DANNY LENNON: Dr. Dominic Dagostino welcome to the show and thank you so much for joining us today.

DOMINIC DAGOSTINO: Thanks for having me Danny. I appreciate it.

DANNY LENNON: It's my absolute pleasure and as I was kind of telling to you offline, I am eager to jump straight into things here because there's so much that I could potentially ask you because I have found your work and the work of your colleagues and related labs to be so fascinating. It is really something that's consistently putting out some really high quality stuff, so I am eager to get into some of that. There's probably two main topics that are of most interest to me and the first one is looking at a lot of your work that is related to cancer treatment, so ketogenic diets, nutritional ketosis and the role of that and other adjunct therapies in treating cancer. Maybe just before we get into the specifics of the research, just to start off, let's talk about why ketosis maybe a useful metabolic state for cancer. What is it about that state of ketosis that is a reason why it first was looked at in the research and why we are potentially seeing it could be useful in some forms of cancer?

DOMINIC DAGOSTINO: Yeah, I think there's quite a few reasons. You might know kind of like the general picture of cancer is that it's a genetic disease and I always thought that too. Actually I didn't know a whole lot about cancer until I

made some observations. I never really had an intention to study cancer but based on some of the observations I wanted to explain them. So I just started going to the experts to explain it, and it gave me a hunch that there was a metabolic component to this. That kind of set off my cancer research program that I have now. About 50% of what we do I would estimate is cancer biology, cancer research.

In regards to nutritional ketosis or the ketogenic diet, there are many avenues through which it could be impacting tumor growth and proliferation and overall survival and we are looking into sort of all these things. We generally like to test things instead of the bottoms up approach which is kind of a big push by sort of agencies like National Institutes of Health and stuff that mechanistically go after things and then develop the therapy. We sort of know things already work, so we test a lot of things from a top-down approach and that would involve dietary energy restriction to intermittent fasting or periodic feeding, ketogenic diet, ketone supplementation, even hyperbaric oxygen treatment and various drugs that are dietary energy mimetics we would say, like common things like Metformin and maybe not so common things like Lonidamine which is like a Hexokinase II Inhibitor.

The overall goal here is kind of to put metabolic stress on the cancer and in addition to that do a pulse protocol that would periodically subject cancer to intense metabolic stress and also oxidative stress. And you could do that with various drugs and even hyperbaric oxygen, and we call that the Press Pulse Protocol and it's adapted from kind of an evolutionary background, ecologists use it for example manage pests on the environment, on a farm or something like that. The Press Pulse Protocol, the ketogenic diet or intermittent fasting or dietary restriction, even the low dose Metformin basically is putting metabolic stress on glycolytic dependent tumor cells. You could argue about 80 to 90% of tumors register very hot on a PET scan and they are very prominent overt phenotype which means they consume excess glucose relative to the healthy tissue surrounding that and that can give some information. It's very useful information for the location and the aggressiveness of

the tumor and oncologists have been using PET for many years to identify the location and aggressiveness of tumor.

We actually use that information to target the cancer cells. We think it's useful. Up until now, oncologists really haven't been doing that and now they are looking into it. The ketogenic diet is a number of things, especially when it's calorie restricted, you have a very sharp decrease insulin levels. A lot of tumor growth is driven by insulin pathway and to where Akt and kinase IGF-1, especially IGF-1, these things through even VEGF and HIF-1-alpha, there's many different things that are driven to drive the hypermetabolic state of these glycolytic dependent tumors that show up hot on a PET scan.

A ketogenic diet first and foremost will lower insulin levels and that will essentially mobilize fatty acids from adipose which are metabolized through beta oxidation in the liver to ketone bodies, beta hydroxybutyrate and acetylacetonate and there's also a lowering of blood glucose typically. Sometimes baseline glucose does not change much but when you are following ketogenic diet, the spikes that you typically get through periodic feeding for example with carbohydrate feeding, will spike glucose and insulin. Those are essentially abolished or significantly attenuated with ketogenic diet – feeding, if the diet is formulated correctly. You have suppression of insulin signaling, suppression of glucose availability through dietary energy restriction, hepatic glycogen depletion and then that transitions the body from a glucose carbohydrate metabolism to a fatty acid and ketone metabolism. Generally, uncomfortable for many people to do this because your brain will crave glucose, so it goes through essentially glucose withdrawal, and then you have some physiological manifestations from that, which could be even things like rapid heart rate, tachycardia, stress response, even elevation of cortisol that happens initially.

But through sustained adherence to ketogenic diet the body will start to produce ketone bodies beta hydroxybutyrate and acetylacetonate and they readily cross the blood-brain barrier and start to compensate

for glucose deficiency in the brain and your brain has remarkable metabolic flexibility and can adapt to using ketones as an energy source. It does not use fatty acids very efficiently. Maybe short chain and medium chain fatty acids can cross the blood-brain barrier, but long chain fatty acids generally do not cross the blood-brain barrier. The liver will convert them into these small ketone molecules which are – I like to think of them as water soluble fat molecules that readily cross membranes via the monocarboxylic acid transporters 1, 2, 3 and 4 and they are kind of located dependent upon the tissue – the brain actually has a lot but blood-brain barrier is also pretty rich in these ketone transporters.

As the ketones get elevated in your blood, there's essentially unrestricted flow of these ketone bodies into the brain and ketones are not only energy metabolite, very effective kind of a high energy metabolite relative to glucose – you can derive little more ATP out of energy production from ketones than glucose, but more importantly from the perspective of our lab as we move forward with different therapies, is that these ketones bodies have epigenetic effects. For example, they function as histone deacetylase inhibitors, class I and II, and that can essentially activate a genetic program that can confer resistance against oxidative stress in various forms actually. That was published by I think Eric Burdon's lab in science.

We actually published an article in Nature Medicine to look at the NLRP3 inflammasome which is kind of a hub initiator. When that's activated you have – many different cytokines become elevated in the blood and it's tightly associated with aging related chronic diseases and several forms of cancer, GI cancer in particular. We know that beta hydroxybutyrate can suppress that and knock that down. It does it in a metabolic independent matter. It does it not through typical classic metabolic signaling. It doesn't do it through that way, it does it through very unique pathway.

Actually, we are hosting a conference called the Metabolic Therapeutics Conference in February which kind of brings in some of these top tier Ivy League investigators. My investigator for that particular study

was Deep Dixit at Yale. He had some interesting research in fasting but he has really delved into mechanistically how these ketone bodies are working. Undoubtedly the signaling properties of ketones are having anti-cancer effects, independent of their effects for metabolism on cancer. We know cancer – there's never been normal mitochondria shown in any cancer cell to my knowledge. Some cancer cells, when they are studied in culture, they will have [inaudible 00:16:52] consumption and what looks to be normal cellular respiration, but when you look at the mitochondria, there are major deficits and molecules like cardiolipin for example. I don't think it's normal in any cancer cell. The mitochondria are qualitatively or quantitatively defective in some way, shape or form and all solid tumors. That defect in mitochondria opens up the ability to be exploited through enhancing ketone metabolism, because unlike glucose, ketones need to be metabolized, specifically in the mitochondria to generate ATP.

If your mitochondria are deficient which is shown in all cancer cells that have been looked at, you have impaired ability of the cancer cell to make ATP from ketones. It's possible cancer cells could be using some of the carbon from ketones for biosynthetic processes. But without energy, without sustaining energy that's an energy-dependent process, you know expanding biomass of the tumors, like shuttling energy substrates to tumor growth. But if the energy is not there to do that, which requires massive amounts of glucose and also tumor cells use glutamine, so we target those things with the ketogenic diet and with ketone supplementation and a toolbox I guess you could say of what we call Pulse Therapy, which are various drugs that when given at specific dosage and timing are relatively non-toxic.

A ketogenic diet is a small piece in the Press Pulse Protocol that we are working on. I think some people interpret our research as like just go on a ketogenic diet and cure cancer. We don't want to promote that. That's not what we do. Actually we've never done a study just to do the ketogenic diet. We always do that – that like sets the stage more or less for us to go and bring in a whole toolbox of other modalities to then weaken the cancer to hit it with other things that

actually increase oxidative stress or compromise tumor metabolism.

DANNY LENNON: Right. That's really, really important points. Just kind of as a recap on some of that just so we have it clear for people here. When we are talking about cancer cells themselves, we are essentially talking about cells that are going to have high glucose metabolism, these elevated rates of glycolysis and there's also mitochondrial dysfunction going on. Then something like nutritional ketosis or fasting or those kind of things that kind of give related effects, can really have those benefits from two ends – number one on lowering say blood glucose which is going to be obviously higher in cancer cells, then also the ketones themselves can have some sort of therapeutic effects. We will circle back to some of that stuff particularly when you mentioned things like Metformin.

But just to kind of return to the point you finished on there, I think it's a really important one because so often when people hear this exciting and fascinating work on the use of nutritional ketosis, and then related issues like fasting to be used in cancer treatments, there's this, like you said, this jump some people make, this incorrect jump where they will start thinking about this as some sort of cancer cure. And that's not what you guys as researchers are putting out. It's more so to think about this as a potential therapy that can be used as an adjunct to other, both standard care interventions as well as a suite of other potential therapies that you are using. Would that be a more accurate picture to paint?

DOMINIC DAGOSTINO: Yeah, exactly. The ketogenic diet more or less just kind of helps us set the stage and ideally it's best if it's calorie restricted too. If you are dealing with a patient population like those with breast cancer – some of the last meetings I got out of, like most of the patients in those groups were – their BMI suggested they were borderline obese, many of them were overweight. So just going and putting them on a ketogenic diet typically causes inadvertent fat loss just through appetite suppression, but if you calorie restrict on top of that you are going to further suppress insulin signaling and also help to limit glucose availability and actually get a nicer elevation of ketones.

Yeah, the ketogenic diet is just one small piece. It could be even a minor piece of the more bigger picture that – or protocols that we are setting up in the Press Pulse Protocol that we are working on. We've actually never done like just a ketogenic diet study, because a lot of that work was already done at least in animal models and we did some initial work – like we used a ketogenic diet as a control to compare it to the ketogenic diet and therapy that we do and we always see an extension of life and suppression of tumor growth. Unless we sweeten the ketogenic diet and they overeat it, and then you can actually increase tumor growth, because – actually if it's the protein is not restricted.

You asked like what's the alternative to the ketogenic diet. Ketogenic diet nicely sets the stage but in oncology a fear of weight loss is a big, big deal, even in patients that's overweight. They will give them lots of basically powdered milk with lots of sugar in it and vitamins and encourage them to eat as much sugar and carbohydrates as possible to maintain their weight throughout the chemotherapy protocols, because you get nausea and throwing up. And I always believe – I said to some of my colleagues of mine that many of the chemotherapeutic strategies work because basically it destroys your gastrointestinal mucosa. You get nausea so you don't want to eat, so you inadvertently lose – it basically forces you and your body into a toxic fasting state because you can't digest the food that you are eating. Your blood glucose and insulin actually will go down or some of the drugs that they use like Dexamethasone or Prednisolone which are kind of used together with chemotherapy to attenuate the nausea can make your blood glucose shoot up and can kind of be a bad thing.

When you talk to someone and talk to hundreds if not thousands of patients, the ones that went through chemotherapy and actually have done well and coming back or in remission, they are typically people who have lost a lot of weight. It's kind of through them into a very toxic calorie restriction or fasting state simply because they couldn't eat food and the food that they ate just went right through them or they through it back up. That needs to be recognized

when we look at very small benefit that chemotherapy offers in some forms of tumors. But we actually focus mostly on brain tumors like glioblastoma and advanced metastatic cancer where therapies are often not even an option sadly.

DANNY LENNON: Right. That actually leads me onto something I was interested to ask. There's obviously vast differences in the types of cancer someone may suffer from and how they actually manifest and their pathologies. It's perhaps then logical to assume that using interventions like nutritional ketosis or calorie restriction or hyperbaric oxygen therapy, etc. isn't going to have the exact same effects across the board for all cancer types. Do we see things like ketosis as a more viable treatment in certain cancers than in others?

DOMINIC DAGOSTINO: Yeah, that's a really good point. Some people who study what I do, just feel like chemotherapy across the board is a bad thing. I can tell you I have actually had students that had leukemia, lymphoma and testicular cancer, all of those things. The liquid cancers which we call the non-solid tumor cancers like leukemia and lymphoma, most of them are highly responsive to chemotherapy. I encourage multiple myeloma – it's highly responsive to the therapies that are out there. What I have seen and I always encourage people to do chemo because in those particular types of cancers, chemo is very effective. I think we've made some good progress there. But their therapies can be greatly augmented with nutritional approaches that we are working on. Testicular cancer is another one, internally grows, kind of slower and fairly responsive to the therapies that are out there.

When it comes to solid tumors, maybe independent of testicular cancer but the liquid cancers would be like the leukemia and most lymphoma, they do that, but when it comes to solid tumors, whether it be lung, kidney, liver, brain, sarcoma and many other types of solid tumors, our therapies have very limited efficacy, especially if that primary tumor has metastasized to other regions of the body, What's generally offered to the patient is just palliative care. Chemotherapy and radiation in the context of a glioblastoma or an aggressive solid tumor that has metastasized, it can do

more harm than good. Chemotherapy is carcinogenic. Actually it damages the mitochondria, it damages the DNA and can cause more mutations in the existing cancer cells and when they come back, they can be more aggressive. Same thing with brain tumor cells and radiation too. Radiation is probably one of the most potent carcinogens that we know of.

We envision a cancer therapy program that when you come on out on the other side you are actually more healthy than when you went in. The Press Pulse Protocol that would incorporate nutritional ketosis and some of the other modalities that we are working with, it actually enhances the health and vitality of your normal cells. We believe that cancer cells are actually not very evolutionarily adept for survival. When you are looking at cancer cells in a Petri dish and you have a Petri dish next to it with normal healthy cells, the normal healthy cells pay their dues so to speak and earn their ability to be on this planet. Whereas the cancer cells are not as evolutionarily adapted to surviving on this planet as the normal cells are. They are not going to go on and live. They are sort of an artifact. The genetic damage that you see in the cancer cells is the downstream epiphenomenon of metabolic dysregulation. They have defaulted back to a metabolic phenotype which in oncology is called the Warburg phenotype to consume to be dependent upon glucose and to some extent glutamine substrate level phosphorylation to maintain their growth and proliferation. So their metabolic requirements are actually much higher than normal healthy cells. When viewed in that context, we can design personalized therapies even based on the type of cancer we think because we can also use some of the genetic information and metabolomic information, proteomic information to design personalized metabolic approaches to managing specific types of cancer. There's a certain commonality.

When you look at two people with glioblastoma and you take out a sample of the glioblastoma, it's tremendously genetically heterogeneous. You could do a thousand different mutations and the other guy maybe have a dozen or two and a totally different mutations. Even the same tumor can have different mutations depending on where you take the biopsy.

Genetically it's tremendously heterogeneous, but metabolically it has the same metabolic phenotype, so the Warburg phenotype which is damaged respiration, there's a qualitative and a quantitative deficiency in the mitochondria that are in human glioblastoma, and they have an upregulation of glucose metabolism and glutamine metabolism.

With that understanding, we can design protocols to target that metabolism to perhaps synergize with existing therapies, but we are really interested in when the patient undergoes the therapy – they are more healthy on the other side of the therapy than they are, than they were going into it. We don't want to ravage the patient with toxic chemotherapy and radiation. We think radiation has been used for a 100 years and we think it's archaic and the protocols have evolved but it's still – we view the Press Pulse Protocol in comparison as putting – it's more of a gentle approach. Instead of thinking of a tumor, some alien theme that we got to cut out, smash with radiation and toxically kill it, we think that you can gradually apply a Press Pulse Protocol to first and foremost just stop the growth. Then you could start to personalize the Press Pulse Protocol to gradually reduce and actually inhibit the growth further on. Some people believe it or not, if they have a tumor in their liver, they could live a normal healthy lifespan if you can manage that. Whereas if you go in there and start cutting things out and giving chemo and radiation, for example if you successfully treat a leukemia, if the child has leukemia and you successfully treat him with chemo, he's 38 times more likely to get another form of cancer later on in life and most likely will. Same with breast cancer, I mean your breast cancer increases dramatically if you are stage 1 or 2 and you have therapies.

It really – the chemo and radiation really take a hit on your genomic stability, of your normal cells, and that can later in life that can compromise you. Because you are doing it in the context of no immune system typically, because you are doing it and your immune system is another whole kind of area of research that I could discuss and get into, and it's relevant to those.

DANNY LENNON: Right. When we are talking about then trying to like based on this understanding of the phenotype, then we have a suite of different interventions we can possibly use, either as throwing them all individually that each can do something or then possibly then this synergistic effect maybe that some of them have together as well. So when we talk about some of these interventions that are being used, I know a lot of your research is centered around things like say hyperbaric oxygen therapy, and we can also talk about some of the metabolic drugs as well, can you maybe just give a brief introduction to people into something like hyperbaric oxygen therapy, why that was used, again what is the kind of idea there, and then some of the other interventions just a kind of brief intro into those?

DOMINIC DAGOSTINO: Sure. Like I said in the beginning, I had never planned to study cancer biology. My original experiment or studies involved building technologies including hyperbaric atomic force microscope and also it's hyperbarically scanning microscope that allowed me to study CNS oxygen toxicity which are seizures. So this technology that was sort of my post doctoral fellowship project allowed me to look at all different types of cells. I was just testing the device more or less to see look at things like membrane viscoelasticity, membrane potential, calcium signaling, real time reactive oxygen species production – these are all things that we could do in our lab with these technologies. I looked at smooth muscle cells and cardiac and primary neurons from the brain.

And one cell line a colleague gave to me and he just told me it was a glioma cell line, I didn't really look too much into it, it's U87MG cell and it was ultimately – it was derived initially from a 44-year-old glioblastoma patient. It was just like another cell line and I was like okay let's see what happens if I stick it inside a hyperbaric chamber and we used the confocal microscope to look at the mitochondria. When I did that, I had a pretty good background of throwing other cells in there – when I put the cancer cells in there, and no one had seen this before because no one has a hyperbaric microscope right, when I put that in there, we used a probe to analyze reactive oxygen species from the mitochondria, I saw that the

mitochondria were emitting huge amounts of superoxide anion on it and they were starting to die and the membranes were starting to blow up. In [inaudible 00:35:39] oxygen that was relatively non-toxic to like primary cortical neurons or primary hippocampal neurons.

So I was like wow this is pretty interesting, and I kind of reproduced it and had other students at the time looking at it. I wasn't really interested in that but we did publish it in Neuroscience and I kind of followed up on it when I found out that they were derived from a – that cell line was derived from a glioblastoma patient and one of my students, a first year PhD student came in and wanted to do a project on ketogenic diet and hyperbaric oxygen together. What that experiment was telling me that cancer cells have damaged mitochondrial function and when you increase the oxygen concentration at the level of the cell, you get proportionately more superoxide anion from a defect in complex 1 and 2, the semi-ubiquinone site, the electron transport chain spits out a lot of superoxide anion, there's also deficiency of cardiolipin in the mitochondria. There's all sorts of mitochondrial impairments in these cancer cells. I didn't know that. All I saw was massive amounts of free radicals to be produced in response to high oxygen.

So that then becomes – and I knew that hyperbaric oxygen was used for radiation necrosis in patients that – and it was actually an FDA approved use of hyperbaric oxygen and there was about 14 different FDA approved uses of hyperbaric oxygen. So I started talking to some of the doctors that were using it and it was like yeah all my patients do so much better. They have radiation and then to help them heal from the radiation, we would give them hyperbaric oxygen, and case report after case report I have talked to people they were like – but they weren't acknowledging the hyperbaric oxygen as something that could further kill off the cancer cells.

What we found in theory, a ketogenic diet would suppress the pentose phosphate pathway which kind of generate the reduced glutathione and maintains the redox status of the cell. So we were thinking that a ketogenic diet could suppress the glycolytic pathways

that were important for the endogenous antioxidant capacity of the cancer cells and that the two may synergize really well together. So you are increasing oxidative stress and at the same time suppressing tumor metabolism. I had also seen at the time that ketones, when I tried to grow the cancer cells in ketones with normal glucose and I would add ketones to the cell culture, they just weren't growing. Actually, LabTech at the time was doing it and I asked, I was like are you doing something wrong, and I was kind of questioning her. But she had so much experience growing cells, she was like, no I don't know, they are just not growing. I looked into it further and I found that there was some evidence that ketones could actually suppress glycolytic pathways in cancer cells.

So I was like okay, who's in experiment here, I didn't have any funding to do it, I had a little bit of departmental funding because I transitioned to a tenure track position, not like 50K funding that I was using for other things. But I had a really smart graduate student come in, her name was Angela Poff, and she's now a research associate with her own grants and everything. And she's like I want to do this project. I was like, well, I am not a cancer biologist, I know physiology and a little bit of cellular biology. But she convinced me to take her on as a grad student and her project was to use hyperbaric oxygen combined with the ketogenic diet. Now we do other modalities too. That was the first experiment we did.

I remember it distinctly I would kind of race into the lab early in the morning to see if any more animals had died. We got to a certain point in the experiment where the animals were eating a high carb standard diet and we had a group eating a ketogenic diet alone and then ketogenic diet and hyperbaric oxygen therapy treatment. At one point, I was like maybe two and a half, three weeks into it, like all the standard diet mice that had tumors in them, that had tumors implanted, they had all died and the ketogenic diet group like about half of them were still alive and the ketogenic diet and hyperbaric oxygen we had like 70 or 80% of them still alive. We were all looking at each other and I said wow there is something to study here.

We went on and kind of reproduced it and we did get a calorie restriction effect with the ketogenic diet, so reviewers were like well, go back and do a calorie restriction control which we did and validated it through reproducing it in the lab. Interestingly, the hyperbaric oxygen alone had only a small anti-cancer effect, at least from the doses that we gave, but it synergizes with the use of the ketogenic diet, so it's an oxidative stress. And James Watson – he just kind of went public saying we need to focus on cancer metabolism for the next generation of cancer biologists out here. And one of the discoverers was Francis Crick of the DNA molecule. And he gave a talk here at the Moffitt Center here a top tier cancer center. And his message was we need to shift our thinking away from cancer as a genetic disease and redirect our thinking as cancer as a metabolic disease and we need to first and foremost develop metabolic based therapies. These can be drugs – pharmaceutical companies completely open to exploring things here, but diet is a big hammer right.

So you can't discuss metabolic therapy without discussing the main impact on your metabolism which is diet. And oxidative stress too, we know that cancer cells have an upregulation of reactive oxygen species that are being produced. So if you throw in agents that cause cancer cells to over-produce oxygen free radicals which would be hyperbaric oxygen, Metformin interestingly does this also, you can push them over their antioxidant threshold if you will and then that can trigger apoptosis and cell death. And that's what we do in our lab. That's kind of the whole idea is very intense metabolic based therapies that are kind of we use like a baseline press and then we have a Pulse Protocol that's kind of more intense with various drug treatments. Some of these drugs also simultaneously will increase mitochondrial ROS production. So it's crippling their endogenous antioxidant capacity and their metabolism with diet and various drugs and then throwing things in that cause them to burst reactive oxygen species and it primes them for apoptosis and cell death.

DANNY LENNON: Yeah, that's super interesting and I think kind of relates back earlier to what you said, it's kind of shifting away from that old mindset of just something

that needs to be kind of just cut out and taken away whereas when we are viewing it through this lens of this kind of metabolic theory of cancer it's always like a slow strangling of the cells to try and get them out of there. Just as we kind of talk about that, I think a lot of this stuff might as well open reading some of Dr. Thomas Seyfried's work on this kind of metabolic theory of cancer and related to that one issue I did want to bring up with you, because you had mentioned it earlier around the importance of the caloric restriction in the context of the ketogenic diet working here. Because I think Dr. Seyfried's, at least some of his research on some animal models had kind of showed that the ketogenic diet when it's consumed in the context of a calorie surplus in fact has then the opposite effect of potentially promoting cancer cells growth and I hope I am accurate in saying that. So first, can you kind of touch a small bit on that and then are there any other contraindications for the patient when it comes to the ketogenic diet?

DOMINIC DAGOSTINO: YEAH, I am glad you brought that up, because that's the big point, and I think in area of – I am not going to say contention between Tom Seyfried and me, we are actually really good friends and worked together on many things, but he is a big fan of calorie restriction and fasting and things and I am too up to a certain extent. When it comes to his specific diet, he has stated many times that the ketogenic diet can cause very rapid tumor growth if it's given unrestricted. He emphasizes very adamantly that the ketogenic diet needs to be restricted to have any form of efficacy at all. The benefit of calorie restriction with the ketogenic diet as opposed to just calorie restricting a carb diet is that you have ketones elevated in your blood, and those ketones make the transition – the ketones make your brain happy basically. It can get more into it but when you are calorie restricting on ketogenic diet your ketones are basically keeping your brain happy. And you do get a little bit more of an insulin suppression and a little lower blood glucose and you don't get any of the glucose spikes when it comes to feeding.

So when it comes to Thomas Seyfried's experiments with the ketogenic diet, it's really important to acknowledge that he was using a ketogenic formula

that was sweetened, it was called Keto Chow. It was made by Nutricia and it was basically hydrogenated fats with a sweetener so the kids that drink this for epilepsy will drink it and be happy and like the taste right. When you mix this stuff up and put it into a paste and you give it rodents, they are going to overeat it anyway right.

I went into this thinking well, yeah, he has a good point there but because it's sweetened and you give it unrestricted they are going to eat and they are going to overeat it and they are going to be giving their bodies a surplus amount of calories which was going to fuel tumor growth. We formulated a ketogenic diet that's sort of along the lines of the standard rodent chow like what's in it. We just manipulated the macronutrients to be a modified Atkins diet which is about 20% protein and the balance being fat. In addition, I was very specific to actually include medium change triglycerides into the diet that I formulated for studies. And I brought Tom in on the study because I wanted to use his model of cancer that no one had a therapy that worked for. So there are just various models out there where people have cured of cancer, but I wanted to use one that seemed impossible to cure cancer, that was aggressive. And that's the model that I chose to use so he was actually on the paper to show that an unrestricted ketogenic diet given ad libitum extended survival in an aggressive model of metastatic cancer, and reduce tumor growth and extended survival.

It's important to note that when they ate an unsweetened ketogenic diet, they calorie restrict to the point where they lose about 5 to 10% of their body weight which really isn't that much. Once we get down the 25% you get problems. But they drop their body weight slightly and they maintain that level of calorie restriction that I believe actually enhances their normal health, it restores their normal health. When you put rodents in a cage and allow them ad libitum access to a high carbohydrate rodent chow they overeat and become like type 2 diabetic of course, they become very sluggish and they become heavier than they would be otherwise if they had to go and forage for their food – whereas if you replace that standard rodent chow with a well-formulated ketogenic diet,

they eat just the right amount. You put them on a treadmill, they will run 25% longer, they live longer. There's all these metabolic benefits, so it actually restores their normal health when you take away their standard rodent chow – their standard rodent chow is actually formulated for maximum health.

So imagine if they are eating like a western diet or something, so we always joke about that. Like the standard diet was through years of research constructed to optimize health, and even decrease tumor burden on animals or prevent the occurrence of cancer. We are actually testing our ketogenic diet. Again, the diet that's like optimal. Imagine, we actually need to start formulating like a western diet and comparing our ketogenic diet to that response. So yeah, calorie restriction is important but I don't think it's as important as some people make it out to be.

DANNY LENNON: Right. Then when we look at kind of anecdotal parallels in humans at least when we see people that try and implement a ketogenic diet, there's obviously a vastly different number of ways people can put it into practice and if we classify one as say the best way to put in where they are choosing good quality foods, keeping plenty of say vegetables to give fiber in there, and overall the breakdown of their fats is going to be good quality so coming from like avocados, olive oils etc.,. But on the other end, someone could still eat "ketogenic diet" that puts them in ketosis but be using foods that are probably on the more process end of the spectrum and maybe poor quality, maybe tons of one particular type of the polyunsaturated fat or etc., and they are going to have maybe vastly different effects on how much that person is going to eat or how much of that may get suppressed etc. So I think it's an important point and just while we are on that to bear in mind that diet quality still matters.

One thing just while we are talking about some of the work that Dr. Seyfried has done, I know he obviously has talked before about something like an extended fasting period can – that's done occasionally can have potential cancer prevention effects or it can be used as a tool in cancer prevention. When we are talking about the set of ketosis, so far we've talked about getting into this ketogenic state being one part of a

potential treatment of certain cancers, but do you think it's viable then to start thinking about it on the same lines as maybe Dr. Seyfried talks about fasting in terms of it being a cancer prevention tool – in that for those who do not have cancer and are currently in good health, do you see any value in perhaps using intermittent periods of ketogenic dieting for those kind of cancer prevention purposes or taking care of say pre-cancer cells or anything like that?

DOMINIC DAGOSTINO: Yeah, that's a good point. And that's an area of research that we want to get into if you notice from – but like nobody does cancer prevention research right, it's hard research to do, it takes a lot of time maybe. But it's definitely feasible to do this kind of research and I think I am glad you brought it up because it has tremendous implications. If we view cancer as a metabolic disease, then that has tremendous implications for not only the way we treat cancer but for cancer prevention. I do believe that intermittent ketosis perhaps in the form of short term or maybe longer term fasting could put the body into a metabolic state that is extremely stressful for existing cancer cells and pre-cancer cells that are really dependent upon these glycolytic pathways. And you could potentially stress the cells and kill them and essentially purge them from your body. And I think that can be an effective strategy.

There's lots of old experiments that showed very nicely that calorie restriction and various forms of fasting, but most of the studies done with calorie restriction showing extension of life but the animals not only live longer – I mean they have far less occurrence of spontaneous tumors. So we are thinking about maybe doing an experiment where there's a variety of inbred strains of rodents out there that at day 200 – or after about a year and a half, so it maybe a day like 400 or whatever. But some of the more other inbred strains will get tumors kind of quicker. Around day 200 you will know that about 30% of them should have various spontaneous tumors. And that particular strain has been classified as getting the tumors for one reason or another.

We could start treating them early on before tumors with you know comparing a standard diet calorie

restriction, intermittent fasting would come in there each day and you give 5.5 grams of food and we know the animal eats typically within an hour or two, and then we could use things like Metformin, Rovamycine and they would have to be big groups, 50-60 animals per group. And then you just follow them throughout their normal healthy lifespan and they will start dropping off towards the end and then start getting tumors and you could do necropsy to confirm it.

But it's a study that I think is so important and I think it needs to be done. And I think Quest Nutrition has really kind of been a pioneer in this and they've helped us out with our cancer research program. Also Scivation who you are probably familiar with, the branched chain amino acids project, they had supported our cancer research. Quest Nutrition, they have a foundation, Epigenix Foundation, that has a keto pet dog sanctuary where they are taking in dogs that have cancer and then treating them with these metabolic approaches and talking about it. A lot of their focus too is that once the dogs go through the cancer treatment therapy, it's really important to not let them go back to a normal diet but to keep them on a diet that we know has anti-cancer effects, and I think that's really important to focus on prevention. Even after someone has gone through treatment, it's more important for them to follow the types of things that I am talking about.

And I think they maybe fasting or intermittently going into ketosis two or three times a year or once a year if you want to do a big fast or something, can be very beneficial, and it's definitely a good thing for your metabolic physiology. It's not normal for people never to go into ketosis. It's not normal for us to be eating three carbohydrate meals per day and then have a restaurant around the corner all the time. It would be normal for us to periodically go into a state of fasting and this probably serves an important evolutionary effect too and help kind of make us more resilient. Because once you actually go into a state of fasting ketosis, it's much easier the second time. It's kind of like your body knows what to do and you've upregulated some of the metabolic machinery and somatic pathways that allow for a smooth transition into that state. And we haven't studied that enough

experimentally but I mean you could talk to people who have dieted for physic shows and things like that, like the first diet is always very hard and then as they do it, they actually have kind of a better handle on it, and it becomes kind of easier for them. I remember talking with Layne Norton, a good friend, about this, and what he's seen in clients too, and that kind of validates kind of what we see in the lab.

DANNY LENNON: Yeah, super interesting for sure and just as you touch on some of the needs that come out from future research, and then this will be our final question, in your opinion what would we need to see emerge in coming research in order for nutritional ketosis, exogenous ketone use, hyperbaric oxygen therapy, all these other treatments that we are seeing with all these great results and continuing the research from your group and others is starting to mount on this, but in terms of it being part of the standard treatment in the frontline and I suppose be accepted by the wider medical community as going from something that's promising to being classified as like a common evidence based intervention, what is the kind of intermediate step that we would need to see emerge from the research for that to happen do you think?

DOMINIC DAGOSTINO: Yeah, I am glad you brought that up. I could probably talk about this for three or four hours. I would try to break it down into the thing that I think is of real importance here, because as we develop these things, I am a PhD basic scientist and I am not a medical doctor, I don't have the liberty to start treating patients, although lots of patients contact me and some of them I have directed to the Moffitt Cancer Center, and they have had some pushback. But now the Moffitt – now, the mainstream cancer centers are realizing that metabolic-based approaches are very good. Actually, one of them years ago, one of the patients that contacted me was Dr. Fred Hatfield who's really big in the power lifting world and written like 50-60 books. He lives in this area and he had got diagnosed by three different oncologists with advanced metastatic bone cancer, and he went through various therapies and he was at the time in really bad shape when I actually went to go visit him in there. He implemented without telling you the details, he implemented some of these approaches

and that was like 2011 or '12. Anyway, he's cancer free. I mean, he's been travelling the world giving seminars at power lifting events for quite a while.

Some people will respond remarkably well and some people depending on the cancer type may not respond or how they implement it. So when it comes to transitioning what we are doing in the lab into the clinic we face a number of challenges. For one thing in meeting with oncology teams, most of them are completely unfamiliar with these approaches, and none of them – very few oncology teams have like a nutritionist at all or nutritionist that's familiar to these approaches. It's usually try to get as much food into the patient to prevent weight loss as possible right. The problem is that the oncology teams are not – I don't want to sound like a conspiracy theorist but the oncology team generates revenue for the institute through giving drugs, chemotherapeutic drugs and radiation therapy, generates enormous amount of revenue for the oncology team.

There is no revenue to be generated by implementing these metabolic approaches yet. Nutrition is just not a whole lot. And actually oncology teams are – they want to pay to have a nutritionist on hand to work with all the patients in a close face to face kind of manner right. They will give them maybe a one-sheeter and say eat as much as you can or drink things that are easy for you to digest. So the insurance companies are not really paying for nutritionists to do this.

Another problem that we face and I will cover the problem and then talk about the solution. Another problem that we face is that the Institutional Review Board, what we call the IRB – the IRB would be very, very hesitant to approve any kind of nutritional or metabolic based therapy that has not you know unlike a drug that goes through various phases of testing and development with a lot of money behind it, they will approve that but they are hesitant to approve a nutritional protocol especially that involve any kind of restriction to the patient, even restricting carbohydrates. It's just kind of very difficult to get that approved. Not unheard of, it's been done, but it's hard to get approved.

So we have the oncology team that's kind of – that's a major hurdle. Getting a nutritionist to work with the patient, insurance won't pay for that, so that's a hurdle. IRB approval, getting ethics approval or review board to approve a therapy, especially in place of a standard care therapy, that's a problem. And then you have patient compliance, you have patients coming in here and be like well, I have never heard of this. So you have to recruit patients. If patients have the option, if you are a glioblastoma patient and have the option to use a drug from Genentech, and then some fasting or ketogenic diet, what are you going to choose, not having any knowledge going into it?

And then I guess probably the biggest hurdle, patient recruitment is definitely a hurdle – probably the biggest hurdle is who's going to fund this. You need to have money coming in from some kind of entity that pays your oncology team, that pays your nutritionist, that pays the patient incentive, and pays for all the resources for the facility. So that's the major – so there's –I could go on and on, but that's like the five major things that are preventing these things and I am talking about getting into the clinic.

Now, despite all those things, oncology teams have actually reached out to us and said they see this, there's chatter about this, and then they dig into the science and then they realize, this makes sense, this makes theoretical sense. And then they have patients that have without any support, they go and do it themselves and they come back and say hey doc, I did this, you gave me no options, I did this and it worked for me, so why aren't you studying this. So that doctor is like reach out to us and say well, my patient did this and is now doing really, really well, so we feel that we need to look into this, can you come down and tell us about the science of it.

So that happens and that's always good to see that happening. But the way that it's going to get into the clinic is as adjuvant to the therapies that they have already given. So if you can convince oncology teams that hey you have a drug that works for a glioblastoma patient, it gives them two or three months of extra life, that's not much. But if they can adapt this protocol

and say hey this can make your therapy work better, that could help us break into the oncology clinic. As long as we have financial backing behind it and patient compliance and IRB approval and nutritionist – as long as we have all those things in place, we can start to get these engineered ketogenic diets which would be a well-formulated ketogenic diet with very careful attention to the types of fats in it, the macronutrient ratio, the calorie amounts are very important. A lot of studies that have been done that don't even count calories, they don't even do – in the fitness world counting macros is very easy no-brainer right, but it's not so much of a no-brainer to like oncologists, because you just don't have a firm understanding of nutrition like your audience probably has.

So that's the idea of transitioning it into the clinic by selling the idea like we can enhance your therapies by using a metabolic program that weakens or compromises a cancer and then you come in with your therapy and then you get less side effects for the patient and a greater therapeutic response, and that's the kind of way that we are selling it. But I think, my vision and hopefully it happens in my lifetime is that the Press Pulse Protocol that I was talking to you about, that we are doing in pre-clinical animal models – is that this more instead of the slash and burn approach with surgery, radiation and chemo, that a Press Pulse Protocol will be gentle, metabolic management of the tumor, to the point where the Pulse Protocol can be dusted and personalized to the patient to where you get eradication of the tumor over time but it's in a much more gentle sort of protracted way where the patient health, they come out on the other side in better health than they did going into it. Unlike the normal standard of care therapy where you come out on the other side hurt and your body is taking a significant hit in ways that it can show up later on even if you do go into remission.

DANNY LENNON: Yeah. It's certainly going to be fascinating to see how this continues to both develop in the research, but like you said, really the exciting part comes as more and more of it can be implemented into kind of clinical scenarios. Dom this has been tremendous so far. Thank you so much for your time and just before we

finish maybe you can let people know where can they find more of your work online, where can they keep up-to-date with the stuff you are doing, if they want to find out more about this type of stuff?

DOMINIC DAGOSTINO: Yeah. Well, I would just like to say thanks for having me Danny. I think it's a great platform for me to connect with. I know you have a big audience already and it seems to be growing faster. I hear people talk about this podcast and this site a lot. So the place to reach me, I guess the best place would be, I have a website called ketonutrition.org and more or less it's not really an interactive website but I have compiled a list of links on there that have for example my academia website is on there and that will link you to my published research including books and articles that you would otherwise really have to pay for. I have kind of loaded them on the site and as of now, they haven't come after me. You can download them for free. And then I have ketogenic diet consultants on there for things like epilepsy and cancer and even performance. I have some people on there in the fitness industry that do some consulting for low carb dieting or all sorts of dieting. Clinical trials, podcasts and various articles on there, I think, it will be a source of information that people can utilize. It goes into more of the things that I was talking about here.

DANNY LENNON: Perfect. And for everyone listen, I will of course link up to that in the show notes so you can check all of that stuff out. With that Dom like I said, thank you so much for taking the time out and thank you even more so for the amazing work you are doing and continue to do going into the future and thanks so much.

DOMINIC DAGOSTINO: Thanks again for having me Danny. I appreciate it.