Spring 2020

Investigations of a Low Carbohydrate Ketogenic Diet as a Possible Treatment for Malignant Brain Tumors

Elizabeth Anaya
Regis University

Investigations of a Low Carbohydrate Ketogenic Diet as a Possible Treatment for Malignant Brain Tumors

A thesis submitted to

Regis College

The Honors Program

in partial fulfillment of the requirements

for Graduation with Honors

by

Elizabeth Anaya

May 2020

Thesis written by

Elizabeth Anaya

Approved by

_____

Thesis Advisor

_____

Thesis Reader or Co-Advisor

Accepted by

_____

Director, University Honors Program

TABLE OF CONTENTS

LIST OF FIGURES

Acknowledgements

I would first like to thank my advisor Dr. Streifel, for her continual support throughout the course of my thesis journey. She has stayed with me through a number of roadblocks and the changing of my thesis topic. Not only has she offered her expert opinions whenever I had questions, but she challenged me to explore new ideas. I can say for certainty that I could not have completed my thesis without her.

Second, I would like to thank my reader Dr. Lucas, who has proved to be a valuable confidant both in my studies and in my personal life. She always gave positive feedback and advice that kept my spirits high. Her optimism was truly admirable. I enjoyed receiving Dr. Lucas' feedback, as well as doing research with her.

Fourth, I would like to thank my family and friends for being my biggest cheerleaders and rocks throughout this process. I would not have been able to succeed without them by my side. In particular my roommates, who would bring me food when I was too "in the zone" during my writing sessions and forgot to eat. Especially my parents who have taught me to work hard and to never give up.

Lastly, my acknowledgements would not be complete without thanking the Regis University Honors Department. Every professor and student in my cohort has had a hand in shaping the person I am today. I could not think of a better group of professors and students to spend these last four years with. I adore all of them and appreciate their support greatly.

Preface: My Food Journey

For as long as I can remember, I have been concerned with the type of food I put into my body. When I was little, I gravitated toward the foods that were high in carbohydrates. I loved pasta, bread, and pizza. If it wasn't for my mother, I do not think I would have ever touched anything leafy or green. Looking back, I know that my diet was not healthy at all. However, I was born into the fat-free era and my parents were feeding my siblings and I according to the food trends of that time. They were told by the United States Department of Agriculture (USDA) that a high carbohydrate diet was normal and even healthy. Sure, fruits and vegetables were important too. However, according to the first food pyramid released by the USDA, more carbohydrates should be consumed than fruits and vegetables combined. Animal products came after carbohydrates, fruits, and vegetables on the pyramid. Fats were at the very top of the pyramid and were only to be eaten sparingly. I remember being told that fats were bad for me and that they were what made people fat. I even remember seeing the words "low-fat" on almost every product in our household: the milk, the cheese, and more. Little did I know that 10 years later I would be flipping this pyramid upside down and drastically changing my paradigm of nutrition. This is my food journey.

Fast forward to third grade, my relationship with food got even more complicated. I was put on a variety of restricted diets to try and determine the source my frequent stomach pain. My doctor advised me to give up certain things like gluten and dairy to see if those would make me feel better. If my stomach pain went away, I was allowed to slowly

introduce foods back into my diet. This was the way they determine food intolerances, with a bunch of trial and error. However, the stomach pain never went away. In fact, the only thing those diets did for me was make me realize how much gluten and dairy were in all of my favorite foods. This was the moment in my life that made me resent the word "diet" and the doctors who put me on them. To me it felt like punishment and I did not see the point. Yet, I still listened to my doctors and pushed through the intolerance tests. I began to fear the words "gluten" and "dairy". Interestingly, during this time of my life, gluten-free and dairy-free products began popping up everywhere as those diets were actually popular.

In high school I was obsessed with eating healthy. I played sports year-round and I was always trying to find ways to maximize my performance in the classroom and on the field. I bought into the organic food trend and even frequently went on juice cleanses to try and detox my body. Juicing became extremely popular during my high school years, as everyone was trying to shed the few extra pounds they had gained from puberty. I convinced my parents that we needed to start juicing like everyone else. Sure enough, my parents bought our first juicer and we began on a rollercoaster ride of hunger and mood swings. Consuming just fruits and vegetables in liquid form was extremely unsatisfying. I finished high school with a distorted view of nutrition and what it meant to eat healthy.

I entered college not really knowing what types of foods to eat and how much to consume. There were so many conflicting food recommendations that it made my choices even more difficult. I had heard horror stories about the "freshman 15", in which many college students gained an average of 15 pounds during their first year. I was on my own

and did not have my parents to tell me what to eat. I began researching nutritional information and trying out a variety of different diets. In doing so, I came across a low carbohydrate ketogenic diet (KD). At first when I tried the diet, I failed. I didn't really know what I was doing or understand the biochemistry behind the diet.

It was not until the summer of 2018 that I was able to put my body into mild ketosis and commit to the diet. That summer I was told by my boxing coach that I had to lose 20 pounds in a short span of six weeks if I wanted to compete. Increasing my healthy fat intake actually helped me lose the weight I needed. I had completely turned the food pyramid upside down and I never felt better. I was in the best shape of my life and I got to eat great foods in the process. That summer I became a believer in the KD. However, even though I had done an immense amount of research before starting it, I knew there was a lot more to be done. I wanted to understand how eating fats could help me lose fat. In addition, I wanted to determine if this diet was sustainable for long-term use.

I chose to do my thesis on the KD in the hopes of answering some of the questions I still had about it. The more I researched, the more I discovered the benefits of a high fat low carbohydrate diet. For instance, I discovered that the KD has long been used to treat epilepsy. In addition, due to its neuroprotective properties, the KD is being investigated as a therapy for traumatic brain injuries, Parkinson's disease, and much more. The possibilities seemed endless. Perhaps the most fascinating thing I came across in my research was the anti-cancer properties that the KD possesses. The KD targets cancer metabolism using the Warburg effect, a topic which I will explain later. I found this topic so intriguing that I knew it was the perfect avenue for my honors thesis.

The second part of my thesis involves cancer. I have always been interested in cancer research. I'll admit that I have a weird fascination with cancer. This fascination stems from wanting to understand its biological complexities. However, it is mixed with a pure hatred for the disease. Cancer is a word that we are all too familiar with. Hearing that someone we know has been diagnosed with it is a common place; yet, it never gets easier to hear. This disease can be different in each individual and is constantly mutating, making it extremely hard to treat. It is a global disease that takes an unfathomable number of lives each year. Personally, I have lost many family members to this horrendous disease, my cousin Emily, both of my grandfathers, and my uncle Carrie. For this reason, I have chosen to dedicate my thesis work to those I have lost. They are my inspiration and the drive that has allowed me to finish this thesis. Thus, my thesis will take a critical look at the KD and the research surrounding its therapeutic uses. I will be specifically combining its neuroprotective and anti-cancer attributes and investigating its effect on malignant brain tumors.

Chapter 1: An Introduction to the Fat-Free Boom

The Evolution of the Food Guides

The U.S. Department of Agriculture (USDA) has been providing Americans with guidelines of what to eat since their first message came out 1916 (U.S. Department of Agriculture [USDA], 2011). Their first messages were centered around which types of foods you should feed young children and were displayed as farmers bulletins. One of the first bulletins categorized foods into five different categories: animal products (milk and meat), cereals (grains), fruits and vegetables, fats, and sugary foods. These bulletins later evolved to include the healthy limits for adults as well. The USDA introduced a new food recommendation, the first pictorial representation of a healthy diet, in the 1940's (USDA, 2011; Imberg, 2015). Their goal was to help citizens get the proper food intake during the Great Depression. There were no serving sizes or amounts of food to eat. However, the lines at the bottom of the bulletin read: "In addition to the basic 7… eat any other foods you want" (**Figure 1**) (USDA, 2011). Their message was clear. The USDA's goal was to combat malnutrition and starvation which were both high level concerns at the time.

The next three food guides that came out were the Food Wheel of 1984 (not pictured), the Food Pyramid of 1992 (**Figure 2**), and the MyPyramid of 2005 (**Figure 3**) (USDA, 2011). Many people liked the MyPyramid because they believed it was aesthetically pleasing, simplified, and fairly easy to follow (Imberg, 2015). In addition, the MyPyramid gave serving sizes of the six different food groups listed, which was a new thing at the time. It was after the 1970's when all of the proceeding food guides began to

carry the same overall message, each one becoming more apparent than the next. The USDA advocated for an increase in grain intake (carbohydrates) and decrease fat intake. The 1970's marked the beginning of a new era, the fat-free boom as many people call it.

Figure. 1 One of the first pictorial recommendations given by the USDA in the 1940's. Seven food groups were pictured including: green and yellow vegetables; oranges, tomatoes, grapefruit; Potatoes and other vegetables and fruits; milk and milk products, meat, poultry, fish, or eggs; bread, flour, and cereals, butter and fortified margarine. Adapted from the United States Department of Agriculture [USDA] 2011.

Unlike the Food Pyramid of 1992, the 2005 MyPyramid does not even give fats a label or category. Only if you look really closely, perhaps squint a little, can you make out the yellow sliver that represents the total fat recommended for a healthy diet. If you look even closer, you might even notice that this category only gets represented by one small

object: vegetable oil. There are no avocados, nuts, coconut, nor fish, all of which contain healthy forms of fat.

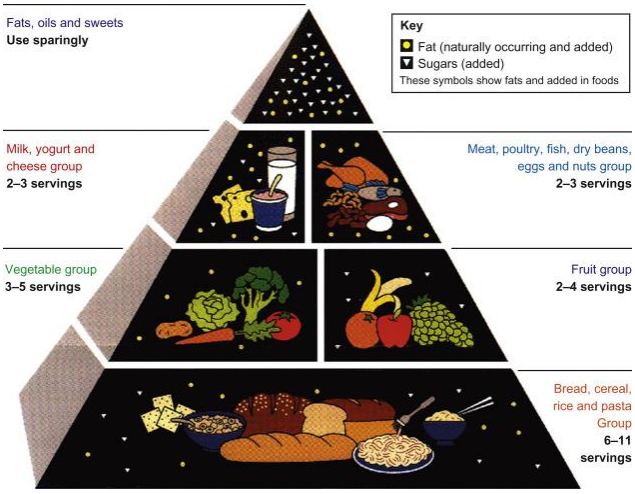

Figure. 2 The food pyramid that was released by the USDA in 1992. It lasted until 2005 when it was replaced by the MyPyramid. It contained six food groups with serving sizes for each group. The groups included: grains, vegetables, fruits, dairy, protein, and fats, oils, and sweets. Adapted from the United States Department of Agriculture [USDA] 2011.

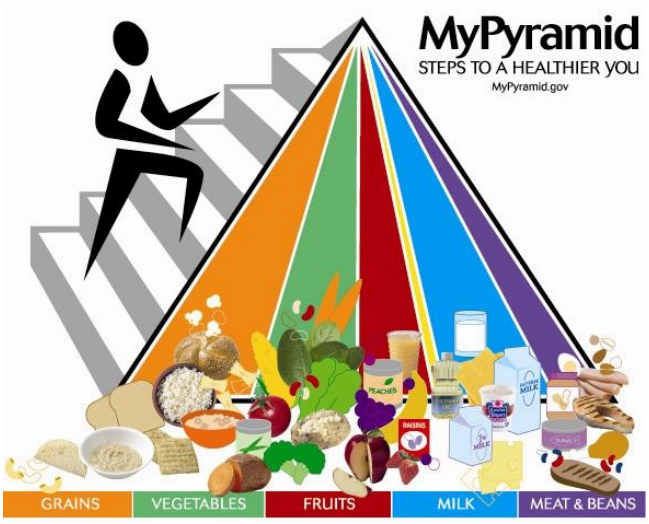

Figure 3. The MyPyramid that was introduced in 2005 after slight alterations from the previous pyramid that was released 13 years prior. The MyPyramid shows the same six food categories as the before, but only five received labels. Serving sizes were taken off and exercise was emphasized, taking a more personalized approach. It was replaced by the MyPlate in 2011. Adapted from the United States Department of Agriculture [USDA] 2011.

Fats were completely taken out of the most current food guide to date. The introduction of the MyPlate (**Figure 4**) was announced in 2011 by Michelle Obama, the First Lady at the time, and Tom Vilsack, the Agriculture Secretary (Imberg, 2015). The USDA claimed that their goal was to remind people to eat healthy and give the food guide a new and modern look. The MyPlate also took a digital platform with all of the serving size recommendations being online. When you go to ChooseMyPlate.gov, you click on each food group read more information about how much a person should eat of each category. Only on the online platform can you find dietary information about fat intake. Although fats are one of the four main macronutrients, they are not represented in the 2011 food guide.

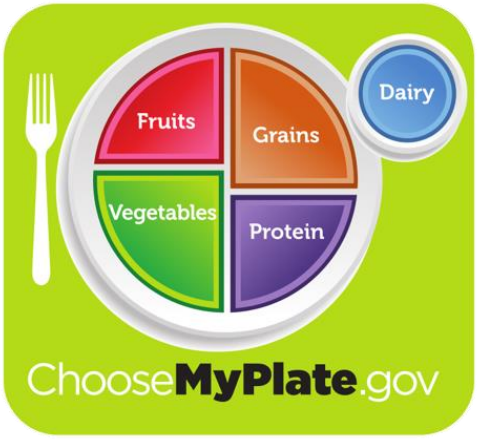

Figure 4. The most current nutritional recommendations from the USDA released in 2011. The MyPlate contains only five food groups including grains, vegetables, protein, fruits, and dairy. Adapted from the United States Department of Agriculture [USDA] 2011.

The USDA is still recommending that people switch to low-fat or fat-free milk options along with reducing the amount of saturated fat and added sugar they intake (USDA, 2011). They claim that through their recommendations a person can work towards

preventing obesity. The fat-free movement began in 1970; yet, it is still seen today. It is clearly visible through the MyPlate recommendations and in grocery stores across the United States. Fat-free and low-fat labels are common place. How did we get here? How did we come to believe the myths that eating fat will make you fat?

Ancel Keys' Seven Countries Study:

The work of Ancel Keys and his research team paved the way for the fat-free boom. In the 1950's, Keys investigated the link between saturated fats and coronary heart disease (CHD) by looking at the following seven countries: Finland, Greece, the United States, Italy, Yugoslavia, the Netherlands, and Japan (Keys, 1966, as cited in Pett et al., 2017; Taubes, 2001). Throughout this iconic Seven Countries study, Keys firmly believed that there was a correlation between saturated fat intake and the number of deaths from CHD. However, Keys and his research team explained that without further investigation the relationship between fat and CHD could not be considered causal (as cited in Pett et al., 2017). Despite Keys' uncertainty his ideas were still widely accepted. The public needed something to blame for the drastic rise in CHD related death and fats seemed like the perfect outlet.

However, not everyone was ready to accept Keys' research. In fact, his study was often criticized for only included data from 7 out of the 22 countries that he had originally surveyed (Pett et al., 2017). It was believed that he and his team excluded data from countries that ate high levels of fats and had low instances of CHD. Making it arguable that he only including countries that fit his hypothesis. Additionally, Keys was also criticized

for dismissing the possible correlation between sugar and CHD (Pett et al., 2017). Many believe that sugar was true culprit behind the rise in CHD. However, Keys and his team did recognize that people who were seen eating a high-fat diet were often also eating a high-sugar diet, making it hard to distinguish individual influence.

Following Ancel Keys:

The Seven Countries study and its findings were widely accepted by the public and government organizations despite the lack of scientific evidence. Keys even made the cover of *TIME* magazine in 1961 (**Figure 5**) (Taubes, 2001). In this issue, Michael Fumento writes about Keys' research findings (1961). In his article titled *Medicine: The Fat of the Land*, Fumento claims that Keys has "proven" the link between CHD and saturated fat. Even though there was no solid evidence to support this claim, Fumento played an important role in convincing the public that fat was the enemy. From this moment on, Keys and his wife were celebrated as the first Americans to follow the new "heart-healthy" diet laid out by Keys (Taubes, 2001). This new-found fame seemed to make Keys an even more credible source in the public eye.

The validity of the Seven Countries Study was heavily debated by many members in the scientific community, each side spending millions of dollars to try and prove the other wrong. Many organizations stood behind Keys including the U.S. Public Health Services that gave Keys and his team $100,000 a year for his research (Fumento, 1961). The American Heart Association along with the International Society of Cardiology as well as many others also gave Keys a substantial amount of money. Keys even received support

from the U.S. Surgeon General's Office who declared “fat the single most unwholesome component of the American Diet” in their 1988 report (as cited in Taubes, 2001). However, only a year later, the Surgeons General’s Office declared that their report was sent out without real evidence to support their claims (Taubes, 2001). Still without real evidence, Keys continued to gain support. As more organizations and governmental agencies joined the movement, fat intake by Americans was dropping rapidly. In a short 30-year span, there was a 16% drop and 15,000 fat-free or low-fat products on the shelves by 2011. Yet, as the average amount of fat consumed by Americans decreased, obesity increased from 14% to 22% (Taubes, 2011). The numbers just weren’t adding up and there was no scientific evidence to prove that an increased fat intake caused CHD. Unfortunately, the fat-free boom was already underway, and it did not seem to be stopping anytime soon.

Figure 5. The TIME magazine featuring Ancel Key on the cover page was released January 13, 1961. This issue contained an article written by Michael Fumento that featured Keys’ research. Adapted from TIME Magazine Article archives 1961.

Chapter 2: Normal Metabolism and Ketosis

Normal Metabolism:

Every living thing, from a single cell to a whole organism, requires energy in order to do work and perform everyday functions. If you take away an organism's energy supply, the organism cannot survive. This well-known and often overlooked fact will become extremely important later when exploring the keto diet as a possible cancer therapy. However, it is first important to understand how the human body gets its needed energy and how this energy is allocated. Metabolism can be defined as the formation and destruction of molecules through a series of specific and interconnected chemical reactions (Berg et al., 2015, p.424). These chemical reactions provide energy for mechanical work, ion transportation, and macromolecule synthesis within a living organism. Human metabolism is extremely complex and filled with many interconnected pathways, all vital for survival. Yet, there are three main macronutrients that will be the focus of this section: proteins, carbohydrates, and fats. In addition, not every metabolic pathway will be extensively explained. Only the vital information needed to understand metabolism manipulation will be discussed.

Under normal conditions, the human body prefers to run off of glucose as the main source of energy. In fact, certain cells can only run on glucose (Berg et al., 2015). The majority of glucose in the body is provided for by dietary carbohydrate intake. Carbohydrates are synonymously known as saccharides (sugars) and can be broken down into glucose or other simple sugar molecules (Berg et al., 2015; Byerley, 2019). Glucose

is the prominent carbohydrate in the body. It can be transported throughout the body via the bloodstream and turned into adenosine triphosphate (ATP) for cellular work. If glucose is not used by cell, it can be stored in the liver as glycogen, or used to synthesize fat.

The amount of glucose in the blood is efficiently controlled in healthy individuals (Byerley, 2019). When glucose levels are high in the blood, insulin is released by insulin-releasing cells in the pancreas. Insulin is a hormone that tells cells to take up glucose from the blood in order to use it for macromolecule synthesis. In addition, when glucose levels are low, glucagon-releasing cells in the pancreas release the hormone glucagon. This signals the cells to stop taking up glucose from the blood. Additionally, the liver responds to this signal by beginning to use up the glycogen storages (Berg et al., 2015). The body will also begin to synthesize glucose on its own from other non-carbohydrate sources. This process is called gluconeogenesis (**Figure 6**) (Berg et al., 2015; Byerley, 2019; Cahill, 2006). Gluconeogenesis prevents the body from running out of glucose. Gluconeogenesis involves the formation of oxaloacetate from non-carbohydrate sources. Oxaloacetate can then follow the anabolic pathway of becoming glucose. Glucose is indeed vital for the certain cells and parts of the brain that can only use glucose for its energy needs. However, with gluconeogenesis the body can make enough glucose to support these parts of the body, even in the absence of carbohydrate intake (Cahill, 2006). The body's ability to undergo gluconeogenesis is vital for the body during times of starvation or low-glucose intake.

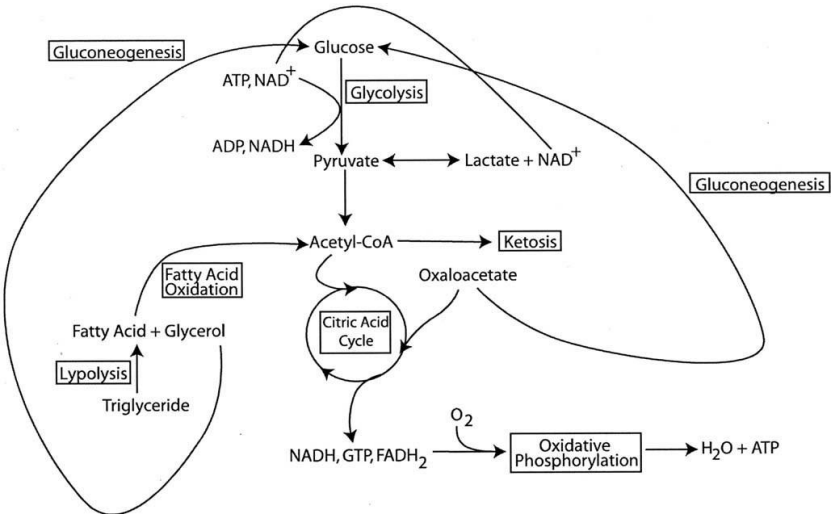

Figure 6. A pictorial representation of glucose metabolism and gluconeogenesis. Adapted from Pogozelski et al., 2005.

The second important dietary macromolecule is protein. When the human body ingests proteins, they can be broken down further into amino acids. Amino acids are not only the building blocks of all of the proteins in the body but can be further synthesized into other important biomolecules (Byerley, 2019). Many amino acids do not need to be dietarily ingested, as the body can synthesize 11 of them. However, there are 9 amino acids that cannot be synthesized (Berg et al, 2015; Byerley, 2019; Youdim, 2013). These amino acids are known as essential amino acids and are the reason why protein is an important part of our diet. There is a specific amount of protein that a person must intake in order to get all of their essential amino acids, often represented as EAA (essential amino acid requirements) (Youdim, 2013). Yet, a person can also ingest too much protein. This is because protein, itself, cannot be stored for future use. Instead, excess protein results in an amino acid build-up that must be broken down in the liver. When amino acids are broken down in the liver, a product called ammonia is formed. Ammonia contains nitrogen and is

toxic to the body. Ammonia must be converted into urea and excreted from the kidneys via the urine (Berg et al., 2015; Byerley, 2019; Den-Bor et al., 2011). Thus, too much protein can cause the buildup of ammonia, causing a variety of problems and even death. Protein intake is therefore limited by the catalytic efficiency of the enzyme responsible for the production of urea. Most importantly, the body can not run on protein alone.

Dietary lipids are also extremely important to the body. Fats, phospholipids and sterols are the three types of lipids that are essential for everyday function. Phospholipids make up cellular membranes and assist in the transport of hydrophobic molecules through aqueous solutions around the body (Byerley, 2019). Sterols also have a vital function in the body. The majority of animal sterols are in the form of cholesterol. Like phospholipids, cholesterol also helps make up the cellular membrane. In addition, cholesterol is the precursor of many hormones in the body; it is used to make many sex hormones, bile salts and even vitamin D. The last important lipid is fat. Fats are energy-packed macronutrients and are composed of a glycerol backbone and fatty acid tails. They are extremely important in the human body, despite some popular beliefs. Fat storage in the body is capable of sustaining the body under conditions of starvation (Cahill, 2006). Furthermore, fats protect our internal organs and help regulate our body temperature (Byerley, 2019). Fats even provide the body with extra padding in certain areas to prevent injury when you fall. They are also needed in order to metabolize fat-soluble vitamins including A, D, E, and K.

Similarly, to amino acids, there are essential fatty acids that the body needs to ingest. The two essential fatty acids are alpha-linolenic acid and linolenic acid which are omega-3 and omega-6 fatty acids respectively (Byerley, 2019; Youdim, 2013). These

essential fatty acids actually help regulate cholesterol in the body. In addition, they have been shown to decrease inflammation, heart disease, decrease autoimmune disease response, and promote brain health (Byerley, 2019). Fatty acid metabolism is important in normal metabolism and even more so when glucose levels are depleted. They can combine with glycerol to form triglycerides and be stored in adipose cells. When energy is needed by cells, triglycerides can be broken down into fatty acids, excreted, and transported to other tissues. Within the cell, fatty acids are metabolized in the mitochondria and broken down into acetyl CoA, which can be used to produce needed energy (ATP) for the cell via oxidation phosphorylation (**Figure 6**). Similar to glucose, fatty acids can also be used for the production of ATP and play an important role in the body.

Metabolism During Ketosis:

When the body does not have an adequate supply of glucose, due to carbohydrate restriction, fasting, and or intense exercise, the body relies on a wide range of pathways capable of sustaining the body. As previously mentioned, fatty acids are extremely important when glucose levels drop. So much so, that fatty acids are the primary source of energy during times of starvation or times of low carbohydrate intake (Youdim, 2013). When glucose levels are low enough, the liver can convert excess acetyl CoA from fatty acids into ketone bodies. Ketone bodies are water-soluble units of energy that are capable of being transported through the blood to energy seeking tissues (Berg et al., 2015). When there is an elevated number of ketones in the blood, a person is said to be in ketosis. The three ketone bodies include: acetone, acetoacetic acid, and β-hydroxybutyric acid. Ketone

bodies are important because fatty acids themselves cannot cross the blood brain barrier to be utilized by the brain. However, ketones are transportable units of energy that can pass the blood brain barrier and be used by the brain (VanItallie & Nufert, 2003).

When glucose levels are low, the body also undergoes gluconeogenesis. You may recall that this is the process of synthesizing glucose from non-carbohydrate sources. For example, the use of free amino acids can be used to synthesize glucose through a variety of pathways (Den-Bor et al., 2011). Some amino acids can be transformed into pyruvate, while others are synthesized into oxaloacetate directly. Alanine is an amino acid that can be converted to pyruvate, while aspartate can be converted more directly into oxaloacetate. Regardless of whether the amino acid gets turned into pyruvate or oxaloacetate, they can move through gluconeogenesis and become glucose. Glycerol, the byproduct of triglyceride metabolism, can also go through gluconeogenesis. However, although glucose can be converted into glycerol during glucose catabolism, this reaction cannot be reversed. Instead, glycerol must first become pyruvate. Other non-carbohydrate sources used in gluconeogenesis include recycled pyruvate, recycled lactate, and ketone bodies, which can be converted to oxaloacetate (Cahill, 2006). As you can see, there are a plethora of non-carbohydrate sources capable of undergoing metabolic transformations to glucose. During starvation, gluconeogenesis is important in the continual supplementation of glucose to red blood cells, and certain cells in the brain that cannot use ketone bodies.

Our bodies' ability to make ketone bodies has allowed humans to go long periods of time without food (White & Venkatesh, 2011). Without fats, the body would only be able to last at most 24 hours without food (Berg et al., 2015; Cahill, 2006). However, fats

in the form of ketones allow the body to last up to two months for people considered to be normal weight and up to one year for people considered to be obese (Freeman & Kossoff, 2010). Triglycerides are more compact and thus contain a higher amount of energy per unit space than their glycogen counterpart (Berg et al., 2015). In fact, ketones can be considered a more efficient form of energy, resulting in more ATP production per mole of substrate. Many people even attribute fats as having a vital role in the survival of the human race (Ben-dor et al., 2011). Researchers have come to this conclusion both through their research on starvation and their investigations of the diet of early hominins.

In order to understand the effects of starvation on the human body, a study was done in 1911, where Agostino Levanzin agreed to be put on an extensive 31 day fast for research purposes (Benedict et al., 1915). At the time of this ethically questionable study, little was known about human metabolism during starvation. Benedict et al., determined that during starvation, the body enters ketosis in order to provide energy to the brain and other cells. However, it wasn't until the 1950's and 60's that therapeutic starvation become popular and thus resulted in an increase in research on the subject and a better insight into starvation (Cahill, 2006).

In 1965 Cahill et al., put six healthy individuals and two diabetic individuals on eight day fasts to collect data on the fluctuations in metabolic substrates. They discovered a discrepancy in the amount of glucose still being metabolized by the brain even without carbohydrate intake. Since the patients were fasting, they were not getting any glucose intake and their glycogen storages were already depleted after the first 48 hours. They determined that 80 grams of glucose a day was being produced via gluconeogenesis. The

majority of these 80 grams was being synthesized using recycled pyruvate and lactic acid precursors from glucose-reliant cells. The rest of the glucose was synthesized from adipose secreted glycerol, amino acids, and even some from ketone bodies (as cited in Cahill, 2006). This goes to show that a high-fat, low-carbohydrate diet can be sustainable. A combination of ketones and glucose from gluconeogenesis provides enough energy for the whole body. In addition, the researchers further concluded that the ketone body β-hydroxybutyrate is the most efficient fuel source for the brain and the heart (as cited in Cahill, 2006).

Reliance on Fat:

Since the 1970's the stigma surrounding fats has become painstakingly clear. It was believed that consuming fat is what makes a person fat and that too much fat can cause heart attacks. However, Ben-Dor et al., explain how this is far from the truth. Many early hominins relied heavily on animal fats as their primary source of fuel (Ben-Dor et al., 2011; Freeman & Kossoff, 2010). In fact, *Homo erectus* was known to mainly hunt large animals with high fat content such as the elephant (Ben-Dor et al., 2011). Fat was vital for early hominin survival, and possibly what is missing from diets today. Research on human starvation has shown the efficacy associated with ketone body utilization (Cahill, 2006; Cahill et al., 1965). Further research even suggests that fat is the desired source of energy for the human body, especially by the brain and heart (Ben-Dor et al., 2011; Cunnane & Crawford, 2003; Cahill, 2006).

The human brain is extensively larger than that of our ancestors and other non-human primates, with the exception of the Neanderthals (Cunnane & Crawford, 2003). As our brain increased in mass, the brain became the single-handed biggest consumer of metabolic energy (Ben-Dor et al., 2011). In fact, an adult human brain uses 20-25% of all metabolic energy produced by the body, with newborns using 70-75% (Ben-Dor et al., 2011; Cunnane & Crawford, 2003). These numbers are extensive compared to that of other animals. To put it into context, a black bear's brain uses less than 5% of their total metabolism (Ben-Dor et al., 2011). A newborn's brain requires more energy than an adult because their brain is actively developing. In addition, in order to supply the needed energy to the brain, babies are born with mild ketonemia and around 500 g of fat available for use (Cahill, 2006; Cunnane & Crawford, 2003). Premature babies are underweight and do not have this fat accumulation. This is believed to be the reason why premature babies are at risk of neurological developmental delays (Cunnane & Crawford, 2003). They have a lack of high energy molecules (fats), which are also needed for lipid synthesis in the brain that provides structure.

In order for our brains to increase in size, certain factors had to come into play. Most importantly, the brain needed an increased in blood supply for oxygen and nutrient consumption (Cunnane & Crawford, 2003). Before the evolution of a bigger brain, just as much energy was needed by the gut (Ben-Dor et al., 2011). These two big organs actively competed for energy. As the brain began to increase, the body could not sustain two energy hogging organs. In order to accommodate a larger brain, the gut needed to shrink. The biggest reduction in gut size was attributed to the evolution of a shorter large intestine

(colon) (Ben-Dor et al., 2011). A humans' large intestine now only makes up 20% of the overall gut, whereas chimpanzees' makes up 52%. A smaller gut required a higher input from energy rich foods, making the consumption of fats vital to survival. Fats contain more energy per unit of weight than carbohydrates and proteins combined. Therefore, a person would need to eat less fat in order to get their daily energy needs. Both protein and carbohydrates provide 4 kcal/g worth of energy, while fats provide 9 kcal/g (Youdim, 2013; Byerley, 2019). The higher calorie content of fats makes them ideal for fueling a human's energy hungry brain.

Fats are considered a more efficient fuel source than carbohydrates and proteins. Additionally, both proteins and plants have a "physiological ceiling" that puts limits on their consumption due to digestion capabilities (Ben-Dor et al., 2011). It is well known that a person cannot survive on protein alone. A person can even get protein poisoning from overconsumption. Protein poisoning can also be referred to as mal de caribou, rabbit starvation, or fat starvation. It is the result of not enough energy production, often from an unbalanced dietary ratio of fat and protein. Energy is required in both the conversion of protein into glucose and in converting the toxic byproduct of protein metabolism into urea. Both of these energy requirements result in a negative energy gain. To produce half a gram of glucose from protein alone, one full gram of protein would be needed (Cahill, 2006). Even though the individual is eating, they would likely still feel hungry. If more protein is ingested, there will not be enough energy to get rid of the toxic ammonia (Ben-dor et al., 2011). A considerable strain is put on the liver and the person is at risk of severe health problems and even death.

Likewise, there is also a physiological ceiling for the amount of raw plants person can intake. Before the utilization of fire for cooking, early hominids often wasted an immense amount of energy trying to digest raw plants. Many raw plants contain toxins and other components that take energy to digest (Ben-dor et al., 2011). Thus, *H. erectus* and other hominids were not able to consume a high amount of plant derived products. They were not able to get enough needed energy from plant intake alone. To this day, many African tribes still prefer to hunt and consume large animals, even during times of increased vegetable availability (Ben-dor et al., 2011). However, the physiological ceiling of plant intake has increased over the years. Food preparations, not limited to cooking, have allowed for the breakdown of harmful toxins and further the digestive process. In addition, some present-day human populations, have adapted genetic mutations that allow for an increased copy number of the salivary amylase gene. Humans now have anywhere from 2-16 copies of this gene, allowing for better digestion of plant products. Some humans have thus evolved to be able to consume larger amounts of plants without hitting the nutritional ceiling. However, Ben-dor et al. explains that although humans are capable of eating more plants than earlier hominids, plants alone in many cases are incapable of sustaining the entirety of the human body.

The Keto Diet (KD):

The keto diet (KD) is heavily debated amongst nutritionists, other health professionals, and even biochemists in regard to its efficacy. They often disagree about the safety and sustainable of the diet. In addition, whether it can be used to lose weight or even

therapeutically to treat a wide range of diseases and ailments. On the one hand, we have those who believe that the KD is the upcoming cure for many diseases. Whereas, on the other hand, we have those who believe that the KD is not safe nor sustainable and actually causes a plethora of health issues including coronary heart disease. In reality, the KD is often misunderstood and is neither a magic cure, nor the cause of health issues. Instead, the KD is a powerful tool that has the potential to improve health conditions. For instance, the KD has been shown to decrease, if not eliminate seizures in patients with epilepsy (Cross & Neal, 2008; Maalouf et al., 2007; Thiele, 2003). More recently, the KD has been shown to improve the conditions of people with cancer (Ji et al., 2020; Nebeling et al., 1995; Seyfried et al., 2009, 2010; Zhou et al., 2007), traumatic brain injuries (White & Venkatesh, 2011), and many other neurological and metabolic diseases (Maalouf et al., 2007).

The KD is centered around changing the body's metabolism and where it derives its energy from. As previously mentioned, the body prefers to run on glucose unless glucose levels are low. During a state of ketosis, the body will use fatty acids in the form of ketone bodies as its primary energy source. More specifically, ketosis is characterized by "having blood ketone levels >.5 millimolar/L" (Charlie Foundation, 2019). The KD is a way of increasing ketone body production by the liver through the restricting of dietary carbohydrates. It is important to note that a ketogenic diet does not mean complete elimination of carbohydrates. It is highly recommended and encouraged to consume adequate amounts of fruits and vegetables, both of which contain carbohydrates (Charlie Foundation, 2019). Often, variations of the KD differ in the amount of recommended carbohydrates (**Figures 7a-f**). The Charlie Foundation shows and explains six different

variations of the KD including: the classic ketogenic diet, the modified ketogenic diet, the MCT Oil diet, the modified Atkins, the low glycemic index diet (LGIT), and intermittent fasting. Although all of these diets have the ability to increase ketone production and utilization by the body, one may be preferred over the others by an individual. For example, the classic KD is usually a 4:1 or 3:1 ratio of fat to protein and carbohydrate intake (**Figure 7a**), while, the modified KD has ratios of 2:1 or 1:1 (**Figure 7b**). The Charlie Foundation offers visual representations of these diets in the form of a plate.

The classic keto diet was designed in the Mayo Clinic for the treatment of epilepsy in 1923. Since then, different variations emerged as adjustments were made for the individual. Each variation is different in its macronutrient ratio (fat divided by the addition of protein and carbohydrates). In addition to these variations, the Charlie Foundation explains that nutritional supplements, electrolytes, hydration, activity level, and possibly fasting are also important aspects of the KD therapy.

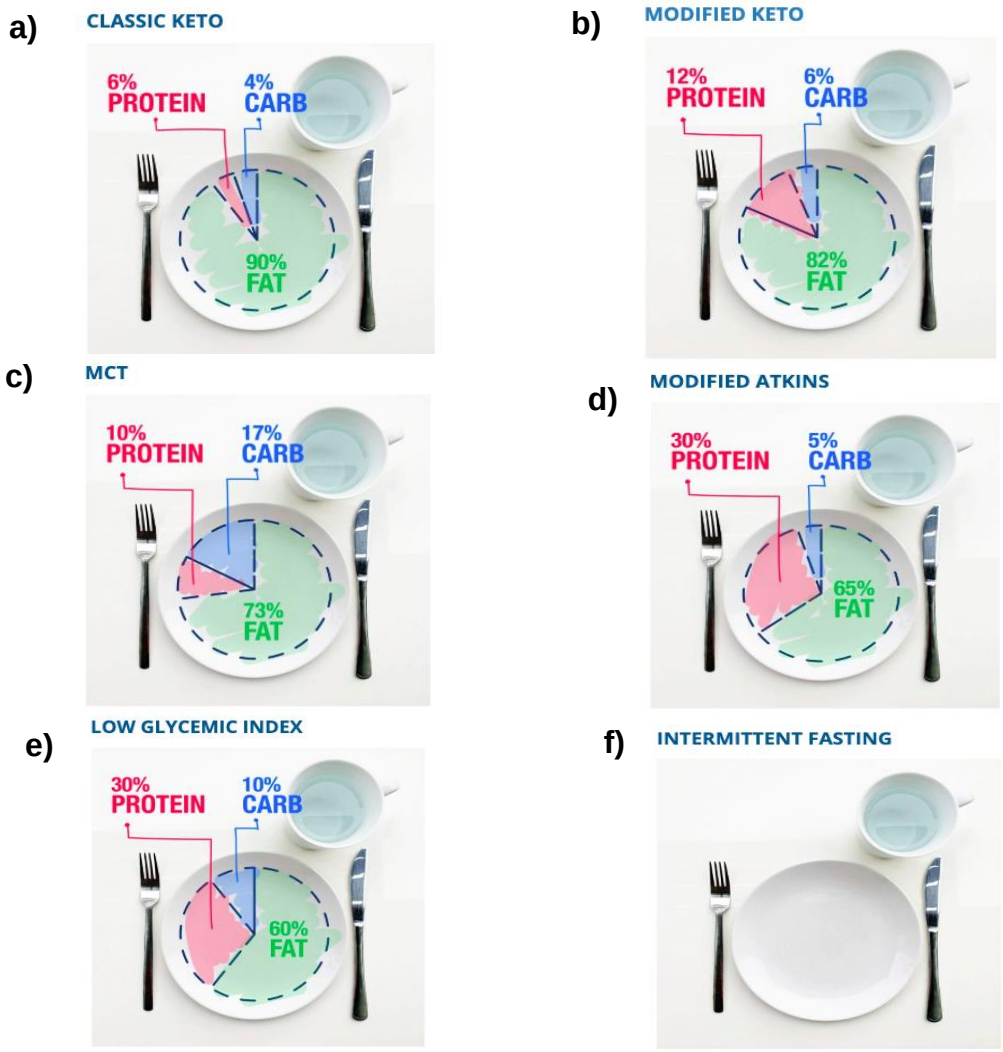

Fig. 7 The six variations of the KD with different macronutrient requirements. **7a** is the classic keto diet that was created to treat epilepsy. 90% of caloric intake should be from fat, 6% from protein and 4% from carbs. **7b** is a modified keto option, created as an easier and more sustainable option with 82% of calories being from fat, 12% from protein and 6% from carbs. **7c** is the MCT variant that relies on fat supplementation of medium chain triglycerides. In the MCT variant 73% of calories are from fat, 10% from protein, and 17% from carbs. MCT supplementation allows for higher carb intake. **7d** is the modified Atkins diet where 65%of calorie intake comes from fats, 30% from protein, and 5% from carbs. Lower carbs allow for more protein consumption. **7e** is the low glycemic index variant where 60% of calories come from fat, 30% from protein, and 10% from carbs. In addition, only carbs with low glycemic index are consumed. **7f** is intermittent fasting that puts the body in ketosis during the fasting state but has no macronutrient restrictions during the fed state. Adapted, with permission from the Charlie Foundation (2019).

When ketones were first discovered in the 19th century, they were found in the urine of diabetic patients, specifically those in a diabetic coma (Vanltallie & Nufert, 2003). This condition soon came to be known as diabetic ketoacidosis, leading to the popular belief that ketones were bad for the body and a result of the incomplete catabolism of fat. Even though this belief is outdated, there is still a fear of higher than normal levels of ketones in the blood. Some people still hold the notion that long periods of ketosis will lead to ketoacidosis and possibly death. However, in a healthy, non-diabetic individual, ketone levels cannot reach the levels needed to induce ketoacidosis (Vanltallie & Nufert, 2003; Cahill, 2006). The human body is efficient at utilizing ketone bodies and has mechanisms in place to prevent ketoacidosis, as previously discussed. In fact, even after a week or more of fasting, ketone body levels of β-hydroxybutyrate are around 4.4-5.5 mM/L; whereas, β-hydroxybutyrate levels seen in diabetic ketoacidosis are usually 23.0 mM/L or higher (Vanltallie & Nufert, 2003). If you recall, ketosis happens with ketone body levels of .5mM/L (Charlie foundation, 2019). Therefore, a person in mild to moderate ketosis should not worry about ketoacidosis. Ketosis can be a safe state of metabolism in non-diabetic patients with proper insulin signaling.

Ketones in the Brain:

Fats are extremely important to the brain. They are vital for many brain functions including nerve impulse transmission, memory storage, and tissue structure (Byerley, 2019). Pete Aherns, head of the Diet-Heart Review Panel along with many other qualified experts, were one of the first groups to publicly voice their concern about limiting fats

during the fat-free boom (Taubes, 2001). They were worried that decreasing fat intake would hurt the body significantly, especially the brain. The brain itself is 70% fat. In addition, ketone bodies are capable of supplying 75% of the brains needed energy and with an increased efficacy in comparison to glucose (Berg et al., 2015, White & Venkatesh, 2011). Two out of the three ketone bodies can be used as fuel sources for the body: acetoacetate and β-hydroxybutyrate (Berg et al., 2015). Interestingly, the heart and renal cortex actually prefer ketone bodies over glucose as an energy source. During starvation, acetoacetate is the preferred energy source for the brain. More recently, ketone bodies have been associated with having neuroprotective effects that can be used in treating epilepsy, Alzheimer's and Parkinson's disease. The KD may also help protect the brain after traumatic brain injuries and strokes; as well, used in managing the symptoms of autism (Freeman & Kossoff, 2010). However, the mechanism in which the KD works to protect the brain has been poorly understood in the past (Cross & Neal, 2008; Freeman & Kossoff, 2010). Many scientists have aimed at attaining a better understanding of these mechanisms, in order to perfect such treatments.

Glutamate is an extremely important neurotransmitter (Lewerenz & Maher, 2015). It has excitatory properties, meaning it can propagate neuron firing. However, excess glutamate is also associated with many neurological diseases including, but not limited to, stroke, epilepsy, trauma and Alzheimer's disease. Overstimulation by glutamate results in excitotoxicity and neural death through the formation of reactive oxygen species (ROS). Researchers examined the antioxidant activity of β-hydroxybutyrate and acetoacetate, specifically in reducing glutamate excitotoxicity

(Maalouf et al., 2007). They hypothesized that ketone bodies protect the brain in many neurological diseases by reducing the amount of free radical formation and thus combating oxidative stress. They concluded that ketones increased the $NAD^{+/}$NADH ratio which leads to increased NADH oxidation. Because of this, ketones are thus capable of reducing glutamine-induced ROS and increasing mitochondrial respiration in neurons. This research provides one of the first mechanistic explanations for the neuroprotective effect seen by the KD.

The Keto Diet and Epilepsy:

The Keto diet has long been used for the treatment of epilepsy. Raising a person's blood ketone level is one of the oldest treatments used to control seizures and is even well documented in the Middle Ages (Thiele, 2003). However, up until the 1920's, the diet consisted of inducing starvation through fasting. The fasts would last for as long as the person could stand it (Thiele, 2003; VanItallie, 2003). After this point, an interest in the mechanisms of starvation led to the creation of the KD. The Mayo Clinic discovered that the therapeutic advantages of fasting could be mimicked through the manipulation of macronutrients. Russell Wilder, out of the Mayo Clinic was the first to coin the name "keto diet" in 1923 (Charlie Foundation, 2019; Thiele, 2003; VanItallie, 2003). The KD was extensively used up until the late 1930's when a new epileptic drug came out called diphenylhydantoin (Thiele, 2003; Freeman & Kossoff, 2010). A drug is often easier to prescribe and monitor than a diet and was thus preferred over the KD. However, the KD reemerged as new evidence showed its ability to treat drug-resistant forms of epilepsy.

The reemergence of the KD in treating epilepsy occurred during the early 1990's and can be pinpointed to a well-known case, which later sparked the creation of the Charlie Foundation for Ketogenic Therapies (Charlie Foundation, 2011). Charlie Abrahams, an 11-year-old boy developed a hard to treat form of epilepsy in 1993. Many medications and procedures had no effect on his condition. Charlie was having multiple seizures a day, and nothing seemed to be helping. As a last resort, Charlie was put on the KD for five years. As a result, he never had another seizure. Charlie's story not only reintroduced the KD into the field of epilepsy treatment, but it caused a massive increase in research on the KD. Since then, references associated with using the KD to treat epilepsy saw an almost 200 percent increase (Charlie Foundation, 2011; Freeman & Kossoff, 2010). The KD is also now used in countries where epilepsy medications are too expensive and/or not available. However, in places where medications are available, the KD is often seen as a last resort to many neurologists. Yet, when surveyed, about 50% of families claimed that, if they were given the option, they would have tried the KD first (Freeman & Kossoff, 2010). Yet, medications are often easier to prescribe, and strict diets are hard for kids to follow and parents to implement.

The KD has proven to be one of the best therapies for hard-to-treat epilepsy with regards to efficacy (Freeman & Kossoff, 2010; Maalouf et al., 2007; Thiele, 2003). A study was conducted in 2001 on a group of 150 epileptic children with hard to treat epilepsy (Freeman & Kossoff, 2010). The children were put on the KD for one year and data was collected from the 83 individuals that successfully stayed on the diet for the full 12 months. In addition, researchers did a 3-6 year follow up. They concluded that at 12 months, all 83

were more than 50% seizure-free, with nearly half of them being 90-100% seizure-free. At the follow up, they determined that many children remained seizure free even after stopping the diet. Others were even able to get off their seizure medications. Similar results can be found in several recent clinical trials testing the efficiency of the KD as an anticonvulsant treatment using cohorts (as cited in Cross & Neal, 2008). These 21 clinical trials conduct was reviewed in Cross & Neal (2008). They determined that every single one of the 21 clinical trials reported the KD as being an effective and safe treatment for epilepsy.

Until recently, there were no class one studies that tested the efficacy of the KD in epilepsy treatment (Cross, 2008; Freeman & Kossoff, 2010; Thiele, 2003). Neurological studies are categorized with different ratings (1-4) based on the type of study conducted. Class one is the highest category containing the strongest evidence. For example, in order to be class one, a study must be randomized, controlled, and follow a variety of other rules. Whereas, a class four could be an expert opinion or case report. The first class one study investigating the efficacy of the KD as a treatment for epilepsy finally emerged in 2008 (Neil et al., 2008). This study was conducted on children with drug-resistant epilepsy. Researchers found that 38% of the children studied had a greater than 50% decrease in seizures compared to 6% in the controlled group. In addition, 7% of children had a greater than 90% seizure decrease compared to 0% with the control. It was concluded that 45% of children participants, nearly half saw a greater than 50% decrease in their seizures. This is a significant decrease in seizures of children compared to the controls. It further supports

the use of the KD as a treatment for epilepsy. In addition, it shows the importance of having class one clinical trials as a way to validate research, especially in neurological studies.

Chapter 3: Effects of the Keto Diet on Cancer

Introduction to the Problem:

One out of every five men will develop cancer during their lifetime, with one out of eight dying because of it. In addition, one out of every six women will develop cancer during their lifetime, with one out of every eleven dying from it (Bray et al., 2018). The International Agency for Research on Cancer (IARC) published these shocking global cancer statistics in September 2018, demonstrating the magnitude with which cancer affects the global population. The 2018 statistics covered 36 cancers in 185 different countries. They determined that 18.1 million cancer cases occurred in 2018, with 9.6 million of these resulting in death. It is important to note that these numbers are estimates for a single year and only include data from 185 countries, meaning that there are some unaccounted for. There is no doubt that cancer rates are rising and higher than they were just a few decades ago. However, this is not due to lack of research and advancements. In fact, the majority of the increase in cancer incidence comes from the increase in human lifespan and better detection (Jones, 2015). As science and medicine have advanced, it has led to the increase in average human lifespan. In turn, this increase also means more time for cells to divide and pick up errors along the way i.e. cancer. Cancer is now the leading cause of death in the U.S. and second worldwide (Byerley, 2019). In contrast, cancer survival has doubled from 1970 to 2010 (Jones, 2015). This means that we are making progress in the "war against cancer". With every scientific advancement, including new chemotherapy drugs, precision radiation, and improved surgical techniques, comes great

strides against cancer. However, a lot of work is still needed to be done as cancer is extremely complex and different in every individual.

The race for combatting cancer officially started when President Franklin D. Roosevelt signed the National Cancer Institute Act in 1937 (Kalberer, 1975). This new act established the National Cancer Institute (NCI), which later became a division of the National Institutes of Health in 1944. This division conducted studies to help understand the causes of cancer and improve cancer treatments and diagnosis. However, cancer was still affecting two out of every three American families in 1970. In order to accelerate the progress of cancer research, President Richard Nixon signed the National Cancer Act of 1971, which increased the yearly budget for the NCI. In addition, an increased budget allowed for the NCI to compartmentalize its research and create five unique and separate cancer divisions: Control and Rehabilitation, Biology and Diagnosis, Treatment, Cause and Prevention, and Research Resources and Centers. Since then, the National Cancer Institute has made great progress in fighting back against cancer. Without these initial legislative steps and funding, cancer survival rates would not have had the major increases in the last 30 years. However, we are not done fighting, not even close. Cancer is still the leading cause of premature death in the U.S. according to 2015 statistics from the World Health Organization and the first or second leading cause in 90 other countries (**Figure 8**) (Brey et al., 2018).

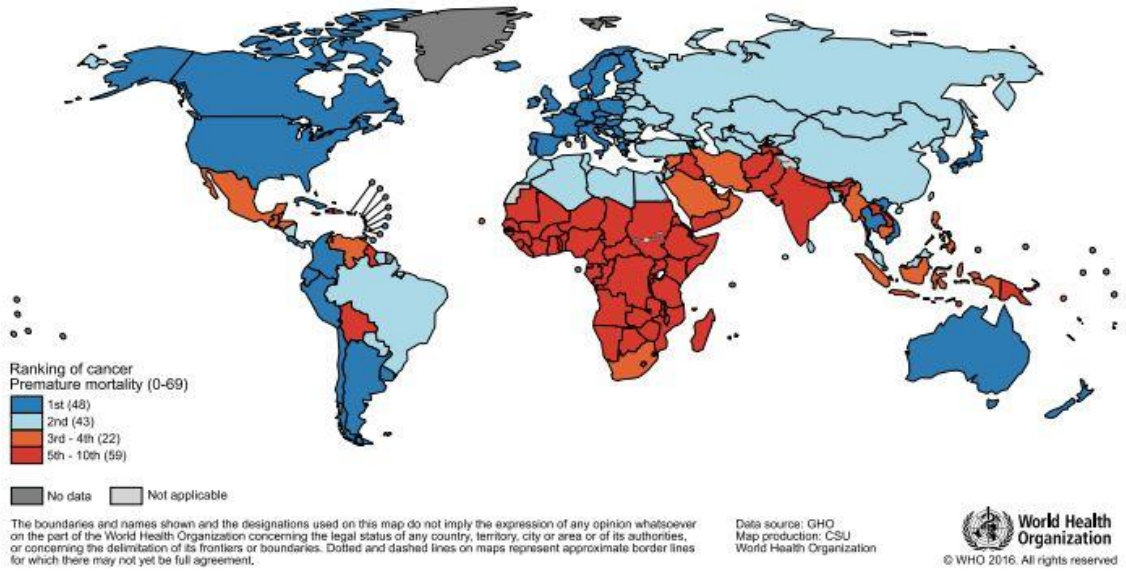

Figure 8. A visual depiction of cancer prevalence around the globe. According to the key, dark blue indicates that cancer is the leading cause in premature mortality in ages 0-69. Light blue indicates that cancer is the 2nd leading cause in premature mortality, orange being 3rd-4th and red being 5th-10th in that area. Adapted from the World Health Organization (as cited in Brey et al., 2018).

What Is Cancer?

Cancer is not a single disease; rather, it is an assortment of diseases. These diseases are all uniquely different, even between individuals with the same cancer. In fact, cells within the same tumor can be different and contain different mutations (National Cancer Institute [NCI], 2015). However, there are commonalities that define the disease as cancer. Cancerous cells often starts out as a normal cells but gains a variety of mutations in their genetic makeup (DNA) along the way. There are six core attributes that are needed in order for a cell to become cancerous. They are often known as the core hallmarks of cancer (Hanahan & Weinberg, 2011). They include: sustaining proliferative signaling, resisting cell death, inducing angiogenesis, enabling replicative immortality, activating invasion and

metastasis, and evading growth suppressors. All of these mutations allow for the cell to survive and proliferate uncontrollably. They no longer listen to the signals that tell the cell to stop dividing or to undergo apoptosis (cell death). In addition, they gain the ability to evade the immune system often because of the microenvironment the establish (NCI, 2015). Cancer cells also have the ability to aggregate and clump together, forming a tumor. In order for a tumor to continue growing and get its needed nutrients, such as glucose and oxygen, a process called angiogenesis occurs. In this process, new blood vessels form and that allow the tumor to consume more nutrients from the rest of the body. Aggregate cells are considered benign tumors until they become invasive. Some cells even spread throughout the body via the bloodstream or lymph system. Cancer cells have the ability to form new tumors in different locations then which they originated and wreak havoc on the body.

Because cancer requires changing the genetic makeup of the cell, cancer is considered a genetic disease and can be inherited (NCI, 2015). This is one of the ways in which a person can get cancer. However, cancer may also arise from a bunch of random mutations that occur over time. Certain environmental factors can even cause cancer such as UV ray exposure from the sun, exposure to radiation, cigarette smoke, and more. Cancer can also be caused by viruses that cause mutations in the genome (Hanahan & Weinberg, 2011). Thus, cancer does not have a single cause and can originate from a variety of different sources.

Cancer Metabolism and the Warburg Effect

Cancer is also a metabolic disease. This understanding has led to a whole new realm of cancer research and possible treatments (Berg et al., 2015). Like any other organism, if it is deprived of energy, cancer can no longer grow and will eventually die. However, cancer was not originally thought of as a metabolic disease. The study of metabolism in cancer cells was revolutionized by Otto Warburg through his discovery in 1920 (Berg et al. 2015; Hsu & Sabatini, 2008). Warburg's discovery came to be known as the Warburg effect and is well-known by cancer researchers everywhere. Warburg discovered the major differences between normal cell and cancer cell metabolism, which allowed for the expansion of cancer drug targets and imaging technologies. However, although the Warburg effect is a well-accepted model for explaining cancer metabolism, debate still ensues in determining the mechanisms behind this change in metabolism. Understanding the basis of Warburg's discovery and the research thereafter is crucial for understanding cancer as a metabolic disease.

Warburg received a Nobel prize in 1931 for his work on cellular metabolism and respiration (Miles & Williams, 2008). The Warburg effect is centered around cancer's unique change in metabolism (Warburg, 1956). Warburg determined that cancer cells increase anaerobic glycolysis even in the presence of abundant oxygen, resulting in an increase in lactic acid production. In his famous paper *On the Origin of Cancer Cells,* Warburg not only provides evidence to this change in metabolism but provides a hypothesis for its origin and perhaps the first mechanistic theory. Although many aspects about cancer

metabolism are still unknown, Warburg helped pave the way for an abundance of research on cancer metabolism.

Normal cells use glucose as their primary source of energy. The glucose that enters the cell goes through three catabolic pathways under complete glucose oxidation (Viel, 2017). These three pathways include glycolysis, followed by the entrance into the citric acid cycle, and then oxidative phosphorylation. Completion through these three pathways requires oxygen and results in maximal ATP (energy) production. Alternatively, when oxygen is not available for use, such as under hypoxic conditions, glycolysis can be used solely to provide energy to the cell. However, unlike oxidative phosphorylation that results in the production of 34-36 ATP, glycolysis only results in 4 ATP and pyruvate as a byproduct (Viel, 2017; Berg et al., 2015). The process of fermentation occurs during hypoxic conditions, a process in which the byproduct pyruvate is converted into lactic acid until enough oxygen is present. Therefore, a higher level of anaerobic glycolysis would be associated with of high levels of lactic acid and vice versa.

Warburg discovered a huge differential increase in the fermentation levels of cancer cells than those in normal cells (Warburg, 1956). Shockingly, the fermentation levels of cancer cells were at almost the same level as a highly proliferating yeast species. In addition, the more cancer cells that were aggregated into a tumor, the higher the fermentation levels were. Upon closer investigation, Warburg learned that high levels of fermentation coincided with extremely low levels of oxidative phosphorylation, indicating incomplete cellular respiration by cancer cells. Furthermore, cancer cells will use glycolysis even under conditions with enough oxygen to sustain oxidative phosphorylation.

These results were the same across all cancer cell types that Warburg studied and thus led to Warburg's two hypotheses. First, he believed that a broken metabolic system is what caused normal cells to turn cancerous. Second, he believed that a broken metabolism could happen through oxygen deficiencies in the cell. Once the cell's metabolism was broken, it became irreversibly cancerous even when reintroduced to an oxygen-rich environment. Warburg came up with two hypotheses during the course of his research, although the hypotheses themselves were later disproved.

Warburg based his hypotheses on an earlier experiment done by Goldblatt and Cameron (Warburg, 1956). These two researchers successfully transformed heart cells into cancer cells by subjecting them to cycles of oxygen deprivation (Goldblatt & Cameron, 1953). It took a long time to induce cancer; however, they were successful in creating cancer in their tissue culture, compared to that of their control. To Warburg, this phenomenon explained why other carcinogens resulted in the formation of cancer (Warburg, 1956). For instance, he believed that radiation caused cancer due to the disturbance in oxygen circulation, providing the same conditions that Goldblatt and Cameron displayed in their experiment. However, Warburg noticed that even after respiration damage, cells did not become cancerous right away. He attributed this to their need to gain mutations that could increase their fermentation levels Everything in Warburg's hypothesis seemed to add up. However, other hypotheses evolved when cancer cells that did not have broken respiration and were fully capable of oxidative phosphorylation were found (Viel, 2017). This meant that broken respiration due to oxygen deprivation was not the cause of cancer, nor were cancer cells forced to switch over to their

reliance on glycolysis. Instead, it seemed as though this abnormal metabolism was beneficial to cancer's proliferation and growth.

Warburg answered many questions that scientists had about cancer cell metabolism. However, what he did not successfully define the mechanism of which these changes occurred. The question later became a matter of how and why? How do cancer cells change their metabolism to rely heavily on glycolysis for survival, and why is it beneficial to them? The Warburg effect baffled scientists. It was hard to believe that cancer cells would mutate into a less efficient producer of energy. However, as more research surfaced new hypotheses were introduced.

One popular hypothesis purposed that glycolysis utilization was driven by the conditions of the microenvironment surrounding the tumor, including low oxygen and low pH (**Figure 9A**) (Viel, 2017; Hsu & Sabatini, 2008). As previously mentioned, tumors need access to high amounts of nutrients including glucose and oxygen. The bigger the tumors get, the more nutrients they need to sustain the growing mass. With an increase in oxygen uptake by the tumor, the surrounding microenvironment becomes hypoxic, meaning without oxygen (Hsu & Sabatini, 2008). As a response, the hypoxia-inducible factor 1 (HIF-1) becomes highly expressed (**Figure 9b)**. HIF-1 has the ability to sustain the cell during hypoxia by inducing angiogenesis, as well as increase glucose uptake in the cell and promoting the increase in glycolysis (Berg et al., 2013; Hsu & Sabatini, 2008, Miles & Williams, 2008; Viel, 2017). However, even with angiogenesis, the tumor microenvironment is still hypoxic (Hsu & Sabatini, 2008). This is because the newly formed blood vessels that form via angiogenesis are not efficient and or reliable enough to

increase oxygen levels that are capable of alleviating the shift towards glycolysis. Sparse and unreliable oxygen is one of the drivers for cancer cells to shift towards glycolysis. This way, relying on glycolysis would allow cancer cells to get energy without relying on oxygen.

In addition to low oxygen, the microenvironment is also acidic, meaning it has a low pH (**Figure 9A**) (Viel, 2017; Hsu & Sabatini, 2008). The pH is lowered by lactic acid production as a byproduct of increased anaerobic glycolysis. Additionally, a low pH is thought to be beneficial for cancer cells because it is associated with a decrease in immune system response (Viel, 2017). Normal cells cannot survive acidic environments. Whereas, cancer cells gain mutations that make them better adapted to acidic environments. Cancer cells thus have a better chance of evading immune cells if they switch to glycolysis reliance.

Another hypothesis purposes that cancer cells are more concerned with increasing their biomass than attaining ATP (Doherty & Cleveland, 2013; Teicher et al., 2010; Viel, 2017). Although ATP production is important to cancer cells, it might not be the most important thing. In order for cancer cells to increase their biomass, they must produce large quantities of macromolecules such as proteins and nucleic acids (**Figure 9c**) (Viel, 2017; Hsu & Sabatini, 2008). These macromolecules are vital for tumor growth and proliferation. The synthesis of these macromolecules needs large amounts of ATP, carbon, and electrons provided by NADPH. Glycolysis is thus preferred over oxidative phosphorylation in cancer cells due to the following conditions. First of all, although glycolysis does not normally produce as much ATP as oxidative phosphorylation, cancer cells upregulate glycolysis so much so that they can produce the same amount of ATP (Warburg, 1956). Additionally,

with an increase in glycolysis, cancer cells also get an increase in the intermediates of glycolysis, such as glucose-6-phosphate. This increased intermediate can be used to produce more NADPH through pentose phosphate pathway (Berg et al., 2013). Secondly, during oxidative phosphorylation, there is a tradeoff for the amount of ATP produced. The six carbons contained in glucose are lost and exhaled as CO2. However, in glycolysis, the carbon can be preserved and can be used for biosynthesis of the important macromolecules (Viel, 2017). All of the following conditions are induced by glycolysis reliance and benefit cancer.

Consequently, there are also a few trade-offs for cancer's overactive metabolism. For instance, by increasing metabolism the number of toxic metabolites that can cause oxidative stress also increases (Hsu & Sabatini, 2008). These reactive oxidative species are extremely harmful to cells in large quantities. They can destroy biomolecules and cause cell death. However, cancer found a way around this. Molecules capable of reducing these toxic molecules would also be needed in high quantities to prevent cancer cell death. Reducing agents such as NADPH are needed in order to counteract this negative effect (Viel, 2017; Hsu & Sabatini, 2008). Glycolysis is then preferred because it results in the increased production of NADPH. Therefore, it can be concluded that sole reliance on glycolysis would benefit a cancer cell greatly, despite the possible trade-offs. Glycolysis

provides a cancer cell with both the needed energy, biosynthetic molecules, and the correct microenvironment for proliferation and growth.

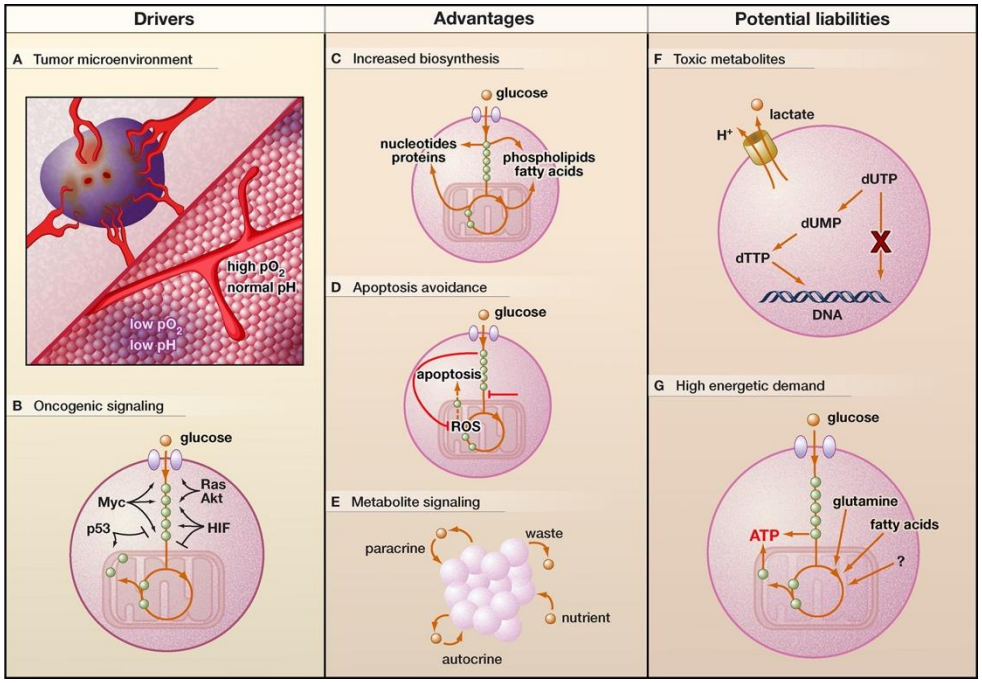

Figure 9. the drivers, advantages, and potential liabilities for glycolysis reliance in cancer cells. **A)** shows the tumor microenvironment including low oxygen and low pH as a possible driver. **B)** shows the signaling of oncogenes, including HIF which results in angiogenesis. **C)** shows how biosynthesis is increased with upregulated glycolysis. **F)** shows the toxic metabolite production resulting from glycolysis that acts as a possible liability. **D, E)** other advantages discusses including apoptosis avoidance and metabolite signaling. **G)** Shows a high energetic demand as a potential liability. Adapted from Hsu & Sabatini, 2008.

Drugs Targeting Cancer Metabolism

If a person goes into the doctor with a bacterial infection, the doctor will often prescribe antibiotics in order to kill the foreign invader that are causing the body harm. In addition to antibacterial drugs, there are antiviral and antifungal drugs that target specific

organisms. All of these drugs have one thing in common. They all target the differences between our human cells and the organisms we are trying to get rid of. However, what do you do when it is your own body turning against you? Your own cells become merciless monsters that steal all of the nutrients in your body and leave you weak and vulnerable. And even when you think you figured out a way to stop these out-of-control cells, they mutate and become even more aggressive than they were before. This is the exact problem that physicians and scientists have when trying to treat individuals with cancer. In fact, because of this, current treatments often harm other healthy cells. Some of these treatments even end up doing more harm than good. However, new treatments and medications are being discovered every day and old ones are improving. With each study and paper that gets published, the closer we get to finding a cure.

Warburg was not the first to study the metabolism of cancer cells. In fact, many scientists have been discovering drugs that target cancer metabolism for decades. One of these scientists was Sidney Farber, who in 1947 targeted nucleotide synthesis with aminopterin, the first chemotherapy drug (Leungo et al., 2018). Aminopterin is similar in structure to folate, a molecule needed for the synthesis of DNA and RNA. Although aminopterin may look like folate, it does not serve the same function and actually inhibits the synthesis of DNA and RNA. Thus, aminopterin and the later discovered methotrexate and pemetrexed, that are currently used today, were called antifolates. Similarly, they started a whole class of drugs coined “antimetabolites”, all of which inhibited the synthesis of new DNA or RNA in the cell. They were chemotherapeutics that worked well on preventing cell proliferation. However, the downside was that they were not specific to just

cancer cells. These antimetabolites affected all proliferating cells, including non-cancerous ones such as hair follicles and the cells that line the stomach (Leungo et al., 2018; Teicher et al., 2012). In addition, chemotherapy does not work on all cancers because some gain resistance.

Lactate is another popular metabolic target for cancer therapies (Doherty & Cleveland, 2013). As discussed previously, lactate plays an important role in cancer metabolism. Lactate, a byproduct of glycolysis, is important in lowering the pH in the microenvironment. Interestingly, lactate is also a signaling molecule. It activates an inflammatory response in order to gain the needed growth factors that the immune cells secrete. These growth factors help tumor progression and angiogenesis (Doherty & Cleveland, 2013). The importance of lactate to cancer cells has been exploited in many cancer therapies. One approach is by inhibiting the enzyme responsible for the production of lactate from its pyruvate precursor. This enzyme is known as LDH or lactate dehydrogenase (Doherty & Cleveland, 2013). Studies have shown that inhibition of LDH helps prevent metastasis by re-establishing anchorage-dependent growth. Cancer cells usually have anchorage-independent growth, meaning they can grow anywhere; whereas, normal cells have to be touching a surface in order to grow. Inhibition of LDH was also shown to directly inhibit growth in certain cancers; as well as, increase the production of toxic oxygenated species. Cleveland & Doherty suggested that this could be due to the decrease in glycolysis which produces the majority of reducing agents (Doherty & Cleveland, 2013).

Understanding cancer metabolism has even allowed for better cancer screening techniques (Miles & Williams, 2008). Fluorodeoxyglucose positron emission tomography (FDG-PET) is one popular screening technique that was made possible by Warburg's discovery. This technology allows for the measurement of glucose metabolism and blood flow in certain tissues (Hsu, 2008; Miles & Williams, 2008; Teicher et al., 2012). Because cancerous cells have an increased uptake of glucose, they can be differentiated from normal cells using this method. Cancerous cells usually also have an increased blood flow due to the formation of new blood vessels. However, blood flow is not always a good indicator with FDG-PET screening technology because it can be different for different types of tumors (Miles & Williams, 2008; Teicher et al., 2012). For instance, for brain/spinal cord tumors, there is not a correlational relationship between glucose uptake and blood flow (Miles & Williams, 2008). Even though FDG-PET is not perfect and is variable, it offers insight to tumor microenvironments which can be helpful for determining treatments. For instance, a tumor with increased glucose uptake but low blood flow could indicate an adaptation of that tumor to hypoxia conditions. Lastly, FDG-PET can complement other screening technologies such as CT scans, ultrasound, and MRIs for more accurate information. CT scans can even prevent blood flow measurement inaccuracies due to an increased blood flow volume of tumors around organs with high blood flow, such as the heart (Miles & Williams, 2008). FDG-PET technology is very important in cancer screening and could not have been made possible without Warburg's discovery. All of the above treatments show the importance of cellular metabolism in cancer treatment. Cancer

metabolism is vital to understanding how cancer works and provides evidence for why metabolic treatments are an important avenue that needs to be explored more.

Brain Tumors and Brain Cancers

The Cure Brain Cancer Foundation is an organization in Australia that conducts brain cancer research and promotes awareness (Cure Brain Cancer Foundation, 2017). In their 2017 report on brain cancer statistics, they claimed that brain cancer was the leading cause of death for children in Australia than any other disease. It is well-known that brain cancer is extremely hard to treat. The brain is one of the most important, if not the most important, organ in the body. It is responsible for sending signals out to the rest of the body. The brain has to be protected and any injury to it can be catastrophic and prevent certain bodily functions. Due to the importance and fragility of the brain, it makes sense why the survival rates are extremely low for brain cancers. The 2017 report continued by stating that all cancers on average have seen a 20% increase in 5-year survival; whereas, brain cancer has only seen 1% increase in 5-year survival over the last 30 years. Furthermore, brain cancer costs more to treat per person than any other cancer (Cure Brain Cancer Foundation, 2017). These shocking statistics show the severity with which brain cancer can affect a population. Even though these statistics are from Australia, similar statistics can be found in other parts of the world. For example, predicted at the beginning of 2019 that around 3,720 children in the U.S. would be diagnosed with brain tumors by the end of the year (American Society of Clinical Oncology [ASCO], 2019). For adults in the U.S., nervous system cancers are the 10th leading cause of death. These statistics are not just

numbers, they are people's lives. They are someone's brother, mother, father, friend, or partner.

A brain tumor can either be primary, meaning it originated in the brain, or metastatic, meaning it originated from somewhere else in the body (Cure Brain Cancer Foundation, 2017). Metastatic brain tumors, synonymously known as brain cancer are more often life-threatening than primary benign tumors (NCI, 2015). Benign tumors can be life-threatening if they are in important parts of the brain. Malignant brain tumors are highly invasive; some can even anchor themselves into the brain with their long filaments. Pediatric brain tumors are also different from tumors in adults. A child's brain is not fully developed, making treatment options for children different. Tumors can also be categorized in four different grade levels. The levels go from least to most harmful chronologically. The Cure Brain Cancer Foundation (2017) lists the following parameters for grading tumors:

- similarity to normal cells (atypia)
- rate of growth (mitotic index)
- indications of uncontrolled growth
- dead cells in the centre of the tumour (necrosis)
- potential for invasion and/or spread (infiltration) based on whether or not it has a definitive margin (diffuse or focal)
- blood supply (vascularity).

With these parameters in mind, the tumor is graded and put into a level category. The more progressive tumors have a higher stage level and likely indicate a harder to treat tumor. It

is important to know the stage of the tumor in order to provide the best treatment options for the patient.

Treatments for Gliomas

The current treatments for malignant gliomas, tumors of the brain or spinal cord, typically consist of surgery, radiation, and chemotherapy. These treatments have, for the most part, not changed in over 50 years (Seyfried et al., 2009). Stagnant treatments are most likely the cause for the low survival rate increase compared to the survival increase of other cancers. Current therapies are sufficient in controlling short-term disease progression. However, these therapies often make things worse for the patient in the long run, both through disease progression and direct effects of the treatments. Thus, the evidence points towards a lack of efficacy in the current treatments of malignant glioma. The implementation of new treatments or the improvement of old treatments are therefore needed in order to increase survival rates for brain tumors.

The first line of defense against malignant glioma tumors is physical removal. Surgical removal of the tumor is highly dependent on the location of the tumor. If the tumor is in vital areas of the brain or the tumor cannot be safely removed, surgery will not proceed, and the tumor is considered an unresectable tumor (Cure Brain Cancer Foundation, 2017). Surgery is often suggested as the first treatment because removed tissue can be sent for a pathological examination. A pathologist can facilitate the creation of a new and more targeted treatment plan. In addition, surgery can provide pain relief and make the tumor smaller so that less radiation and chemotherapy will be needed. However,

the cutting of tissue during surgery releases growth factors to promote cell growth for healing (Seyfried et al., 2009). In turn, these growth factors increase cancer cell proliferation as well. Tumors are likely to grow back with cells that can contain completely different mutations than that of the first tumor.

The second line of defense is in the form of chemotherapy, which is done after surgery to kill the remaining cancer cells (Cure Brain Cancer Foundation, 2017). It can be done by itself or in conjunction with radiation therapy. Chemotherapy will not be given to patients if they are too weak. Chemotherapy can be administered via an injection through an IV, an injection directly into the liquid around your brain, orally, or implanted into the brain during surgery. However, chemotherapy has adverse effects that can shorten lives and make things worse. Seyfried et al., concluded that chemotherapy drugs have severe and highly toxic effects (2009; 2010). Bevacizumab and irinotecan are two chemotherapy drugs that are associated with lower toxicity compared to others; yet, in one study they resulted in 6% fatalities (Vredenburgh et al., 2007, as cited in Seyfried et al., 2009). Fatalities were a direct link to chemotherapy toxicity that caused blood clots and/or stroke. In addition, 38% stopped treatment in the study due to issues with toxicity. This is just one out of the many studies that Seyfried et al., argue as evidence against the use of current treatment options for malignant brain tumors. However, out of surgery, chemotherapy and radiation, they believe radiation to have the most adverse side effects.

Radiation therapy is also known as radiotherapy or radiosurgery, which is a slightly more precise form. Nonetheless, it is a process by which high levels of radiation are used to target a location of tissue with the goal of reducing tumor growth (Cure Brain Cancer

Foundation, 2017). Radiation therapies not only harm the targeted tissue but produce oxidative damage to healthy surrounding tissues as well (Seyfried et al., 2009). Necrotic tissue also elevates glutamate levels in surrounding areas. Glutamate can be metabolized into glutamine, which is a fuel source for brain tumor cells. In addition, the cancer cells that are able to survive radiation are often stronger than the ones in the original tumor.

The majority of cancer treatments, especially for malignant gliomas, can be extremely toxic. Yet, they have been used for the past 50 years. Instead of using new treatments or improving the current ones, treatments usually consists of trying combinations of these drugs. For instance, 99% of individuals are also given dexamethasone, a steroid that is supposed to reduce inflammation from surgery/radiation (Seyfried et al., 2010). However, it also increases the amount of glucose in an individual's blood. As we know, glucose fuels the cancer cells and thus increases its activity and proliferation. The problem lies within the industry that provides cancer therapy, a patients' willingness to accept toxic treatments, and a physician's willing to prescribe them. In order to break this cycle, new non-toxic and effective therapies must be recognized tested for efficacy and accepted.

The KD and Brain Cancer

The neuroprotective effect of the KD is well-known and highly documented due to its therapeutic uses in treating drug-resistant epilepsy (Seyfried et al., 2009). Current research on the KD aims to use these same neuroprotective effects and extend them to other neurological diseases. One of the newest treatments being explored is on malignant brain

tumors. The KD approach as a cancer therapy deals with taking away the main source of energy for the highly proliferative tumor cells, while still providing energy to normal cells. The KD exploits the Warburg effect with the goal of starving out cancer. Ketone bodies can be produced during a low-carbohydrate, high-fat and moderate protein diet. These ketones bypass glycolysis all together and restrict the energy available to cancer cells. Unlike cancer cells, most normal healthy cells are capable of using ketones for energy. According to Zhou et al., a partially restricted KD is the only approach that has the capability of specifically targeting cancer cells while sparing and even protecting healthy cells (2007).

Cancer cells are sensitive to sudden changes in their surrounding microenvironment and will either not be able to grow or die if conditions are unfavorable (Seyfried et al., 2009). Many researchers have hypothesized that the KD can be used to create an unfavorable microenvironment for cancer cells in the brain; in addition to creating a neuroprotective effect for normal brain cells by increasing ketone bodies in the blood (Seyfried et al., 2010; Seyfried et al., 2009; Ji et al., 2020; Zhou et al., 2007). Current treatments for malignant brain tumors are not providing long-term solutions for patients. Instead, the KD could act as a new method of treatment, as it has promising preliminary results.

It took many years, funding, support, and research to implement the keto diet as a treatment for epilepsy. The use of the KD as treatment for malignant brain tumors on the other hand has not been heavily studied since it is a fairly new idea. However, efficacy of

the KD in treating malignant brain tumors is supported in cell cultures, rat models, and a few human case reports. These results are just the beginning.

In a recent study, Ji and colleges aimed to determine the effect that a KD would have on glioma stem-like cells (Ji et al., 2020). They grew up cell cultures on Beta-hydroxybutyrate (BHB) medium at different concentrations in order to mimic a state of ketosis. They hypothesized that in addition to having an anti-cancer effect, a KD could provide a protective effect on normal cells. Results from this study showed that glioma stem-like cells grown in BHB medium had reduced proliferation, higher levels of apoptosis, reduced tumorigenic capacity, reduced glucose uptake, inhibited glycolysis, as well as the disturbance of other hallmarks of cancer and overall cancer cell growth **(figure 10B)**. In addition, they determined that normal neural stem-like cells were not harmed and actually had increased proliferation **(Figure 10A)**. This study demonstrated the specificity that a KD would have in targeting cancerous cells. Not only does the KD itself lower glucose levels through carbohydrate restriction but results in the production of ketone bodies. This can be seen in **Figure 10A** where results indicate that a 10mM BHB, Glow medium were preferable for the growth of normal neural stem-like cells. While, in **Figure 10B-C**, results indicate that this medium inhibits glioma stem-like cell growth (Ji et al., 2020). The ketone body BHB is thus shown to have anti-tumor properties as well as neuroprotective properties.

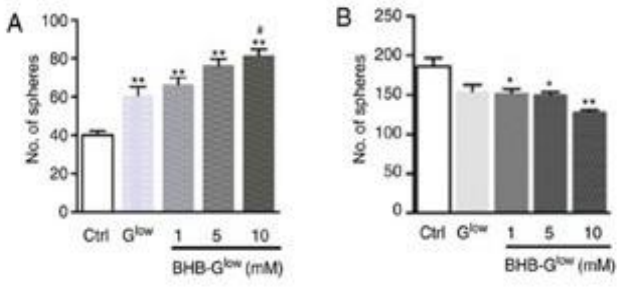

Figure 10. results from BHB manipulation on glioma stem-like cells and normal neural stem-like cells. **A)** Contains manipulations done on normal neural stem-like cells. The control cells were grown up in normal medium; Glow cells were grown in low glucose medium; the BHB Glow cells were grown in low glucose medium treated with different concentrations of BHB. BHB concentrations increase numerically with the highest concentration being 10mM. **B)** Contains results from manipulations done on glioma stem-like cells with the following conditions seen in A. Adapted from Ji et al., 2020.

Ji and colleges show promising results in the battle against malignant brain tumors. However, their research like many others before them was done on cell cultures. As many scientists may know, cell cultures, although they are a necessary preliminary step in determining treatment efficacy, they are not fully indicative of human trails. One of the next steps would be to see how the KD works against live animal models. Animal models can be used as a way to better understand the effect of an agent on the human body. It should be known that animal models themselves are not perfect either. Yet, the allow researchers to see treatment responses in a living organism.

In a study done by Seyfried et al., 2010, researchers used rat model in order to determine the effect of diet on glioblastoma multiforme, a common malignant brain tumor. The diets they used were either calorically restricted (R), or unrestricted (UR) and either a standard diet (SD), or a ketogenic diet (KD). They concluded that a (R) diet reduced tumor growth by lowering all nutrients, including glucose levels that inhibit glycolysis and overall

proliferation (**Figure 11**). In addition, a (R) diet further stressed out cancer cells by reducing blood flow and oxygen consumption. Interestingly, they saw that both restrictive diets (SD-R and KD-R) were effective in treating malignant brain tumors in rats (**Figure 11B**).

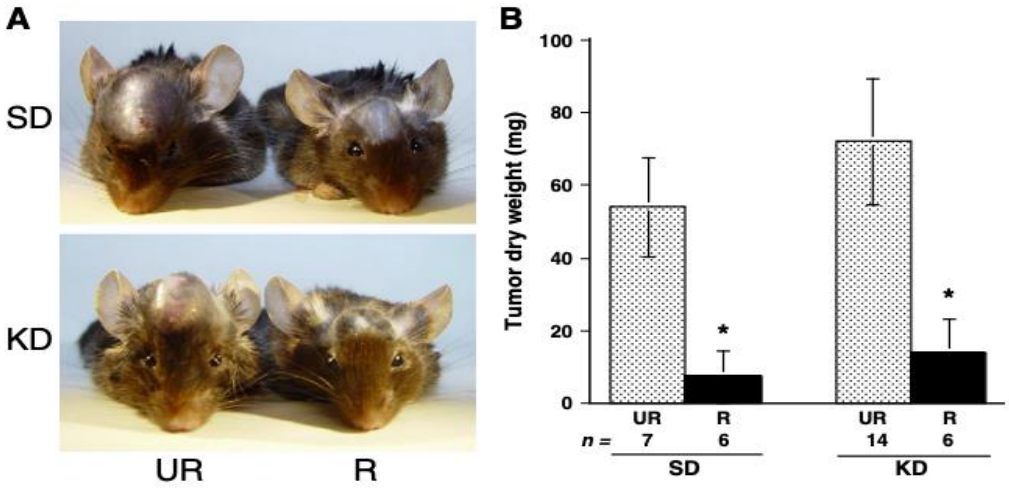

Figure 11. results from four dietary manipulations aiming at controlling malignant brain tumors in rats. SD-UR is the control with a standard and unrestricted diet. Both a standard diet and a ketogenic diet were tested with calorically restricted diets (SD-R and KD-R respectively). A ketogenic diet was tested on its own with unrestricted caloric intake KD-UR. **A)** offers tumor visualizations after diet manipulation. **B)** shows quanitative data collected on tumor size after dietary manipulations. Adapted from Seyfried et al., 2010.

However, researchers also saw that an unrestricted keto diet (KD-UR) was not effective in reducing tumor growth (Seyfried et al., 2010). They hypothesized that the human body would respond better to the standard KD than a rat would. Theoretically, a human brain is more efficient at utilizing ketones due to our evolutionary ability to withstand long periods of starvation (Cahill, 2003). This would allow us to starve out cancer cells while still supply energy to our big brains. Regardless, the study done by Seyfried showed that restricting

tumor access to nutrients through metabolic manipulation was effective in reducing tumor growth. They further believe that a restrictive diet worked due to its ability to reduce glucose available to the cancer cells (Seyfried et al., 2010).

Nebeling and colleges were one of the first researchers to use the KD as a treatment for malignant brain tumors in humans (Nebling et al., 1995). Although their sample size was small and only consisted of two people, these two case reports show great promise. Their first patient was only 18 months old at the time of her diagnosis of stage IV anaplastic astrocytoma. She received traditional treatments of chemotherapy and radiotherapy. She had poor responses to all conventional treatments. Instead, these conventional treatments made her lose a considerable amount of weight and resulted in hospitalization from a drug associated seizure. After seven months, six chemotherapy treatments and no response, treatments were stopped. At this point she was put on the KD as a last resort by Nebeling and colleges. During just the first eight weeks, improvements were made, a variety of skills were learned, and imaging showed a 21.77% decrease in glucose uptake by the tumor. She continued on the KD for 12 months and tumor progression halted. In addition, she began to develop socially and physically. Her family believed that her overall quality of life improved as well.

The second patient in the study was six years old at the time of her diagnosis of stage II cerebellar astrocytoma (Nebling et al., 1995). There were some neurological complications that ensued after her two surgeries. She underwent radiation therapy for three months and started on chemotherapy shortly after. Similarly to the first patient, she also experienced toxicity associated with the chemotherapy that resulted in hearing loss,

and low magnesium levels. She continued to receive chemotherapy but was placed on the KD for further treatment. A 21.84% decrease in tumor glucose uptake was seen at week eight. Both patients went into remission and continued to enjoy a good quality life. The researchers concluded that the KD was safe enough to administer to children and that the results were promising. However, they did recognize that more research was needed to confirm the efficacy of the KD in treating malignant brain tumors. Additionally, they recognized that at this moment the KD cannot replace current treatments; rather, it should first be used as a supplemental therapy. By using the KD in addition to current treatments, physicians would be able to reduce the amount of radiation and chemotherapy levels given to a patient and thus minimize their toxic effects.

Another case report was conducted by Zuccoli et al., 2010. The patient was 65 years of age and had been diagnosed with a stage IV glioblastoma multiforme. Before this case report, a KD has not been used on an elderly patient in order to determines its efficacy in treating glioblastoma multiforme (Zuccoli et al., 2010). Metabolic manipulation through the use of a KD along with caloric restriction was administered for 14 days. After the 14 days, standard treatments of radiation and chemotherapy began. All treatments, including the KD were stopped after two months when brain scans were clear (**Figure 12**). The patient remained healthy for the whole two months and had no neurological complications. Researches believed that the KD supplemented the conventional therapies, allowing for better treatment of the tumor. They explained that regression of a glioblastoma in that short of a timeframe was extremely unlikely for an elderly patient treated with conventional treatments. Zuccoli et al. attribute this regression to the use of metabolic manipulation,

especially because similar regression was seen in children who were put on the KD with the goal of treating malignant brain tumors.

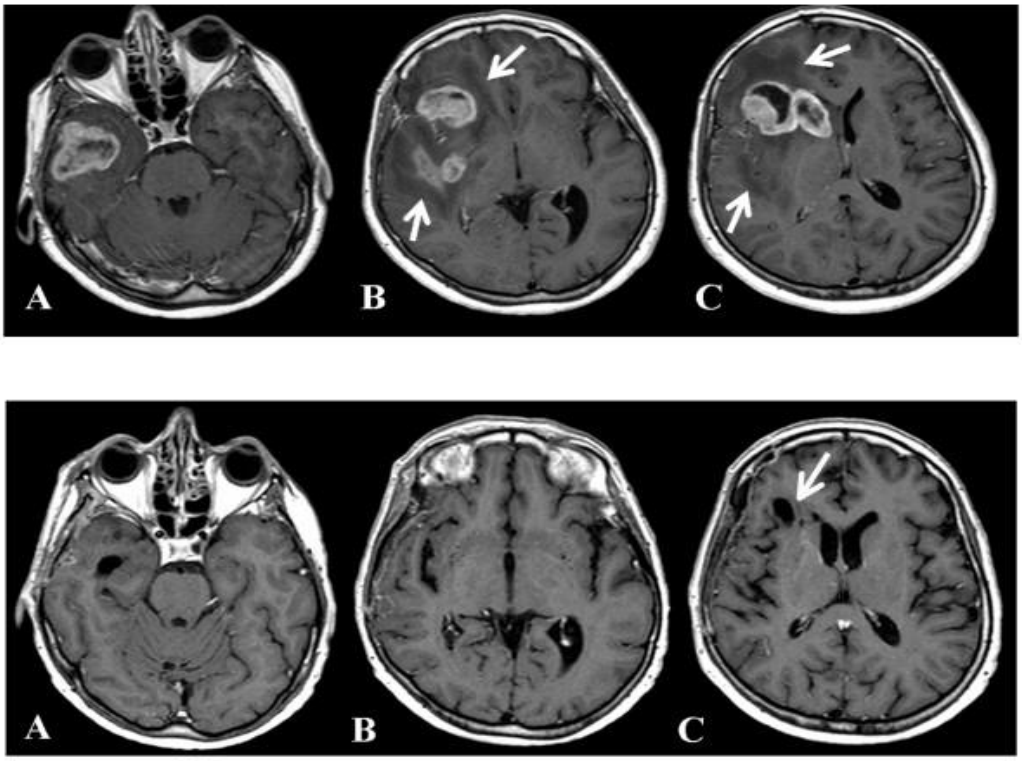

Figure 12. brain scans from before and after using a KD as a supplemental treatment of glioblastoma multiforme. The top pictures show brain scans of a 65-year-old female at time of diagnosis before treatment. The bottom pictures scans proceeding two months of treatments. **A,B,C)** are different views of the brain. Adapted from Zuccoli et al., 2010.

All of these above studies have shown that metabolic manipulation can be effective in treating malignant brain tumors (Ji et al., 2020; Nebling et al., 1995; Seyfried et al., 2010; Zuccoli et al., 2010). However, they all recognize that more research is needed in order to implement the KD as a treatment for malignant brain tumors. Seyfried et al., 2010 explains the discrepancies that may arise when model organisms are used in scientific

studies. In their specific study, they explain how ketone utilization in the brain is different between rats and humans. Human trails are thus the necessary next step.

Chapter 4: What Now?

Personal Opinion:

It took many years and plenty of research to prove the efficiency of the KD in treating epilepsy. The use of the KD in treating other neurological diseases is not as well supported (Ji et al., 2020; Nebling et al., 1995; Seyfried et al., 2010; Zuccoli et al., 2010). For example, there have not been any approved clinical trials in the United States to test the efficacy of the KD in treating malignant brain tumors (Seyfried et al., 2009). Current research has shown promising results in preliminary findings through cell cultures (Ji et al., 2020), rat models (Seyfried et al., 2010), and human case reports (Nebling et al., 1995; Zuccoli et al., 2010). Although more research is needed for wide range implementation, I believe that patients should be aware of the of the KD as a supplemental therapy. In addition, it should be given as an option if conventional treatments are ineffective. Current treatments are not sufficient enough for treating malignant tumors on their own. It is vital to improve current treatments in order to reduce patient toxicity and improve long term patient survival.

The KD has great potential for treating malignant brain tumors. Current, research supports the safety of administering the KD to all age groups, both young (Nebling et al., 1995) and old (Zuccoli et al., 2010). By restricting carbohydrate intake, glycolysis would be suppressed in all cells. Ketone bodies would then be produced as a result. The majority of the body's healthy cells would be able to utilize ketones as a form of energy, including many parts of the brain. Cancer cells, which rely on glucose for the majority of their energy would starve. In addition, the production of ketones, specifically BHB,

would have a neuroprotective effect. Thus, the KD could be the key to treating malignant brain tumors while still supporting healthy brain activity and normal cell growth.

As previously mentioned, cancer is the leading cause of premature death in the U.S. and second worldwide (Byerley, 2019). In addition, malignant brain tumors are the leading cause of death for children in Australia (Cure Brain Cancer Foundation, 2017) and the 10th leading cause of death for adults in the United States ([ASCO], 2019). Malignant brain tumors are also extremely hard to treat which results in poor survival (Cure Brain Cancer Foundation, 2017). In fact, glioblastomas, in adults, result in a less than 5% five-year survival rate with the median being 9-12 months (Ji et al., 2020). This is why we should care, as a society and as individuals. More research is needed in order to change these statistics. Preliminary results for the use of the KD as a therapy are promising not just for malignant brain tumors, but other cancers as well. For instance, similar preliminary results were seen in cell lines of breast and colon cancers (Fine et al., 2009). The inhabitation of cancer proliferation could be seen across most cancers. Thus, by expanding research, the KD therapy can be more widely used.

However, I do understand that no treatment or therapy is right for everyone. Diets are harder for physicians to monitor and drugs can often be seen as more convenient to administer (Thiele, 2003; Freeman & Kossoff, 2010). The KD is a lifestyle change that might be hard to adapt for some people; as it is not always easy to limit carbohydrate intake. In addition, every treatment has side effects including the KD. Luckily, many of the side effects of the KD have been well documented through its use in treating drug resistant epilepsy. Some of the side effects that patients experienced during treatment

include hypercholesterolemia, mineral deficiencies, acidosis, constipation, and weight loss (Kossoff & Hartman, 2012). However, many of the side effects of the KD can be avoided and or easily treated. First, different variations of the KD can be used depending on the patient in order to minimize side effects while promoting efficiency. Second, vitamin supplementation helps prevent many of the side effects as well. Since the KD has been used in treating epilepsy, standard protocols have also been achieved that help minimize side effects. It is important for patients to consult their doctor before any dietary manipulation.

Regardless, it is essential to move forward with research, with the goal of improving the KD as a potential therapy. This makes research the first thing that needs to occur in order for implementation. Specifically, class one clinical trials are needed for testing the efficacy of the KD in treating neurological diseases. The KD has been successfully implemented as a therapy for drug resistant epilepsy because of the class one clinicals that exist. Additionally, in order to be able to do research, there needs to be more funding available. This might require changing the stigma surrounding the KD as well as other high-fat diets. Public misconceptions can be a huge roadblock for research capabilities. I believe that educating the public on the possible benefits of the KD as a therapy is important. Often times the public has a hand in dictating scientific research, as we saw from the fat-free boom era. Ancel keys gained public support which resulted in funding for his research (Taubes, 2001). Without these steps, it will be hard to move forward towards the utilization of the KD. I firmly believe that the KD will be a useful therapy in treating malignant brain tumors. Together we can change the statistics.

Works Cited

Ben-Dor, M., Gopher, A., Hershkovitz, I., & Barkai, R. (2011). Man the fat hunter: the demise of Homo erectus and the emergence of a new hominin lineage in the Middle Pleistocene (ca. 400 kyr) Levant. *PLoS One*, *6*(12).

Benedict, F. G., Goodall, H. W., Ash, J. E., Langfeld, H. S., Kendall, A. I., & Higgins, H. L. (1915). *A study of prolonged fasting* (No. 203). Carnegie institution of Washington.

Berg, J. M., Tymoczko, J. L., Gatto, G. J., & Stryer, L. (2015). Chapter 15 Metabolism: Basic Concepts and Design. In Biochemistry (Eighth, pp. 423–448). New York, NY: W. H. Freeman and Company.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.

Byerley, L., (2019). Chapters 3-7. In APUS: An Introduction to Nutrition. American Public University System: The LibreTexts libraries.

Cahill Jr, G. F. (2006). Fuel metabolism in starvation. *Annu. Rev. Nutr.*, 26, 1-22.

Cahill, G. F., Herrera, M. G., Morgan, A., Soeldner, J. S., Steinke, J., Levy, P. L., & Kipnis, D. M. (1966). Hormone-fuel interrelationships during fasting. *The Journal of clinical investigation*, *45*(11), 1751-1769.

Charlie Foundation. (2019). “How to Get Started with the Ketogenic Diet.”

Charlie Foundation for Ketogenic Therapies, The Charlie Foundation. Retrieved from (https://charliefoundation.org/).

Cross, J. H., & Neal, E. G. (2008). The ketogenic diet—update on recent clinical trials. *Epilepsia*, *49*, 6-10.

Cunnane, S. C., & Crawford, M. A. (2003). Survival of the fattest: fat babies were the key to evolution of the large human brain. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *136*(1), 17-26.

Cure Brain Cancer Foundation. (2017). "Facts and Stats". Adapted from (https://www.curebraincancer.org.au/page/8/facts-stats).

Doherty, J. R., & Cleveland, J. L. (2013). Targeting lactate metabolism for cancer therapeutics. *The Journal of clinical investigation*, *123*(9), 3685-3692.

Freeman, J. M., & Kossoff, E. H. (2010). Ketosis and the ketogenic diet, 2010: advances in treating epilepsy and other disorders. *Advances in pediatrics*, *57*(1), 315-329.

Freeman, J. M. (2009). The ketogenic diet: additional information from a crossover study. *Journal of child neurology*, *24*(4), 509-512.

Fine, E. J., Miller, A., Quadros, E. V., Sequeira, J. M., & Feinman, R. D. (2009). Acetoacetate reduces growth and ATP concentration in cancer cell lines which over-express uncoupling protein 2. *Cancer cell international*, *9*(1), 14.

Fumento, Michael. (1961). "Medicine: The Fat of the Land," *TIMES*, January 13.

Goldblatt, H., & Cameron, G. (1953). Induced malignancy in cells from rat myocardium

subjected to intermittent anaerobiosis during long propagation in vitro. *The Journal of experimental medicine*, 97(4), 525-552.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. cell, 144(5), 646-674.

Hsu, P. P., & Sabatini, D. M. (2008). Cancer cell metabolism: Warburg and beyond. *Cell*, *134*(5), 703-707.

Imberg, K. (2015). The History of USDA Nutrition Guides. Retrieved November 11, 2019.

Ji, C. C., Hu, Y. Y., Cheng, G., Liang, L., Gao, B., Ren, Y. P., ... & Wan, F. (2020). A ketogenic diet attenuates proliferation and stemness of glioma stem-like cells by altering metabolism resulting in increased ROS production. *International Journal of Oncology*, *56*(2), 606-617.

Jones, G. (2015). Why are cancer rates increasing?. *Cancer Res*.

Kalberer, J. T. (1975). Impact of the National Cancer Act on grant support. *Cancer research*, *35*(3), 473-481.

Kossoff, E. H., & Hartman, A. L. (2012). Ketogenic diets: new advances for metabolism-based therapies. *Current opinion in neurology*, 25(2), 173.

Lewerenz, J., & Maher, P. (2015). Chronic glutamate toxicity in neurodegenerative diseases—what is the evidence?. *Frontiers in neuroscience*, 9, 469.

Luengo, A., Gui, D. Y., & Vander Heiden, M. G. (2017). Targeting metabolism for cancer therapy. *Cell chemical biology*, *24*(9), 1161-1180.

Maalouf, M., Sullivan, P. G., Davis, L., Kim, D. Y., & Rho, J. M. (2007). Ketones

inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience*, 145(1), 256-264.

Miles, K. A., & Williams, R. (2008). Warburg revisited: imaging tumour blood flow and metabolism. *Cancer Imaging*, *8*(1), 81.

Neal, E. G., Chaffe, H., Schwartz, R. H., Lawson, M. S., Edwards, N., Fitzsimmons, G., & Cross, J. H. (2008). The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *The Lancet Neurology*, 7(6), 500-506.

National Cancer Institute [NCI]. (2015). "What is Cancer?". Retrieved from (https://www.cancer.gov/about-cancer/understanding/what-is-cancer#drivers-of-cancer).

Nebeling, L. C., Miraldi, F., Shurin, S. B., & Lerner, E. (1995). Effects of a ketogenic diet on tumor metabolism and nutritional status in pediatric oncology patients: two case reports. *Journal of the American College of Nutrition*, *14*(2), 202-208.

Pett, K. D., Willett, W. C., Vartiainen, E., & Katz, D. L. (2017). The seven countries study. *European heart journal*, *38*(42), 3119-3121.

Pogozelski, W., Arpaia, N., & Priore, S. (2005). The metabolic effects of low-carbohydrate diets and incorporation into a biochemistry course. *Biochemistry and Molecular Biology Education*, *33*(2), 91-100.

Seyfried, B. T. N., Kiebish, M., Marsh, J., & Mukherjee, P. (2009). Targeting energy metabolism in brain cancer through calorie restriction and the ketogenic

diet. *Journal of cancer research and therapeutics*, *5*(9), 7.

Seyfried, T. N., Shelton, L. M., & Mukherjee, P. (2010). Does the existing standard of care increase glioblastoma energy metabolism?. *The lancet oncology*, *11*(9), 811-813.

Taubes, G. (2001). The soft science of dietary fat.

Thiele, E. A. (2003). Assessing the efficacy of antiepileptic treatments: the ketogenic diet. *Epilepsia*, *44*, 26-29.

Teicher, B. A., Linehan, W. M., & Helman, L. J. (2012). Targeting cancer metabolism.

U.S. Department of Agriculture [USDA]. (2011). "A Brief History of USDA Food Guides." Retrieved from (https://www.choosemyplate.gov/eathealthy/brief-history-usda-food-guides).

VanItallie, T. B., & Nufert, T. H. (2003). Ketones: metabolism's ugly duckling. *Nutrition Reviews*, *61*(10), 327-341.

Viel, A., (2017). HarvardX: Principles of Biochemistry. Retrieved February 6, 2020, from (https://youtu.be/0ypJL27iIf8).

Warburg, O. (1956). On the origin of cancer cells. *Science*, *123*(3191), 309-314.

White, H., & Venkatesh, B. (2011). Clinical review: ketones and brain injury. *Critical Care*, *15*(2), 219.

Youdim, A. (2013). Overview of nutrition. Merck Manual online.

Zhou, W., Mukherjee, P., Kiebish, M. A., Markis, W. T., Mantis, J. G., & Seyfried, T. N. (2007). The calorically restricted ketogenic diet, an effective alternative

therapy for malignant brain cancer. *Nutrition & metabolism*, *4*(1), 5.

Zuccoli, G., Marcello, N., Pisanello, A., Servadei, F., Vaccaro, S., Mukherjee, P., & Seyfried, T. N. (2010). Metabolic management of glioblastoma multiforme using standard therapy together with a restricted ketogenic diet: Case Report. *Nutrition & metabolism*, *7*(1), 33.