# Dr. Dominic D'Agostino: Emerging Applications of Nutritional Ketosis

**Transcribed from a presentation delivered at the 2019 CrossFit Health Conference at Monona Terrace in Madison, Wisconsin, on July 31, 2019:**

Can you all hear me okay? Yeah? Okay.

So, just as a show of hands, how many people here follow a carbohydrate-restricted diet? Okay. And how many follow what you'd call a high-carb diet for your training or lifestyle, or whatever? Okay, so it's good to know. I didn't know coming into a CrossFit community because, you know, you hear about the athletes — How many don't care, yeah. That's good. That's a good point. Well, yeah, yeah —

Well, before I begin, I'd like to thank Greg and Karen for the invitation. It's great to be here, to be among a community that's very passionate about what they do. I teach at the medical college — medical students, and I want to kind of direct them towards this event next year, too. And the organizers too. I want to thank the organizers. Not all conferences have such great logistics and great people behind it. And I can tell the people behind it doing the logistics are very passionate about this event, and it really shows with the organization.

So, today I'm going to be talking about emerging applications of nutritional ketosis. And uh, and I'm gonna focus, you know, talk about the original application of the ketogenic diet, which was to control epilepsy. And before drugs sort of came on the scene, the ketogenic diet was really the standard of care for epilepsy. And then later on as drugs emerged, it then became used for drug refractory or drug-resistant epilepsy. And that led me down the path of using nutritional ketosis with the observation that fasting ketosis can manage seizures. I became interested in the ketogenic diet and ketone supplementation, which I'll be talking about, and using that for a very specific application, which is central nervous system oxygen toxicity seizures, which limits Navy SEAL safety and, and operational activities based upon the closed circuit rebreathers that they use. And in the process of studying nutritional ketosis for oxygen toxicity seizures, we had developed a number of technologies funded by the Department of Defense that allowed us to look at a range of different cell types — one of those cell types was the cancer cell type — and, and made some observations that led me down a path at looking at the Warburg effect and cancer as a metabolic disease, and that connected me with Tom Seaford back 10 — 10 years ago.

And it led to several students doing Ph.D. dissertations on the topic of cancer. So, I'm going to touch on, sort of, what we do from an operational perspective supporting the Navy, and also our work with NASA, and also touch upon — I know they seem very dissimilar — but touch upon our cancer research, too.

Before I begin, because I don't want to forget and I'd like to acknowledge the people who have supported the research that I'm going to show you today and even the idea of using nutritional ketosis, the ketogenic diet, and ketone supplementation: the Office of Naval Research, which is part of the Department of Defense. Really, they were generous enough to buy into my idea that nutritional ketosis could delay or mitigate oxygen toxicity seizures. So, it was about 15 years ago, and it took about a couple years to convince them to fund the work, so about 12 years ago, they started funding this work. And we also partner with NASA on various missions, extreme environment missions I'll talk about, NAVSEA, which is funding a ketogenic diet study at Duke University: diving research subjects on a ketogenic diet while they're exercising and doing cognitive function tasks, and we do EEG measurements, and we actually push them to the edge of a seizure. So, that's funded by NAVSEA. So, the animal work that I do in the lab actually is transitioning to human lab work and also operational stuff. And we have funding from DARPA for our next NASA project, and we have NIH funding with my collaborator looking at Alzheimer's disease, looking at ketone supplementation in various models of Alzheimer's.

So, we have industry support too, in some cases internal support from the University of South Florida, and we also partner with a lot of foundations, 501c3 foundations, for things like kabuki syndrome or glucose transporter type 1 deficiency syndrome, maybe things you haven't heard about. But they're diseases that are very responsive to the therapeutic effects of nutritional ketosis.

And my university asked that I do a disclaimer, disclosure, and my conflicts of interest. So, this information is not medical or nutrition advice. I'm not a registered dietician. I'm a Ph.D. scientists. The presentation includes data on ketone technologies that are patented, and those patents are held by the University of South Florida. And we have licensing partners that have commercialized some of the patents. Royalties from those commercialized products go back to the University of South Florida. I also get royalties, which I donate back to the university to fund some of the research that I'll be showing you today. And I'm also co-owner of a company, Ketone Technologies LLC. I don't have any ketone supplements. I don't have any supplements of my own, but the university has licensing partners. Our company does partner with NASA, and we do some other things, so we support education and also research activities.

So, before — when I got into this, before I started studying ketones, we needed to develop tools to understand the fundamental changes that occur in the brain and then the muscles of people, astronauts, and Navy SEAL war fighters that are in these extreme environments. So, environments where the oxygen partial pressure is high, or the total pressure of a gas is high or low.

So, we created a number of environmental chambers, and here's one of them — this was — my postdoctoral fellowship project was to create a hyperbaric atomic force microscopy. And an AFM actually has a scanning resolution of an electron microscope, but you can image living tissue with it. And you can get down into looking at proteins on the membranes and assess membrane lipid peroxidation. I also got another Department of Defense grant to put a confocal microscope in there that would allow us to look at — to measure simultaneously — membrane changes in response to graded levels of oxygen and pressure and simultaneously measure the production of oxygen free radicals as a function of oxygen levels in the cells and in tissue. And this allows us to understand fundamentally how these extreme environments affect cells, tissues, and organs. And we, we also have adapted these technologies for use inside a hyperbaric chamber, where we actually do physiological measurements, and we implant a radio telemetry device inside rats where we can measure EEG, diaphragmatic EMG, to look at respiration, an ECG to look at heart rate, things like heart rate variability, and we create an environment inside these environmental chambers that simulates military dive operations or an operational setting. And then we collect, in this case telemetry, 14 channels of data. And then what we're looking at is we're — we're administering a drug, or most of my work has been on ketones, and we want to enhance or delay as a countermeasure — to delay the deleterious effects of low oxygen or high oxygen that causes oxygen toxicity seizures.

And I started looking at and — and still do — we look at cells, and one of the cell types that I was looking at was a U-87 glioblastoma cell. And these were first originally derived from a 44-year-old brain tumor patient. So, I was looking at fibroblast, smooth-muscle cells, various types of primary neuronal cultures from the hippocampus and the cortex, and I observed that as you increase the level of oxygen over time, there was a proportional increase — this is time on the x-axis here and the level of superoxide production — and the slope of the line will indicate the level of superoxide production, which is the primary free radical produced by primarily the mitochondria in response to high levels of oxygen or stress or mitochondrial damage. And we saw that neurons, and basically metabolically active cells, have a pretty high slope, but these cancer cells here were really off the charts. And I didn't understand — I was looking at all the different cell types and going — looking at the data and saw that cancer cell mitochondria and the mitochondria are these punctate staining things here, these little balls. This is the nucleus of the cell, and the mitochondria were over-producing oxygen free radicals in the presence of high oxygen. And this was very — a very interesting phenomenon to me. And as the level of oxygen got higher and higher with hyperbaric oxygen, I saw the mitochondria starting to disappear, and then the cells would explode. So, I never saw neurons explode, or I never saw muscle cells explode in the presence of high oxygen, but I saw these cancer cells exploding under hyperbaric oxygen conditions. And I didn't know why it was happening, and that led me to looking at the, the

Warburg effect, which is essentially — and I'll talk about it in a little bit — damaged mitochondrial function or oxidative phosphorylation with gradual, sort of, compensatory fermentation, and the energetic processes are switched away from the mitochondria and more towards glycolytic effects. And I'll talk about that shortly.

So, we also observed that, and colleagues basically observed that, if you fast a rat for 24 or 36 hours, they could withstand the effects of high-pressure oxygen — up to five atmospheres of oxygen that typically produce a seizure in five minutes. They could go about 15 minutes or 20 minutes. So, this motivated us to develop various strategies to induce therapeutic ketosis. And I'll talk about the ketogenic diet and talk about ketone esters and also ketone salts and the different supplemental forms of ketones that are on the market.

So, the work that I did in cells and tissues and also in whole animal studies over the last couple years transition to work that I personally have done as a crew member in NASA extreme environment mission operations, where I lived underwater for 10 days in a hyperbaric environment, and I measured blood, urine and saliva, and tissues. I measured my skin microbiome, gut microbiome, all these things. We collected a lot of data on this mission, and it's fantastic because we can work with astronauts, and it helps NASA understand how living in these extreme environments can — how we can develop countermeasures for deep, deep space operations.

And we also have work that we're doing, as I mentioned, at Duke University, where we put subjects inside a hyperbaric chamber. We have them pedal a bike. We measure EEG, and you see a couple — you see all the lines coming in there? We have blood coming from the person directly to a mass spec, where we look at blood gases and blood metabolites, and it's a very advanced setup, and it allows us to really push people to the limits, and simultaneously, they are doing cognitive function tests and piloting, basically an airplane — a simulation. So, we look at their performance on various tests. And my wife is a cognitive neuroscientist, so she's very interested in behavioral studies. So, when I was on the NASA mission and she was a crew member in, in the last NASA NEEMO mission, mission 23, but on my mission she started experiments looking at cognitive function, using the NIH toolbox, like reaction time, decision-making, all these things. So, everything I'm showing here, from the cells to the, the mission, to the work at Duke — we're basically understanding what these extreme environments do at baseline, and what these extreme environments do to our safety and performance in the context of nutritional ketosis or therapeutic ketosis.

And that can be achieved with a diet, that can be achieved with intermittent fasting, or ketone supplementation. And we're sort of looking at all these different ways to

induce therapeutic ketosis. But I'm gonna take a little bit of a step back and talk about some of the work and some of the people that inspired me to go down this path. When I was trying to explain the observation that was made from some Navy research scientists demonstrating that fasting could prolong an animal's exposure to high-pressure oxygen and delay a seizure by up to 200 to 250%, before even I started studying this.

So, I connected early on when he was still alive, Dr. George Cahill at Harvard, and was asking him about this study here. Prior to 1967, and even now, people, some people think that glucose is the primary fuel — the only fuel — that the brain can use for energy. And so, prior to 1967, this was sort of in the textbooks. Work that George Cahill did on subjects that were fasted for 40 days, and these are primarily overweight obese subjects, demonstrated that brain energy metabolism changed significantly. And what the brain was using for fuel changed significantly.

So, after about a week to 10 days, as you'll see on the graph over here, brain energy metabolism is derived, primarily about 60% or more, from the ketone bodies: beta-hydroxybutyrate and acetoacetate. And these are elevated only after fairly extreme starvation — they called it starvation — or prolonged fasting. So, starvation would be a situation where their health was declining fast. These subjects appeared lucid. They did well in various psychological tests, and their blood measurements indicated that they're relatively healthy, even after 40 days of fasting. In an extension of the study that could never be approved by IRB today, they injected these fasted subjects with twenty IUs of insulin to push their blood glucose down into a range that I teach is basically fatal hypoglycemia. So, the — you know in some of the lectures that I teach to the first-year medical students, we teach about when your blood glucose gets down below — to about 30, you go into a coma, a little bit lower, you go into a seizure, and then you die. So these subjects experienced severe hypoglycemia in the range of one to two millimolar, so down to a level where the meter probably wouldn't even read it. Like, I've pushed my level down there and the meter just flashes low, so it doesn't even measure at these low levels.

And they had — no harmful effects were observed in this extreme hypoglycemia, because, presumably, the ketones were functioning to preserve brain energy metabolism and cognitive function. And so they had no symptoms of hypoglycemia, which was amazing. And, you know, I — I teach in my lecture and I always sort of add, you know, this study in there that in the context of a normal diet — yes, at this level of hypoglycemia — would be fatal if your ketone levels are not elevated.

So, to me, this was a really remarkable observation, that elevating your blood ketones could sort of make you bonk proof, and in the event that you go hypoglycemic from insulin shock or, you know, peripheral glucose utilization is

exceeding the capacity to maintain blood glucose levels, the ketones are sort of like insurance to prevent any hypoglycemic episodes. And I see this in my student — and I'll show you some of his data later. Andrew Koutnik, who's a Ph.D. student, soon to defend, he studies cancer cachexia, but he's Type 1 diabetic and uses low-carb and the ketogenic diet to manage his Type 1 diabetes. When I first started giving these talks I said, “If you have Type 1 diabetes, don't ever even think about carb restriction or the ketogenic diet,” so, I've changed my tune about that. There are now publication, authored by David Ludwig from Harvard, to show that carbohydrate-restricted diets may be optimal for those even with Type 1 diabetes.

So, a little bit about ketones in general. Ketones, we know, are energy substrates, and now more recently in the last four or five years, we come to understand them as a powerful signaling molecule that functions almost like a hormone. There's a ketone receptor. There are several ketone receptors. Ketones function as histone deacetylase inhibitors. Ketones function to suppress inflammatory pathways that — in a way that's completely independent of their metabolic function. So, they have important signaling properties. Clinical ketosis is defined as a blood level of ketones that's above 0.5 millimolar. So, that takes about — if you stopped eating now, If you're not on a ketogenic diet regular — if you stopped eating now, it takes about 24 hours for your blood ketones to really get up into the clinical ketosis range. So, you could achieve it with fasting, and if you're low carb you would get into ketosis faster, if you were, if you're doing intermittent fasting. Some people do urine ketones. That's okay. It's not optimal, but a urine ketone level of 15 milligrams per deciliter on the ketostix that you can buy at any CVS or Walgreens, will correlate to about 0.5-mmol ketone level.

Nutritional ketosis, I define it as a dietary strategy to elevate ketones into, into this range, and that typically involves a carbohydrate consumption — fibrous carbohydrates, non-starch, non-sugar — of less than 10% of the total diet. And generally speaking, 25-50 grams of carbohydrates — that is the level where you start to get into nutritional ketosis and start to get some of the benefits of nutritional ketosis.

Ketoacidosis — and this comes up a lot with the medical students, or if I teach at a clinical conference. All the hands go up, “What about ketoacidosis?” — Ketoacidosis is a very specific metabolic derangement associated primarily with the absence or insufficient amount of insulin in the body. So, without insulin, when our ketones level rise, that actually releases a small amount of insulin, which helps your body fine-tune ketogenesis in the liver. So, without insulin we have runaway ketogenesis. And in the context of high glucose, high ketones can be very detrimental to health and create an acidotic situation that, if it's not corrected with fluid electrolytes and insulin injection, will lead to a coma and death. So, you know — So, even — I was

gonna say, even people with Type 1 diabetes can successfully do the ketogenic diet if they manage their insulin correctly.

Keto adaptation is a time-dependent physiological adaptation to using fatty acids and ketones for energy. So, we know that the more you do the ketogenic diet, or carbohydrate restriction, the more you adapt, the easier it gets and the more benefits you derive from it, from those adaptations. So, part of what we're doing in a lab is actually measuring those adaptations, looking at the gene transcription, looking at the presence of the monocarboxylic acid transporters, which are upregulated about 50% after about two weeks in rodent models after being in nutritional ketosis. The glucose transporters are also changing, and it seems to be a tissue-specific changes in the brain and the muscle, and we haven't reported on that yet, but we're putting that together for publication. And the last thing I want to highlight is exogenous ketones. Because this term did really not exist years ago, like 10 years ago, and now they're on the market. I get a lot of questions about it, and there are a lot of research — a lot of research being done. I think there's at least a half-dozen registered clinical trials on clinicaltrials.gov right now, looking at exogenous ketones. And there are many forms of exogenous ketones. There are electrolyte mineral salts. There are at least three to four different types of ketones esters. There's a monoester, a diester, and a triester. And so, there's various forms, and we are investigating how these different forms — the different applications of these forms and how they can be formulated and what therapeutic applications they may have.

So, there are different strategies that one can use to get into a state of nutritional therapeutic ketosis. There is starvation — that's not fun — but there's caloric restriction, there's intermittent fasting, there's the ketogenic diet, which is a specific macronutrient ratio. The classical ketogenic diet, the four to one, was about 87% fat. So, now we know — I'm just coming from an epilepsy conference — that the modified ketogenic diet, which is what, what you see here, or at least the ratio is about 75% fat, 20% protein and a minimal amount of carbohydrates, like 5%, seems to have similar seizure protection in adults and maybe in pedi — in some pediatric populations too, depending on the disorder. So, these things — basically, starvation, and the ketogenic diet, and intermittent fasting — mobilize fats. The fats go to the liver, and it stimulates beta-oxidation of fats with the continuous suppression of insulin, so, only in the context of mildly depleted liver glycogen and continual suppression of the hormone insulin, and blood glucose drops a little bit too.

But the homeostatic mechanisms that maintain blood glucose are very powerful. So, blood glucose really does not bottom out. It might stay in the, the 70 to 80 milligram per deciliter range, even with no carbohydrate consumption for many people. So, these ketone bodies are produced: beta-hydroxybutyrate, acetoacetate.

Acetoacetate can spontaneously decarboxylate to acetone. Interestingly, acetone has some interesting functions on various ion channels, which can affect brain function. And this is difficult for patients to achieve, so we developed various forms of exogenous ketones and also acknowledged that fats — ketogenic fats, like medium-chain triglycerides, which go directly to the liver by a hepatic portal circulation and are oxidized very fast to ketone bodies — they're not packaged into chylomicrons like the long-chain fats. And then ketone salts and ketone esters are metabolized partially in the liver but mostly spill into the blood and then rapidly elevate ketone levels. Like a ketone ester, if a person consumed it right now on stage, by the time I get off stage it'll look like I fasted for 10 days. So, you can achieve a level of ketosis in the five to six mmol range, go from zero to five to six within 30 minutes.

So, there are very powerful agents that we're studying, but as the science sort of advanced, we understand these molecules not only as energetic substrates for the brain, the heart, and even skeletal muscle, but they have powerful signaling properties in the brain and in other tissues. So, I'm not going to go through all the signaling pathways here, but you can see in this panel below, that there are many different signaling pathways that are impacted by nutritional ketosis.

So, changing our metabolic physiology significantly changes brain energy metabolism and also the neuropharmacology of the brain. So, one of the studies that we did in Angelman syndrome, we take brain tissue out, and we look at all the different neurotransmitters, and the ratio of glutamate to gaba changes significantly. It activates glutamic acid decarboxylase, which makes a very — an excitatory amino acid transporter, an inhibitory or brain stabilizing neurotransmitter known as gaba. So, gaba also has anti-seizure effects. So, it's one of a dozen different mechanisms, and that's what's really fascinating about the ketogenic diet. It doesn't just work through one mechanism. It works through many different mechanisms in synergy. And that's why there are no drugs that really can mimic the ketogenic diet, because it works through so many different mechanisms.

So I've given talks at pharmaceutical companies where they want me to give a talk on the mechanism of the ketogenic diet so they can sort of reverse-engineer some drug to target a mechanism. And when I throw up, you know, that panel there that shows all the mechanisms, I think they get depressed, because they realize a drug's really not possible. There is a compound called 2-Deoxy-D-glucose, and it inhibits — by inhibiting glycolysis, you can stimulate some of these pathways, and now there is a clinical trial as a ketogenic diet in a drug. So, it's basically a glucose antagonist. So, that — there are, and we are doing cancer research with that compound too.

So, here's the biogenic or bioenergetic effects of ketones. And in this case, it's just the brain. So, the liver is the site of ketone production. So, under situations of

suppressed glucose gluconeogenesis in the liver and also suppressed insulin signaling and mild glycogen depletion, the liver produces these ketone bodies as acetyl-CoA from beta-oxidation of fatty acids accumulates. And the acetyl-CoA condenses together to form acetoacetate and then beta-hydroxybutyrate. Beta-hydroxybutyrate is the primary ketone body in circulation because it's more resistant to degradation, whereas acetoacetate can be spontaneously decarboxylated. But beta-hydroxybutyrate needs to be broken down to acetoacetate to be used as fuel, so keep that in mind. But acetoacetate is essentially the oxidized form of beta-hydroxybutyrate.

Not to get too, like, technical here, but I just want to highlight that, that in the context of — so the point is the liver makes ketones but does not use ketones as an energy source. It lacks succinylcholine transferase, and that's very interesting because cancer cells also lack this enzyme. Many — most cancer cells lack this enzyme that allows the cancer cells to use the ketone bodies for fuel. So, the liver lacks — succinylcholine transferase, and that's sort of like an evolutionary, sort of, advantage, because the liver is a very greedy organ. If you're hungry and you eat something, the liver will take what it wants — amino acids and glucose and things — and then, you know, put back into circulation, you know, what you can use. But the liver is very greedy. But in the case of ketones, the liver doesn't really use ketones as fuel, it produces it.

So, these ketone bodies spill into circulation, and they bypass many of the regulatory steps associated with the glucose being able to support bioenergetic functions. So, in the case of impaired glut1 glucose transporter 1 deficiency or glut3 deficiency — that's the transporter on the membrane of neurons — in the case of those being deficient or not functioning, or in the case of Alzheimer's disease or traumatic brain injury, the glut3 transporter gets internalized inside the cell, and then the glucose can't get into the cell. But ketones bypass that process. It even bypasses pyruvate dehydrogenase complex. So, if you have p — pyruvate dehydrogenase complex deficiency syndrome, it is treated with the ketogenic diet. So, for kids that have this, they need to be on the ketogenic diet. PDH is also impaired in Alzheimer's disease and also traumatic brain injury.

So then, by virtue of bypassing these regulatory sites, ketones can preserve brain energy metabolism. And that's a very important thing for a number of different disease states that we study, including glut1 deficiency. We're also looking at — yeah a number of different neuro-metabolic disease states where the ketogenic diet is very therapeutic. So, in Alzheimer's disease — so we know Alzheimer's disease is characterized as glucose hypometabolism. And the hallmark characteristic of Alzheimer's disease is a decrease in intensity of a fluorodeoxyglucose PET scan. So, there's less of an intensity showing radioactive glucose present in the liver. There's less uptake of glucose.

It's also interesting to observe that a number of studies showing that cerebral blood flow increases about 30% during acute hyperketonemia. So, even in a very short order, if I consume ketones right here within about 15 to 20 minutes, you can measure with Doppler blood flow measurements that blood flow is increasing in the brain. In some people, that actually gives them a headache. If they consume a large dose of like a ketone ester, it's increasing blood flow too much.

So, with that kind of metabolic effect on the brain and some of the things — signaling things I was talking about, you can imagine that there's emerging applications of the ketogenic diet. And I saw this early on, that when I started studying this it was really only pediatric epilepsy, but I just saw, you know, so many different opportunities, I — at the time that I started talking with George Cahill and Richard Veatch and Theodore Van Italie — all the icons, people I consider icons — they encouraged me to go down this path, and I was a young untenured professor, and I realized that, you know, I was gonna really delve into this to make this my career.

So, so this — these are the applications for ke — and I didn't even — there are many applications that I didn't even put on here, like the ketogenic diet for acne, or polycystic ovary syndrome, or we study various psychological effects of ketones too. And we're getting into that.

So, I am just going to focus primarily on cancer and some of the work that we do in an operational setting from the NASA NEEMO missions, but just look at the emerging applications. And on the left here are really things that have strong evidence in the literature, and weight loss, and weight management, Type 2 diabetes — I think we can say there's strong evidence for that — obviously, inborn errors of metabolism. The last — prior to coming here, I was in Chicago meeting with the doctors who actually give ketones intravenously through — with all these different neuro-metabolic disorders, and they can bring children to life by giving them ketones when they have specific metabolic disorders. And things like Lennox-Gastaut syndrome — it's been used for decades for that disorder and epilepsy. And you know, I have it in the emerging applications, but I think Type 1 diabetes, too, there's emerging data from people out there using it, like the group TypeOneGrit on Facebook. My student, my Ph.D. student is part of that group, and it was — there was a publication that essentially resulted from, from that group. So, there's more data emerging.

And in cancer, 10 years ago, I think there was one or two studies on clinicaltrials.gov, and now I look this week, there's over 30 clinical trials on using the ketogenic diet in cancer studies. So, this is a very emerging field, and I think you're

gonna see with the clinical trials now, a lot more studies, results from those studies hitting PubMed.

So, it was observations that we made in the cell types that we studied under hyperbaric oxygen therapy, and also with supplemental ketones, we observed that ketones decrease proliferation in these cancer cell types. That led me down a path — I wasn't supposed to be studying it. I was supposed to be studying oxygen toxicity seizures, because we had a contract with the Navy, and I was full-time on that contract — but I was obsessed with these observations that we made in cancer cells. And the only thing that really explained the observations that we saw, especially the damaged mitochondria and the overproduction of oxygen free radicals as we increased oxygen concentration, and no one had saw that before, because no one has a microscope inside a hyperbaric chamber. So, these are some novel observations and I needed to explain them.

And it led me — it connected me with several people, including Tom Seyfried at Boston College. And I read his review shortly after connecting with him, “Cancer as a Metabolic Disease,” which he published in *Nutrition and Metabolism*. And then he's got a book by the same name, *Cancer as a Metabolic Disease.* I have published at least seven articles or studies with Tom Seyfried, and we work together in different capacities. But he explained to me the Warburg effect — which I had taken cancer biology in college and I had never heard of it before — that cancer mitochondria or cancer metabolism is fundamentally different from metabolism of healthy cells.

So, essentially the Warburg effect in one sentence is damaged mitochondrial respiration, and there's compensatory fermentation. So, the basic energy processes that allow a cell to maintain its bioenergetic potential would be oxidative phosphorylation. The mitochondria is making about 88 to 90% of the ATP, the energy currency in the cell, in neurons, and heart, and skeletal muscle too. And as the — as a person or the cells are exposed to a number of different agents — they could be chemicals, radiation, inflammation, hypoxia, insulin resistance, and hyperglycemia — produces a very ripe, ripe environment for the mitochondria to be damaged and the mitochondria DNA to be damaged.

The nucleus has very robust DNA repair mechanisms. The mitochondria does not have as robust DNA repair mechanisms. So, if a cell is bombarded with things like radiation or carcinogenic agents, the capacity for the mitochondria to repair itself is not as, as high as — not as robust as the, the nucleus ability to repair DNA. So, the mitochondria take a big hit, and as mitochondrial function is impaired by progressive damage to environmental agents, viruses, for example, can cause cancer, and the viruses that cause cancer impair mitochondrial function. So, mitochondrial function goes down, cellular ATP levels go down, and the nucleus of the cell essentially can sense the bioenergetic potential of the cell — can sense the

ATP levels, and it senses that the cells in an energetic crisis. And when it's in — when it gets to this threshold, I would say, you know when you — and every cell is different, every person is different. I mean, there's a lot of variables here, but there becomes a threshold where progressive damage to mitochondrial function causes a cascade of events to activate a number of genomic pathways that stimulate the cell to increase glycolysis. And various oncogenes are associated with increased glucose metabolism.

So, a normal cell then transforms — and understanding, too, that embryonic cells that are proliferating and growing fast also have a glycolytic phenotype, but normal cells that are not proliferating primarily get their energy from mitochondrial oxidative phosphorylation. When the mitochondria are damaged by a number of different agents, I believe that hyperglycemia, hyperinsulinemia, mitochondrial syndrome is a major driver for this mitochondrial damage. When the cell transitions from an oxidative phosphorylation energy pathway to a more glycolytic pathway through mitochondrial damage, then there's a point of no return. It transitions from a normal cell to a healthy cell. And it's — it's debated, but it's not fully understood if a tumor cell can transition back to a healthy cell. Generally, we don't believe that that can happen. Maybe in some cases it can, but when a normal cell — when it's activated and an aquagenic program and drivers are kicked on, it becomes a tumor cell. And there are a number of factors that can drive the Warburg effect and actually make that tumor and expanding biomass to a large solid tumor. And drivers of the Warburg effect can kick on invasiveness and metastasis where those tumor cells get in circulation and then metastasize, and then it becomes sort of an irreversible process.

So, these are the drivers of the Warburg effect, and maybe, I guess, Tom Seyfried would say the initiators of the Warburg effect. So, the — the metabolic theory of cancer sort of posits that it's the initial damage to the mitochondria that's the enabling factor that essentially transitions a normal cell to a cancer cell. There are genes involved, no doubt, but the metabolic control of those genes is likely sort of the root cause. And now the geneticists, you know, in years past it was really just linked to genetic alterations, but now we have an appreciation and an understanding, and NIH directed research to understand how metabolism is directing those gene pathways to actually initiate carcinogenesis and the factors associated with cancer progression.

So, damaged mitochondria — I believe in the theory of — metabolic theory of cancer — is the initial cause and also a major driver. There's a derangement of tumor metabolism. Tumor hypoxia — as a tumor expands, as the biomass expands, the core of that tumor becomes hypoxic, damages the mitochondria more, there's more genetic mutations and that — the, the inside of the tumor takes on a more aggressive Warburg phenotype. So, it's literally fermenting sugar, you know, as it

grows. And really, people with advanced tumors, if they look at the actual tumor, the mitochondria are deficient — are structurally and biochemically abnormal. And according to Tom and some of the colleagues that I've connected with, when it comes to aggressive tumors, they have never found a tumor that has what we would call “normal mitochondria.” So, really the medic — damaged mitochondria are really a major driver of cancer. If your mitochondria are healthy, they call the shots. Healthy mitochondria will keep a high bioenergetic state of the cell. High ATP levels will enhance the fidelity of the nuclear genome such that DNA repair processes will happen and preserve that genomic stability.

So, that's a really important point, I think, that Tom Seyfried tries to make, is that the ultimate tumor suppressors are healthy mitochondria. And there are different ways — exercise, CrossFit, ketogenic diet, low-carb nutrition, intermittent fasting — all these things we know — periodic caloric restriction — enhance mitochondrial function. Things like elevated insulin, glucose, lactate, increased PI3K/AKT/mTOR pathway, is a major driver for cancer. There are drugs being developed that target this pathway, by Lou Cantley, for example, one of our collaborators. And these drugs, interestingly, do not work in the context of a normal diet. They need to be used in the context of a diet that suppresses insulin signaling. So, the ketogenic diet dramatically enhances the effect of these metabolically targeted drugs, the PI3 kinase inhibitors.

Elevated ROS and inflammation — so, reactive oxygen species — overproduction kicks on inflammatory pathways, which can damage the mitochondria and really stimulate this, you know, carcinogenesis. Suppressed anti-tumor immunity. So, as the tumor pumps out lactate and, and lowers the pH, that actually changes the micro environment to prevent your body from recognizing that you have a tumor. And the ketogenic diet increases cancer-associated immunity. So, it helps increase the vigilance of your immune system to recognize cancer and to attack it through a number of mechanisms. And my colleague, Adrienne Scheck at, formerly at Barrow Neurological Institute, has studied that and published on that.

So, in getting back to how we can change — this idea of changing metabolic physiology to not only change brain energy metabolism but to target the Warburg effect, and there are — there's a seminal paper called “The Hallmarks of Cancer,” and there are particular — There's eight things that sort of characterize the hallmarks of cancer, and I'm going to talk about that in the next slide, but keep that in mind as I go through the things that are associated with the ketogenic diet.

So, we know that — about — when we give exogenous ketones, we can lower blood glucose by 50%. There was also a paper, an exercise science study, showing that the ketone ester beta-hydroxybutyrate monoester can cause a 50% reduction in lactate. We know that glucose and lactate are major drivers for cancer growth and

proliferation. We know that there's a reduced proliferation and glycolytic ATP levels in the presence of ketones. Actually, Dr. Feinman here published an article showing that acetoacetate, one of the ketone bodies, could decrease — reduce proliferation and reduce glycolytic ATP production. And there is a reduction in glutamine, c-Myc, and various pathways associated with cancer cachexia. So, ketones directly affect a number of pathways associated with muscle wasting, and that's — that's the project of my Ph.D. student Andrew Koutnik is really studying, sarcopenia and cancer cachexia.

So — and this is a diet. So, this is a diet, and this is administration of a metabolite that your body makes is really changing these pathways. There's a reduction in proliferation and metastasis, and a reduction in a number of different inflammatory pathways and drivers, enhanced — as I mentioned my colleague Adrienne Scheck, looked at enhanced tumor-associated immunity. And the ketogenic diet caused a dramatic increase in the body's ability to recognize cancer. And this is, this is an animal model, that particular study, but they're studying it in humans now. And it also has an anti-apoptotic effect, and anti-angiogenic and a pro-apoptotic effect. So, that's the ketogenic diet, especially in the context of caloric restriction or periodic caloric restriction, kicks on pathways, again, that associated with the body's ability to recognize and attack cancer.

And some of the to — the more interesting things have emerged in the last five years is this — the effect of ketone bodies working through epigenetic mechanisms. So, there's epigenetic enhancement of cellular defenses that are associated with preserving healthy mitochondria and perhaps extending longevity or healthspan over time. And — and there was a study that I was involved in, it was actually published in *Nature Medicine*, looking at the effect of beta-hydroxybutyrate on suppressing an inflammatory pathway associated with driving cancer growth. And that's the NLRP3 inflammasome. So, when that — when that protein complex gets activated, it kicks on and elevates many different inflammatory cytokines, including things like IL-6 and IL-1β. So, ketone body, specifically without metabolic regulation, specifically regulates and suppresses that, that pathway. So, again, you can achieve this physiological state through time-restricted eating, ketogenic diet — the food that makes up the ketogenic diet is very important.

In years past, epilepsy clinics would give hydrogenated fats in the form of a supplement called keto cow, and just, you know, it was just not — they would give Crisco. So, now there's a much greater appreciation for the types of fats and how these diets are formulated. It's also acknowledged that the ketogenic diet does not have to be as strict — 90% fat — as we once thought. So, modified forms of the ketogenic diet, actually a form that I follow which is about 75% fat, is being used clinically now. And there's a number of drugs that I mentioned before, mimic many aspects of nutritional ketosis. And that would be 2-Deoxy-D-glucose, perhaps

3-bromopyruvate — the jury's kind of still out on the utility of that — drugs like lonidamine that inhibit hexokinase 2, metformin has a mild calorie restriction sort of effect — metabolic effect. And we're studying that, too, and we published some research on that.

So, we also published a paper with our colleagues in neuro-oncology at Moffitt Cancer Center. And the sort of the gist of this review — this publication — was that the ketogenic diet could target the Warburg effect, and then that it could be a very powerful, at least an adjuvant to the current standard of care for high-grade glioma. And we're focusing on low-grade glioma, probably only because the — the glioblastoma patients are taken up by the pharmaceutical companies, because they want to use their drug. So, the only patients that really could — we could apply to do an NIH-sponsored research would be low-grade glioma patients. That also gives us a bigger window to assess the efficacy of this therapy, because with high-grade glioma, you know, you got about a year. But with low-grade glioma, you have more time to work with the patient and to work out the kinks of the diet and maintaining that nutritional ketosis.

So — So, the — the message that we wanted to convey in this review, which was, you know, co-authored with just conventional neuro-oncologists and people who really didn't even study the ketogenic diet, but our research and some of those studies that have been out there convinced them that this was a very viable approach, especially for brain tumor patients that have epilepsy. They have seizures, too, so they have two things that the ketogenic diet can work very effective for. They have seizures caused by the brain tumor and a rapidly expanding, highly glycolytic tumor. So, it just makes sense that brain tumor patients — not all cancers are as responsive to ketogenic diet therapies. There are cancers like liquid cancers, like leukemia, lymphoma, testicular cancer — these things are very responsive to the standard of care. Not that they — you shouldn't use the ketogenic diet, but they should consider the standard care the frontline approach. But anyway, with high-grade glioma, there is no effective therapy. So, we believe that a metabolic-based therapy should be the frontline approach and only under certain situations really the standard of care be used.

But the slide that I showed you previously really went through all the different mechanisms and clearly demonstrates by published literature — some of it is animal model studies, some of it some of its inhuman — that nutritional ketosis or therapeutic ketosis targets all the hallmarks of cancer. So, when a drug company is developing a drug to target cancer, they're usually targeting, you know, invasiveness or angiogenesis. They're really looking to target a very specific thing, like Avastin, for example. Avastin just targets angiogenesis. The ketogenic diet, especially if it's calorie-restricted, targets all these things. So, that's an important thing.

And the last sort of slide that I have as I'm coming to the end is this sort of scenario — and this is research that my student who published in *Cell — The Cell Journal*, or review, on the anti-catabolic effects of ketone bodies. Andrew Koutnik, who's Type 1 diabetic, uses nutritional ketosis to manage his Type 1 diabetes, has — did this review and is studying the effects of ketones in the lab and a number of different animal model systems, animal models of cancer cachexia, but also using something called lipopolysaccharide, or LPS, where we give LPS, create massive systemic inflammation, and then we try to mitigate that with a number of things including ketone bodies using a ketone ester. So, ketone bodies, we know, decrease nitrogen excretion. It reduces alanine mobilization. So, the major gluconeogenic amino acid would be alanine. So, alanine goes down quite remarkably if you infuse ketones. And that was a human study where they IV-infused ketones and showed alanine goes down significantly.

And one of the most interesting effects of exogenous ketones is that it caused a dramatic effect at lowering your blood glucose levels. So, in animal models, in — in rats for example that have a blood glucose of a 120 to 150, if you give ketone esters to their maximum tolerable dose, the blood glucose goes down to 30 or 40 milligrams per deciliter. That's not changing their diet. That's just a ketone-induced lowering of blood glucose. So, this has not been published in humans yet, this level — this dose level. I mean, I've studied it my — I've consumed it myself and can show and can demonstrate that, you know, you — I can get glucose levels down to the range where it just doesn't even read on the meter with certain ketone esters. So, I think that's an important therapeutic application, and I know people are studying this now, looking at insulin resistance and diabetes. But it stimulates muscle protein synthesis and increases muscle regeneration and proliferation — we know acetoacetate does. The ketogenic diet has been reported to have anti-cachexic effects at reducing pancreatic, colon, lung cancer, and skeletal muscle catabolism in rodent models.

So, we're interested in partnering with the Moffitt Cancer Center to get a clinical trial in humans. Putting patients on the ketogenic diet can be difficult. So, they're very interested in supplementation. I am interested in both, the ketogenic diet and then supplements essentially as an adjuvant to the ketogenic diet. But they're kind of resistant against using the ketogenic diet.

So, for future directions, what we're really — kind of this idea that we've talked about, and Tom Seyfried, my colleague — we've written and co-authored a review on this, talking about this idea of a press/pulse approach, where a press therapeutic, you know, program would be a daily routine of maintaining a therapeutic ketosis range of — and Tom Seyfried uses the glucose ketone index. If your glucose level is three mmol and your ketone level is three mmol, you would

have a glucose ketone index of one. If your ketones were — if your glucose was four and your ketones were two, you would have a glucose ketone index of two. We feel that keeping into that one to two range, if you look at all the animal model studies, especially for seizures, it's extremely therapeutic. And it knocks down — it hits all those pathways that I just showed you that target cancer metabolism. The drug metformin conceivably could be used continuously. I think Tom Seyfried's a little bit resistant against metformin. It may have some side effects, but exercise, meditation, these things can help you get an ideal glucose ketone index, which we know, we talked about the metabolic zone. So, you bring glucose down to the level of ketones and ketones up. If you stay within that zone, we know, experimentally in animal models, and I think the human data will show this and some of it points in that direction, that you are at the very least slowing — taking the foot off the gas pedal of cancer growth for the cancers that are responsive to — that have the Warburg effect or have a Warburg phenotype, we say.

But that sets the stage for other — other treatment options to be used. And our idea is to use them in an on and off fashion: three weeks on, three weeks off. The stand — I am a proponent of standard of care: chemo, radiation, and immune therapy. I think for many cancers these can be highly effective and well-tolerated. I've communicated with enough patients that they get a much better response if they're on the ketogenic diet and — and the side effects are much less, too, if they're on the ketogenic diet. Hyperbaric oxygen therapy — we're just in animal model studies now, but it's like the studies that I showed you — what I was doing 10 years ago when I was looking at brain tumors, and the cells and the mitochondria were exploding, and the normal healthy brain cells were not. I mean, that was sort of very convincing to me, that high-pressure oxygen was far more toxic to cancer cells than they were to normal healthy cells. And we published that observation in *Neuroscience* but really didn't package it as an anti-cancer effect. It was just like an interesting observation.

IV vitamin C, and David Diamond turned me onto this, that vitamin C is a glucose antagonist. Vitamin C at high levels and mmol concentration can be a pro-oxidant and can actually drive redox chemistry. It can drive the Haber-Weiss reaction and Fenton chemistry and redox biochemistry in a way that actually stimulates reactive oxygen species production and oxidative stress. So, it could be used as a pro-oxidant therapy with or without hyperbaric oxygen therapy, but I think it would work better with hyperbaric oxygen therapy and a whole toolbox of metabolic drugs.

So, we're doing these studies now in preclinical animal models, and we're looking at breast cancer, brain tumors, and lung cancer. We're looking at a variety of different things. So, here are ongoing and future projects. We have oxygen toxicity studies in rats and in humans. We actually have a registered clinical trial — it's being

conducted at Duke —, preclinical animal model work looking at press-post therapies, preclinical cancer cachexia studies. The low-grade glioma clinical trial that I talked about is a future goal. So, we're repackaging an R01 NIH proposal and hope to get that — that funded. We have — we're studying a syndrome called kabuki syndrome, which is a rare genetic disorder, and in the context of kabuki syndrome, we believe that beta-hydroxybutyrate is an epigenetic therapy. So, it's actually stimulating acetylation, and it's modifying the histones' ability to activate various DNA components to therapeutically treat kabuki syndrome. And this was a — we got the mouse model from Johns Hopkins, and we have some encouraging studies going on now. We have a clinical trial with Angelman syndrome with Vanderbilt. And I didn't have much time to talk about it today, but the NASA Extreme Environment Mission Operations is sort of the big project that I'm sort of moving towards and allocating more effort into, basically, moving our research — our laboratory research out into an operational setting where we look at the gut microbiome, we look at strength, we look at body composition, we do metabolic measurements, psychological measurements, we look at sleep, heart rate variability, stress, all these different things. So, I'm really excited about that. We recently got DARPA funding for the next mission to study that.

And I really have to give a big thank — I mean I — I'm here presenting it, but it's really the students, the post-docs and the research associates that are in the trench doing the work, allowing me to be here today. And that includes a long list of growing people, including Kristi, who's here today in the audience. She helps out in the lab, many different projects, and helps to maintain our website, too. And my wife, she has another lab in psychology, and she's not at the College of Medicine anymore, but she's on main campus and she's actually, she has resulted — She has done a lot of publications over the last couple — couple days. I think more than half of my publications over the last year are primarily a result of her efforts. I have all the different references here to point to different — the research, the science that I talked about. A number of different books I want to highlight: *Tripping Over the Truth*, Tom Seyfried's book *Cancer as a Metabolic Disease*. It's really heavy reading, so if you want a little bit lighter reading with a narrative — if you really want the science, *Cancer as a Metabolic Disease* is an amazing book that really is a tremendous effort. I mean, Tom put is heart and soul into that book and it really encompasses a lot of cancer biology and cancer metabolism. *Tripping over the Truth*, I had the honor of writing the foreword to that book and that — Travis Christofferson. He has another book coming out in the next month called *Curable*. So, start looking for his other book coming out.

And my last slide, I'd just like to thank everyone for your attention. I know it's the last talk of the day. And like to thank my assistant, Kristi, for maintaining KetoNutrition.org. It's a website where a lot of the stuff that we do is there. She maintains a blog. We have nutrition consultations, and we blog about a lot of

different subjects, different publications that we like to — to basically take recent articles and package them in more layman's terms, to make it accessible to the public. So, they have more information, easy-to-digest information about nutritional ketosis. And thank you for your attention.