. Additionally, Stafford et al. found that animals fed a ketogenic diet had reduced amounts of reactive oxygen species as well as change in gene expression. Noteworthy changes in gene expression included the ptgs2 (COX2) gene which had reduced expression in tumor cells of mice fed the ketogenic diet. The inhibition of COX2 is associated with increased apoptosis. Data from this study suggests that the ketogenic diet may be beneficial in not only treatment of the tumor cell, but the diet may simultaneously produce changes in gene expression and neuroprotective effects for healthy cells.

Even though the studies discussed in this literature review include different cancer entities, it is notable that the ketogenic diet has been shown to significantly reduce tumor size and progression in combination with a variety of therapies in animal models. It was shown to be an effective complimentary therapy capable of increasing response to chemotherapy and radiation in mice with xenograft models of human lung cancer in the randomized control trial (Allen, et al., 2013). The researchers were able to show a benefit of the ketogenic diet in two different radiation dosing regimens as well as the hypofractionated radiation therapy. Rieger et al. also found that mice that consumed the ketogenic diet in addition to chemotherapy Bevacizumab had significant reduction in glioblastoma tumor size compared to mice that received chemo alone. Additionally, the ketogenic diet in combination with hyperbaric oxygen therapy had significantly increased survival times compared to standard diet and the tumor growth rate was noticeably reduced among mice with metastatic cancer in the Poff et al. study.

The prospective observational studies on human subjects included a small number of participants. Without a control group these two studies were not able to conclude causality between the ketogenic diet and disease progression. The ketogenic diet may not be feasible for all patients as evident by the dropout rates and ability to reach stable ketosis observed in both studies. There may be concern regarding the use of a diet therapy for human cancer patients susceptible to cachexia. The ketogenic diet resulted in significant weight loss in all the above studies with both human and animal subjects. Some potential benefit that was observed included improvement in emotional wellbeing and insomnia. It cannot be ruled out that the placebo effect may have altered results. It is certain however, that allowing patients an active role in their treatment enables improvement in some aspects of quality of life.

Currently there is not sufficient evidence for dietitians to make recommendations in favor of the ketogenic diet for the oncology population. However, if an oncology patient were to attempt to follow the ketogenic diet, it would be the role of the dietitian to provide information and resources for the patient. The dietitian should assist in meal planning to assure appropriate calorie intake for weight maintenance, since cachexia is a concern for this population.

The poor prognosis of glioblastoma, diminished quality of life, and a mean survival of 14.6 months even with treatment makes continued research for more potential options for treatment of high importance. There has been evidence of the potential benefits of the ketogenic diet in cancer therapy. With more research and randomized clinical-control trails using human subjects, the ketogenic diet could be a non-toxic, relatively well-tolerated addition to traditional treatment for glioblastoma and hypothetically all cancer types.

Chapter 3: Methods

The purpose of this study is to evaluate the ketogenic diet as an adjuvant cancer therapy. This study will compare sensitivity of the chemotherapy Bevacizumab and progression free survival among participants with gliomas following a ketogenic diet to those following standard nutrition recommendations for cancer. This will be the first randomized controlled trail examining the effect of the ketogenic diet on treatment sensitivity and progression-free survival in the human oncology population.

Target Demographics

Inclusion Criteria

This study will include adults of 18 years of age or older that have been diagnosed with a malignant glioma, starting chemotherapy. Participants may be included if they have experienced relapse at ≥ 6 months after surgery or ≥ 3 months after completion of radiation therapy. Participants must have measurable tumor-markers in serum or visible tumor in CT/PET/MRI in order to evaluate disease progression. Participants must be in acceptable condition as measured by the Karnofsky Performance Score ≥60.

Exclusion Criteria

Participants will be excluded from this study if they have a history of diabetes or require the use of insulin to manage blood sugar. Pregnant women will be excluded. Patients with open/healing wounds or surgical incisions will also be excluded due to the risk of increased bleeding and delayed wound healing with the chemotherapy. Patients should wait 28 days after surgical wounds have healed before starting Bevacizumab.

Sampling

Subjects will be gathered by convenience sampling. Sampling will take place throughout the Essentia Health system specifically at Essentia Health Cancer Center (Duluth, MN), Essentia Health Cancer Center (Fargo, ND), Essentia Health St. Joseph's Cancer Center (Brainerd, MN). The proposed study will be posted to the Essentia Cancer Center websites in the respective locations. Oncologists in the participating locations will also be aware of the study and can inform eligible patients. Subjects will be included in the study if they meet the inclusion criteria.

A sample size of 46 participants total, 23 per group, would provide statistical significance with a power of 80% according to a clinical sample size calculator (Clincalc, n.d.) as well as take into account the average attrition rate of 42% observed in the human subject studies discussed in chapter two. However, given the low incidence of advanced gliomas and glioblastoma and the extended 3-year recruitment period which yielded 20 participants in the study by Reiger et. al., the recruitment goal for the proposed study will be 20 participants, 10 in each group.

Study Design

This will be a randomized controlled trial with a treatment period of eight weeks and a follow up every month for a total of 6 months. A meta-analysis including three trials in the setting of a newly diagnosed glioblastoma, found that progression status at 2, 4, and 6 months were significant predictors of overall survival. This study suggested that progression-free survival at 6 months is an appropriate primary endpoint for research evaluating treatment regimens for patients with newly diagnosed glioblastoma (Polley et. al, 2010). Patients in the proposed study will receive Bevacizumab chemotherapy prescribed per oncologist recommendations. Participants will be randomized into the experimental diet group or the control diet group.

Experimental Group

The participants in the experimental group will be put on a ketogenic 60-gram carbohydrate restricted diet for the 8-week study period, to coincide with the treatment regimen. In the inpatient setting participants will be provided ketogenic meals three times daily as well as ketogenic snacks as desired. In the outpatient setting patients will be responsible for preparing their own meals. Calories will be unrestricted, and participants will be able to eat as desired. Participants will receive education on the principles of the ketogenic diet from a registered dietitian as well as written information and recipes. Figure 3 shows an example meal plan, using recipes from the Charlie Foundation (The Charlie Foundation, 2018). A Registered Dietitian will check in with participants bi-weekly to answer any questions and provide information as a resource to promote adherence to the diet. Patients will keep a food log to monitor dietary compliance. Following the diet intervention period participants may return to their usual diet.

Figure 3: Example ketogenic meal plan

Meal	Fat (gm)	Protein (gm)	CHO (gm)	Calories
Breakfast: Flax Bread Scrambled eggs with mayo	 42 31	 14 6	 6 1	 458 307
Morning Snack: Berries and Cream	 6	 1	 1	 62
Lunch: Beef Stew with roasted butternut squash Keto flat bread	 21 39	 18 27	 9 4	 297 475
Afternoon Snack: Buffalo Chicken dip with celery	 31	 8	 2	 319
Dinner: Crab Cakes Cool and Spicy Jicama slaw	 43 7	 25 1	 4 3	 503 79
Totals	220 79% total kcal	100 16% total kcal	30 5% total kcal	2500 Kcal

Control Group

Participants in the control group will consume their usual diet. These participants will be provided a general diet with snacks as desired in the inpatient setting and will also be responsible for their own meals at home. Calories will also be unrestricted. Patients will meet with a dietitian and will be provided nutrition education based on traditional recommendations for cancer emphasizing protein and energy intake. The dietitian with work with the participants to individualize a nutrition plan to accommodate the patient's increased needs. This may include small, frequent meals and snacks or nutritional supplements and nutrient-dense nourishments to promote calorie and protein intake. Recommendations are based on the Nutrition Care Manual (Academy of Nutrition and Dietetics, 2018). These participants will also be asked to complete a daily foods log to ensure patients are meeting estimated needs. The dietitian will check in with participants bi-weekly for support for these patients as well.

Data Collection

The primary outcomes that will be measured are 6-month progression-free survival and response to chemotherapy. Secondary variables that will be measured include compliance to diet, stable ketosis, quality of life surveys, changes in blood parameters, and weight loss. Ketosis will be measured by urine ketones 2 to 3 times per week using urine test sticks. Stable ketosis is defined as ketones present in the urine for more than half of the measurements. Serum will be assessed biweekly as well to validate urinary measurements and serum ketosis will be determined with measures of at least 0.6 mmol/L of ketone bodies. Lab parameters and anthropometric data including weight and body mass index (BMI) will be assessed at baseline and every two weeks. Disease progression will be assessed by an oncologist by comparing

baseline tumor-markers in serum or visible tumor in CT/PET/MRI. The validated survey on quality of life, EORTC QLQ-C30, will be given at baseline, after the 8-week diet intervention, and at the completion of the 6-month study. This study will provide evidence on the effect of the diet and whether or not any increase in progression free survival also coincides with improvements in quality of life.

Statistical Analysis

Statistical analysis will include comparisons between the control and experimental group using appropriate statistical methods for continuous and categorical variables. Survival analysis will be measured using the Kaplan-Meier test. Comparisons of mean lab values, weight changes, and changes in the quality of life survey scores between baseline and the end of the study within groups will be analyzed by a paired t-test. One-way repeated-measures analysis of variance (ANOVA) will be used to evaluate the between group changes in variables during the study.

Chapter 4: Discussion

A ketogenic diet while undergoing chemotherapy for brain cancer may improve response to the therapy and result in increased length of survival. The proposed study is a randomized controlled trial to determine whether this is the case.

Anticipated Results

Progression- free Survival

It is anticipated that there will be a statistically significant improvement in 6-month progression-free survival for participants with cancer undergoing chemotherapy on the ketogenic diet compared to control group on the standard diet. Since this will be the first randomized control trial with human subjects who are not at the end stages of their disease, using a traditional cancer therapy and the ketogenic diet therapy, it is difficult to predict the amount of progression-free survival time the experimental group will exhibit. However, the study conducted by Rieger et al, showed a trend of increased progression-free survival, a median of 6 weeks for the participants that were able to reach stable ketosis compared to a median of 3 weeks for those that did not reach ketosis using diet therapy alone (2014). This study allowed for salvage treatment using Bevicizumab at disease progression. When compared to a similar cohort, the researchers found that the patients on the ketogenic diet with chemotherapy had an increased response to treatment. The response rate for those on the ketogenic diet was 85% with a median progression-free survival of 20.1 weeks, compared to a response rate of 65% and a median progression free survival of 16.1 weeks. A reasonable anticipation is that by supplying an alternate source of energy for healthy cells while inhibiting energy production for tumor cells, an improved response to therapy would occur.

Additional research found that mice consuming the ketogenic diet in combination with the chemotherapy Bevacizumab, had a significant decrease in tumor size compared to mice that received chemotherapy alone. In the proposed study, it is be predicted that the experimental group on a ketogenic diet will show a similar outcome, an improved response to the chemotherapy, compared to the control group on a standard diet.

Other studies with a variety of treatment regimens using animal subjects have had similar results. Through diet intervention alone, the Stafford study found an improvement in survival (25 vs 19 days respectively) as well as a significant reduction in reactive oxygen species in tumors from the mice fed the ketogenic diet compared to mice fed the standard diet (2010). Stafford concluded that reduction in ROS as well as changes in gene expression may yield neuroprotective effects that could improve survival. In another study, the ketogenic diet was shown to increase response to chemotherapy as well as three different radiation therapy regimens in mice with xenograft models of human cancer (Allen, et al., 2013). Additionally, the ketogenic diet in combination with hyperbaric oxygen therapy had significantly increased survival times and reduced tumor growth rates compared to standard diet among mice with metastatic cancer (Poff et al., 2013).

Diet Tolerance and Compliance

It is expected that this diet will be well-tolerated, and that intensive RD support and counselling will result in good dietary compliance. It is anticipated that participant surveys will not result in a decline in quality of life related to the implementation of the diet intervention. Previous research has found the diet to have no adverse effects for human subjects. Participants in the Rieger studies did not experience bowel distress. There were improvements in blood parameters including total cholesterol, liver function tests, total leukocytes count, blood glucose

level in participants following a ketogenic diet. These participants also reported improvements in emotional health and insomnia (Schmidt et al., 2011).

Weight loss is anticipated due to the nature of the disease state and the potential for cachexia as well as side effect of chemotherapy. Most of the available research on cancer and the ketogenic diet show significant decreases in weight. The proposed study will attempt to minimize weight loss through RD support and counselling as well as meal plans that meet calorie and protein needs to maintain weight.

Potential Problems

There are several problems that may occur during the proposed study. These will be discussed with potential solutions below.

Recruitment

Recruiting enough participants may be slow as the incidence of Glioblastoma is rare: two to three cases in 100,000 people. To achieve the desired number of participants, the recruitment period may need to remain open until the recruitment goal of 28 participants with would leave room for anticipated attrition. To make sure patients are aware of the study and to increase rate of recruitment, the proposed study will be posted to the Essentia Cancer Center websites in the respective locations. Oncologists in the participating locations will also be aware of the study and can inform eligible patients.

Tolerance and Feasibility

This may not be a feasible diet for all. The ketogenic diet is a drastic diet change from the standard American diet. Participants may find the intervention difficult to comply with, due to changes in eating habits, meal planning, and food preparation. To ameliorate diet compliance issues and feasibility concerns, participants will be provided resources including bi-weekly visits

with an RD, diet education, written information, and recipes. With intensive support, there is increased likelihood for consistent adherence to the diet for the 8-week period.

Attrition

There is potential for participants to drop out of the study, as there is with all studies. Ongoing acceptance of the diet, succumbing to the disease, and quality of life impacts may be a variety of potential reason that participants may not complete the study. A solution may be to remove them from the study, however a reduction in subjects would result in decreased statistical power. The available statistics from these participants may still be used in the study and included in an appropriate portion of data analysis, which would eliminate attrition bias, but the outcomes may show more significance if the sample size reflects the population. Because of potential for attrition, the recruitment goal has been bolstered to account for an average attrition rate of 42% based on the human studies discussed in chapter two.

Unexpected results

If the results indicate that a ketogenic diet does not increase survival as expected, it may be because participants were not compliant with the diet, or unable to reach ketosis. As previously discussed, dietitian support will be available to promote dietary compliance. The use of steroids, typical of treatment of brain cancer, may produce a challenge in effectively lowering blood glucose to allow for ketosis as well. Urine ketones will be measured by participants 2-3 times weekly, so participants will be able to gage any diet adjustments that may need to be made in accordance with ketone production. Additionally, serum ketones will be measured bi-weekly to validate the urinary ketone assessment. Dietitians will assess results of the serum ketone and discuss adjustments in diet as well.

With all these measures in place to counteract potential problems, if the data does not correlate with the anticipated outcomes, then perhaps the proposed study may lay ground work for future research studies or conclude that the diet should be ruled out as an adjuvant therapy in favor of other area of investigation.

Application and Clinical Relevance

This will be the first randomized controlled trail on the ketogenic diet as an adjuvant therapy for brain cancers. The results of the proposed study may help dietitians and oncologists determine if the ketogenic diet is a feasible adjuvant therapy. The results of participant compliance and tolerance should be evaluated to determine if the diet is appropriate for the oncology population. The results of the proposed study may also help determine if the ketogenic diet is effective in improving treatment outcomes in this specific population. It would help provide more evidence for practitioners as well as patients in regard to the ketogenic diet which has been gaining interest as a potentially effective intervention in the oncology population. However, additional research will be needed to replicate findings and strengthen conclusions before using the ketogenic diet as a standard diet therapy for cancer.

Future Studies

More research is needed in the form of randomized controlled trials with human participants to demonstrate the potential benefit of the ketogenic diet as a complimentary therapy for the treatment of cancer. The impact of the ketogenic diet on tumor metabolism in specific tumor entities could prove as an adjuvant therapy in clinical use as an addition to the current standard of care.

Future research studies could evaluate the ketogenic diet in combination with different regimens of radiation therapy or other chemotherapy drugs. It was shown to be an effective

complimentary therapy capable of increasing response to radiation in mice with xenograft models of human lung cancer in the randomized control trial conducted by Allen, et al. The researchers were able to show a benefit of the ketogenic diet in two different radiation dosing regimens as well as the hypofractionated radiation therapy (2013.) Additionally, the ketogenic diet in combination with hyperbaric oxygen therapy had significantly increased survival times compared to standard diet and the tumor growth rate was noticeably reduced among mice with metastatic cancer in the Poff et al. study (2013.)

Future research studies may focus on alternative primary tumors in human subjects such as lung cancer, as there were optimistic results in the study conducted by Allen et al, using mice with xenograft human lung cancer. Other primary cancers that may be further research could include gastrointestinal cancer or head and neck cancer for example. The effectiveness of the diet may vary depending on the point of the disease for each individual. Metastatic cancer may also be evaluated for response to the ketogenic diet in future studies with human subjects as there has been a positive response in the animal study on metastatic cancer in the study conducted by Poff, et al in 2013. There is minimal research available on the ketogenic diet and pediatric oncology patients, this may be an additional area of future research.

Ketogenic tube feeding may be a consideration, especially in the head and neck cancer population. Nutrition support utilizing a ketogenic formula would certainly control for variation in diet compliance among participants. Currently on the market there is one complete product available for ketogenic enteral feeding. KetoCal by Nutricia is available as a powder formula with a 4:1 or 3:1 macronutrient ratio (fat: protein + carbohydrate), or as a liquid formula with a 4:1 ratio. These formulas are designed for use in children over 1 year (Nutricia, 2018). At present there are no ketogenic enteral formulas available on the market designed for adults. Future

researchers may want to consider ketogenic supplements such as KetoCal which comes in vanilla flavor or a fermented yogurt, vegetable oil, and added protein drink as was used in the study conducted by Schmidt et al. Future research may also consider the use of MCT oil to help prevent cachexia earlier on in the course of the disease.

With more research and randomized clinical-control trials using human subjects, the ketogenic diet may be found to be an effective, non-toxic, relatively well-tolerated intervention to traditional treatment for glioblastoma as well as other cancer types, and if not, should be ruled out in favor of other avenues of pursuit.

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Appendix A

Mount Mary University

Institutional Review Board (IRB)

for the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN

UNTIL THE IRB HAS APPROVED THIS PROJECT

Directions:

- Faculty and student researchers, as well as student research advisors, should **read all relevant information on the University IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html
- All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org
- The IRB application must be filed and approved by the IRB **prior** to any Mount Mary University faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
- If there is a cooperating institution, attach a copy of their IRB approval.
- In the case of a student research project, the student may complete the IRB application but the student's research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g. informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as **exempt** from full IRB review, the research advisor may request the student to complete and submit a full IRB application.
- Complete this application using your word processing program (ex. Word), then print it out and obtain signatures from all investigators and advisors. (**Handwritten applications will not be accepted**.) For your benefit, save the completed application on your computer in case it needs to be revised and resubmitted.
- This is a professional document; please check spelling, grammar and punctuation.
- Submit a hard copy of the completed application with required signatures and attachments to Tammy Scheidegger, IRB Chair, Counseling Department. **(Emailed applications will not be accepted.)**
- Allow a **minimum of 10 working days** to process your application. Make sure this time frame is accounted for when considering initiation of data collection and due dates for student projects.
- For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
- Upon receipt of the IRB letter of approval, data collection may begin.

I. Required Documentation (No action will be taken without these attachments.)

Are the following attached to the IRB application?

Consent application	☒ Yes	Applications should include explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix.B.
Questionnaire/Survey Instrument(s)	☒ Yes	If survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question should be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	☒ Yes	Copy of transcript, certificate or other evidence .
Copy of cooperating institution's IRB approval.	☐ Yes	Not required if there is no cooperating institution. .

II. Investigator(s):

Name: Erin Einarson Phone: (218) 330-4947
Affiliation with Mount Mary University (e.g. faculty, student, etc): Student
Email: einarsoe@mtmary.edu

Signature: Erin Einarson Date: 6/29/18

If student, list Research Advisor and complete Section II. Research Advisor must provide requested information and verify.

Department: Graduate Program in Dietetics

Research Advisor's Name: Megan Baumler
Email: baumlerm@mtmary.edu Phone: 14149303116

Research Advisor: Have you completed Human Subject's Training? ☒Yes ☐ No

Research advisor's signature indicates responsibility for student compliance with all IRB requirements.

Signature: ___Megan Baumler_____ Date: 7/9/18
Research Advisor

III. Project Description

Instructions: Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):

The purpose of this study is to evaluate the ketogenic diet as an adjuvant cancer therapy. This study will compare sensitivity to the chemotherapy Bevacizumab and progression free survival among participants with gliomas following a ketogenic diet to those following standard nutrition recommendations for cancer.
2) Relevance to practice/body of knowledge:

This would be the first randomized controlled trial of the effect of the ketogenic diet on treatment sensitivity and progression-free survival in the human cancer patients. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing evidence on the ketogenic diet therapy as an adjuvant therapy for cancer research. In addition, the ketogenic diet as an adjuvant therapy may benefit the oncology population by increasing sensitivity to chemotherapy, improving survival, and reducing the cost of treatment.

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):

This is a randomized control trial with a target of 30 participants. Eligible subjects will be recruited by convenience sampling place throughout the Essentia Health system specifically at Essentia Health Cancer Center (Duluth, MN), Essentia Health Cancer Center (Fargo, ND), Essentia Health St. Joseph's Cancer Center (Brainerd, MN). Following consent, patients will be randomized into the experimental group or the control group.
The participants in the experimental group will be put on a ketogenic 60-gram carbohydrate restricted diet for the 8-week study period. Participants will receive education on the principles of the ketogenic diet from a registered dietitian as well as written information and recipes. A registered dietitian (RD) will check in with participants bi-weekly to answer any questions and provide information to promote adherence to the diet. Patients will keep a daily food log to monitor dietary compliance. Participants in the control group will consume their usual diet and will meet with an RD for nutrition education based on traditional recommendations for cancer, emphasizing energy and protein intake. The participants in the control group with also keep a food log to assess intake compared to estimated needs. The dietitian will check in with participants bi-weekly for support for patients in the control group as well.
Statistical analysis will include comparisons between the control and experimental group, at baseline and at the end of the study, using appropriate statistical methods for continuous and categorical variables. Survival analysis will be measured using the Kaplan-Meier test. Comparisons of mean lab values and weight changes between baseline and the end of the study within groups will be analyzed by a paired t-test. One-way repeated-measures analysis of variance (ANOVA) will be used to evaluate the between group changes in variables during the study.

4) What measurement/data collection tools are being used?

The primary outcomes that will be measured are progression-free survival and response to chemotherapy to be assessed by an oncologist by comparing baseline tumor-markers in serum or visible tumor in CT/PET/MRI.

Secondary outcomes that will be measured include compliance to diet, urinary ketones, changes in blood parameters, and weight loss. Ketosis will be measured by urine ketones 2 to 3 times per week using urine test sticks. Stable ketosis is defined as ketones present in the urine for more than half of the measurements. Serum will be assessed biweekly as well to validate urinary measurements and serum ketosis will be determined with measures of at least 0.6 mmol/L of ketone bodies. The researchers will assess lab parameters and anthropometric data including weight and body mass index (BMI) at baseline and every two weeks. Disease progression will be assessed by an oncologist by comparing baseline tumor-markers in serum or visible tumor in CT/PET/MRI

Is the proposed project "research" as defined by Institutional Review Board requirements?

Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.
A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

Does the research involve human subjects or official records about human subjects?

☒ Yes
☐ No

***If NO STOP here and SUBMIT application*.**

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

☒ Yes
☐ No

If the YES box is CHECKED, proceed to SECTION IV.

If the NO box is CHECKED, STOP here and SUBMIT application.

IV. Exemptions

Are you requesting exemption from IRB review in one of the federally approved categories?
If yes, please reference OHRP website http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html and continue with application.

1) Does the research meet the criteria for exempt category 1 (education)? [45 CFR 46.101 (b) (1)]

Is the research conducted in established or commonly accepted educational settings (e.g. schools, Universities or other sites where educational activities regularly occur)?	☐ Yes ☒ No
Does the research study involve only normal education practices (e.g. instructional strategies, techniques, curricula, or classroom management techniques)?	☐ Yes ☒ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

2) Does the research meet the criteria for exempt category 2 (specific procedures)? [45 CFR 46.101 (b) (2)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?	☒ Yes ☐ No
Is the information obtained recorded in such a manner that human subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)	☒ Yes ☐ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

3) Does the research meet the criteria for exempt category 3 (public officials)? [45 CFR 46.101 (b) (3)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?	☐ Yes ☒ No
Are the human subjects elected or appointed public officials or candidates for public office? **If no, proceed to Category 4.**	☐ Yes ☒ No
Does any federal statute require without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter? (See Appendix B)	☐ Yes ☐ No

*If **all** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

4) Does the research meet the criteria for exempt category 4 (existing data/specimens)? [45 CFR 46.101 (b) (4)]

Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens?	☐ Yes ☒ No
Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)	☒ Yes ☐ No

*If **both** questions are answered **yes,** stop here, proceed to **Section I Required Documentation**, and **submit** application.*

5) Does the research meet the criteria for exempt category 5 (federal program research)? [45 CFR 46.101 (b) (5)]

Does the research involve studying, evaluating or examining federal public benefit or service programs?	☐ Yes ☒ No
Is the research conducted through a federal agency?	☐ Yes ☒ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

6) Does the research meet the criteria for exempt category 6 (taste and food quality)? [45 CFR 46.101 (b) (6)]

Does the research involve a taste and food quality evaluation or consumer acceptance study?	☐ Yes ☒ No
Does the food consumed contain no additives, or a limited amount of food additives at or below a level approved by the FDA or EPA or the Food Safety and Inspection Service of the U.S. Department of Agriculture	☐ Yes ☒ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

If no exemptions apply, continue with application.

V. Additional Project Information

1) What human subjects training has the researcher completed (e.g. course work, online certification)?

Online certification, CITI Human Research Basic Course

2) What process is used for obtaining informed consent (attach the informed consent application)?

See Appendix B for consent application.

3) Does the research include special populations?

Minors under 18 years of age?	☐ Yes	☒ No
Persons legally incompetent?	☐ Yes	☒ No
Prisoners?	☐ Yes	☒ No
Pregnant women, if affected by research?	☐ Yes	☒ No
Persons institutionalized?	☐ Yes	☒ No
Persons mentally incapacitated?	☐ Yes	☒ No

4) If **YES,** describe additional precautions included in the research procedures.

5) Does the research involve any of the following procedures?

False or misleading information to subjects?	☐ Yes	☒ No
Withholds information such that their informed consent might be questioned?	☐ Yes	☒ No
Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects?	☐ Yes	☒ No

6) If **YES**, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

7) Does the research involve measurement in any of the following areas?

Sexual behaviors?	☐ Yes	☒ No
Drug use?	☒ Yes	☐ No
Illegal conduct?	☐ Yes	☒ No
Use of alcohol?	☐ Yes	☒ No

8) If **YES**, describe additional precautions included in the research procedures.
Chemotherapy agent Bevacizumab will be prescribed and managed by the oncologist

9) Are any portions of the research being conducted online?

Survey posted on a website?	☐ Yes	☒ No	If yes, assure anonymity
URL for survey includes information that could identify participants?	☐ Yes	☒ No	If yes, assure anonymity

Invitation to participate sent by email?	☐ Yes	☒ No	If yes, assure anonymity
Items use drop-down box?	☐ Yes	☒ No	If yes, assure that items allow choice of "no response"

10) If **YES**, describe additional procedures.

11) Describe the methods used to ensure confidentiality of data obtained.

Patient information will be kept confidential. Patients will be identified by a number code rather than medical record number or name on any documents or statistical analysis. access to electronic medical records will be secures and only researchers and health care providers involved in the study will be allowed access. Any paper documentation will be shredded in the medical information shred bins and Essentia and any digital records will be deleted after the completion of the study.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)

Risks associated with the diet intervention include weight loss. Weight loss will be mitigated with bi-weekly meetings with the registered dietitian who will work with the participant to individualize recommendations to meet estimated needs while maintaining diet compliance.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

Evidence suggests that a ketogenic diet as an adjuvant therapy may benefit the oncology population by increasing sensitivity to chemotherapy, improving survival, and reduce the cost of treatment. The results of this study thus will benefit the field of nutrition and dietetics in clinical practice by providing evidence on the ketogenic diet therapy as an adjuvant therapy for cancer research.

Appendix B

Consent Form for Participation in a Research Study

Essentia Health / Mount Mary University

A Randomized Control Trial Assessing the Ketogenic diet as an Adjuvant Therapy for Treatment of Glioblastoma

Description of the research and your participation

The purpose of this study is to evaluate the ketogenic diet as an adjuvant cancer therapy. This study will compare sensitivity to the chemotherapy Bevacizumab and progression free survival among participants with gliomas following a ketogenic diet to those following standard nutrition recommendations for cancer.

Your participation will require you to follow an 8-week diet intervention. Participants in the experimental group will be put on a ketogenic 60-gram carbohydrate restricted diet for the 8-week study period. In the inpatient setting participants will be provided ketogenic meals three times daily as well as ketogenic snacks as desired. In the outpatient setting patients will be responsible for preparing their own meals. Calories will be unrestricted. Participants will receive education on the principles of the ketogenic diet from a Registered Dietitian as well as written information and recipes. A registered dietitian will check in with participants bi-weekly to answer any questions and provide information as a resource to promote adherence to the diet. Patients will keep a food log to monitor dietary compliance. Participants in the control group will consume their usual diet. These participants will be provided a general diet with snacks as desired in the inpatient setting and will also be responsible for their own meals at home. Calories will also be unrestricted. Patients will meet with a dietitian and will be provided nutrition education based on traditional recommendations for cancer. The dietitian will check in with participants bi-weekly for support for these patients as well
Participants in both groups with receive Bevacizumab chemotherapy dosed per oncologist recommendations

You will also be evaluated by your oncologist at baseline and bi-weekly during the diet period then monthly for the next 4 months. Participants will be evaluated for progression-free survival and response to chemotherapy. Secondary outcomes that will be measured include compliance to diet, stable ketosis, surveys on quality of life, changes in blood parameters, and weight loss. This will require a blood draw and urinary measurement each time. Additionally, disease progression will be assessed by an oncologist by comparing baseline tumor-markers in serum or visible tumor in CT/PET/MRI

Risks and discomforts

Risks associated with the diet intervention include weight loss. Weight loss will be mitigated with bi-weekly meetings with the registered dietitian who will work with the participant to individualize recommendations to meet estimated needs while maintaining diet compliance.

Potential benefits

There are no known benefits at this point. Evidence suggests that a ketogenic diet as an adjuvant therapy may benefit the oncology population by increasing sensitivity to chemotherapy, improving survival, and reduce the cost of treatment. This research is the first randomized controlled trial using human subjects regarding the ketogenic diet as an adjuvant therapy for glioblastoma. This research will help further the knowledge and information available regarding the ketogenic diet's role in treatment of gliomas

Protection of confidentiality

All records of participation will be kept strictly confidential. Patients will be identified by a number code rather than medical record number or name on any documents or statistical analysis. access to electronic medical records will be secures and only researchers and health care providers involved in the study will be allowed access. Any paper documentation will be shredded in the medical information shred bins and Essentia and any digital records will be deleted after the completion of the study.

Voluntary participation

Your participation in this research study is voluntary. You may withdraw from the study at any time for any reason without penalty. Withdrawal from the study will not jeopardize or otherwise affect any treatment or services the participant is currently receiving or may receive in the future.

Contact information

If you have any questions about this study or would like more information, please contact Erin Einarson at 218-330-4947.
If you have any questions or concerns about your rights as a research participant, please contact the Mount Mary University Institutional Review Board at 414-258-4810.

Consent

I have read and understand this consent form and have been given the opportunity to ask questions. I give my consent to participate in the research study.

Participant's signature_____ Date:_____

Appendix C

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

Your birthdate (Day, Month, Year):

Today's date (Day, Month, Year):

	Not at All	**A Little**	**Quite a Bit**	**Very Much**
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	**A Little**	**Quite a Bit**	**Very Much**
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4

9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your <u>family</u> life?	1	2	3	4
27. Has your physical condition or medical treatment				

interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Appendix D

Items to be Considered for a Budget

Advertising Costs

- Brochures
- Fliers/Posters

Lab Equipment/ Tests

- CT/PET/MRI scans
- Scales
- Lipid panel
- Blood glucose tests
- Serum ketone lab

Pharmacy

- Bevacizumab
- Urine ketone test sticks

Nutrition Services

- Kitchen scales
- Special food items if not already available

Participant Resources

- Nutrition education handouts
- EORTC QLQ- C30 surveys

Staff

- Registered Dietitian
- Oncologist
- Research Assistants