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## An Evolutionary and Mechanistic Perspective on Dietary Carbohydrate Restriction in Cancer Prevention

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## **Abstract**

The confluence of basic cell biochemistry, epidemiological and anthropologic evidence points to high dietary carbohydrate and the associated disruption of the glucose-insulin axis as causes of the current increase in metabolic disorders, metabolic syndrome, hypertension and cardiovascular disease. This hyperinsulinemic state likely contributes, as well, to an increased mutagenic microenvironment, with increased risk for cancer. This critical review discusses these risks in their historical and evolutionary context. The evidence supports the benefits of lowering the glycemic load of the diet as a preventive measure against the development of cancer.

## **Keywords**

carbohydrate restriction, calorie restriction, cancer, cancer prevention, evolution, ketogenic diet, nutritional environment

*Editor's note:*

*Dear Journal of Evolution and Health Reader,*

*Prior to 1944, the entire scientific community "knew" that proteins had to be the units of heredity. No other macromolecule could possibly be responsible for the enormous diversity of expression in life. Certainly, DNA couldn't have been responsible. After all, there were only four bases! Even the word, protein, was designated to mean "primary."*

*However, three men, Oswald Avery, Colin MacLeod, and Maclyn McCarty, published a paper in February 1944 in the Journal of Experimental Medicine that clearly demonstrated that the entire scientific community was simply wrong: DNA was the "transforming" genetic material, not protein. Consequently, this landmark paper drove the race for the discovery of the structure of DNA and spurred the discovery of the mechanisms that underlie heredity, cell expression and evolution.*

*Similarly, this paper presents an alternate hypothesis that is in contrast to the conventional wisdom of cancer mechanisms and therapies. As such, the reader should be aware that some of the literature the author's used for references were speculative in nature. Caloric restriction and carbohydrate restriction are not the same yet are difficult to separate in the literature. Therefore, keep a healthy skepticism as you read. Indeed, the word "Perspective" in the title demonstrates that the authors are themselves aware that the article needs to be tested for its validity!*

*An evolutionary basis for cancer mechanisms is a unique perspective not seen elsewhere. It is the Journal of Evolution and Health's hope that the article will result in further testing of this interesting hypothesis.*

*Sincerely,*

*David C Pendergrass, PhD*

*Editor-in-Chief*

## INTRODUCTION

The multiple genetic changes [1,2] that characterize the cancerous state limit the ability to target isolated molecular pathways. At the same time, diet as an effective treatment, widely and often enthusiastically held in popular and social media, holds limited influence in medicine, at least as a sole treatment. In practice, diet is considered an option which usually fails and therefore must be subservient to pharmacology. Nutrition as cancer prevention may, however, be better received.

Calorie restriction (CR) as cancer prevention was proposed and tested in animal models more than 55 years ago [3-5]. Studies of CR in humans have generally addressed cancer treatment [6-9] but, most recently, carbohydrate-restricted diets, ketogenic diets, and so-called paleo diets which have *de facto* reduction of carbohydrate (CHO) as a common feature, have shown much promise for prevention [10]. It is understood, however that the discussion has outstripped both understanding of underlying mechanisms and the limited experimental support [11]. In this critical review, we provide background and recent results supporting the interest in CHO restriction in cancer prevention.

## RATIONALE

The rationale for diets based on limiting CHOs and/or generating a ketogenic environment derives first, from our evolutionary past which provided greater variation in the availability of total nutritional sources. However, even in times of plenty, our ancestors had limited access to dietary sugar or purified starch, a circumstance made worse during times of intermittent starvation, quite likely during the ice ages in northern Europe or on the Asian Steppes [12.] The evolutionary pressure for maintaining physiologic stability under extremes of dietary deprivation is evident.

Total caloric restriction has shown efficacy in the treatment of cancers in animal models [7,13,14] but the studies raise questions of the extent to which outcomes are due to *de facto* limitation of CHO. If CHO is 70% of total calories, for example, calorie restriction is primarily CHO restriction

and will be associated with the changes in the insulin and/or ketosis that may accompany CHO deprivation. While there are numerous intracellular control mechanisms, the insulin-glucose axis exerts a predominant effect and provides a stimulus for excessive growth and mutagenesis [15] as well as support for excessive biomass production required during the progression of cancer [16]. The hyperinsulinemic/hyperglycemic state also may appear as increased mitochondrial reactive oxygen species which, in turn, are likely to have a mutagenic effect [17–19]. Reduced insulin signaling, therefore, is expected to have beneficial effects in tumor suppression and is not excluded as the effect of total caloric restriction.

Support for the importance of insulin signaling comes from epidemiologic associations between several types of cancers and obesity, hyperinsulinemia, and hyperglycemia. In studies of intracellular signaling pathways, insulin is consistently found to modulate tumorigenesis [20]. Reduction in insulin signaling may also provide a parallel approach to cancer inhibition *via* the systemic effects of ketosis which develops only under low systemic insulin levels. KBs in cell culture studies have been demonstrated to act as metabolic inhibitors of glycolytic ATP production and cell growth in several cancer lines [21,22] and also have demonstrable action as signaling molecules with broad cancer inhibiting effects [23–25].

## EPIDEMIOLOGIC ASSOCIATIONS OF CANCER, OBESITY, AND DIABETES

Historically, obesity as a major health problem is recent. It can be argued that excessive, cheap sources of CHO, coupled with the recommendations by health agencies to consume high carbohydrate diets, have contributed to hyperinsulinemia and accompanying widespread metabolic syndrome, obesity, type 2 diabetes and lipid disorders. (The U.S. Department of Agriculture, the American Diabetes Association, and the American Heart Association have advised consuming CHOs at about 55-70% of total caloric intake until recently, now revised slightly downward).

However, obesity, hyperglycemia, and hyperinsulinemia have now also been associated with an increased risk of a variety of cancers [26–31]. Obesity has even surpassed smoking as the greatest behavioral risk factor for cancers in the U.S. Multiple studies have now established CHO restriction as an effective, if not superior, way to treat obesity and type 2 diabetes [32–38].

Obesity leads to several physiologic states that accommodate cancer induction and growth. Excess adipose tissue behaves as an endocrine organ, secreting inflammatory factors and hormones that create a hospitable environment for malignant cells both locally and systemically. Adipose cells secrete tumor necrosis factor alpha (TNF $\alpha$ ) and interleukin 6 (IL-6), and both are inflammatory mediators that have been shown to promote cancer progression through activation of pro-malignant pathways, including PI3-K/Akt, MAPK, and nuclear factor-kappa  $\beta$  [39,40]. The inflammatory state produced by adipose tissue can increase cellular proliferation, tumor survival, and metastases [41]. Systemically, the increase in inflammation can blunt the normal immune response to cancer cells while released cytokines up-regulate fibroblasts, vascular endothelial cells, and macrophages in the extracellular matrix [42]. Finally, high level of C-reactive protein (CRP), another marker of adipose tissue-derived inflammation, is predictive for poor survival in patients with metastatic cancer [43].

The global effect of excess adipose tissue also inhibits the body's ability to effectively modulate insulin and glucose levels. Obesity is accompanied by decreased insulin sensitivity, insulin resistance, and corresponding elevated levels of insulin and serum glucose [44]. Obese individuals also experience a parallel increase in insulin-like growth factor 1 (IGF-1) [45]. As data reveal that cancer cells exhibit increased glucose consumption, [46] it is not surprising that this metabolic state corresponds with poor outcomes in cancer patients [47–50]. Elevated serum glucose and corresponding elevated levels of insulin and IGF-1 provide both stimulus and sustenance for tumor proliferation, and altered glucose metabolism predisposes patients to metabolic dysregulation, favoring a malignant phenotype [16,51], as well as stimulating cellular proliferation while providing cancer cells protection from apoptosis [50,52].

Along these lines, residents of mainland Japan, who consume a more Western diet consisting of an abundance of food experience significantly higher rates of cancer and shorter life span when compared to residents of Okinawa [53]. Furthermore, the Mongolian population has an extraordinarily low incidence of breast cancer even compared to other Asian countries which is particularly interesting given their almost exclusive reliance on red meat and dairy products for energy intake [54]. Also of interest, while data are mixed, some studies in anorexic patients and

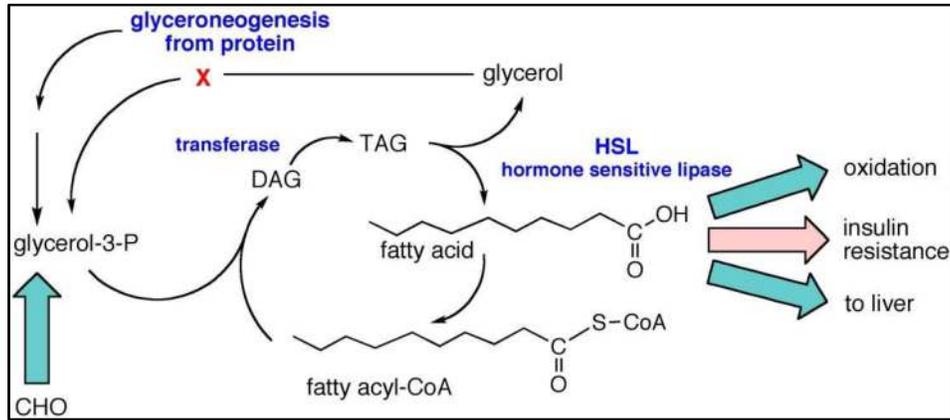
those who encounter intervals of minimal food consumption – similar to those of our ancient ancestors – reveal a lower risk of cancer [55,56] even when correcting for the overall reduction in life expectancy. Patients with a history of food overconsumption experience a decrease in cancer incidence and mortality after undergoing a surgical intervention to limit food intake [57–60].

Thus these data indicate that energy restriction and traditional diets that do not promote obesity can have an impact on cancer development.

### THE GLUCOSE-INSULIN AXIS IN METABOLISM

The modern “Western diet” is generally considered to consist of highly refined sugars and starches. The key factor is the rate of absorption and low-glycemic-index carbohydrates and added dietary fiber are widely recommended. In practice, these are secondary considerations since approximately 90% or more of the CHOs in the Western diet, i.e. between 250 and 400 grams per day, represent highly absorbable and digestible starches and sugars. At these levels of intake, what follows may be understood to refer to essentially all ingested CHOs.

Dietary CHOs elevate serum glucose concentration, thus stimulating insulin release from the beta cells of the pancreas. A primary effect of insulin secretion – kinetically and thermodynamically – is the repression of fat breakdown (lipolysis) in fat cells (adipocytes). Insulin is generally tissue building or anabolic, and stimulates protein, CHO and lipid synthesis. As is more widely appreciated, insulin represses hepatic production of glucose, the process of gluconeogenesis [38–40], directly or indirectly by the inhibition of glucagon [61]. This is likely the major mechanism for the clearance of blood glucose while the generally appreciated recruitment of GLUT4 receptors to the cell surface of peripheral cells is likely secondary [62]. As shown in Figure 1, glucose itself provides the substrate for re-synthesis of triglyceride (TAG) in adipocytes. Because adipocytes do not express glycerol kinase, the glycerol-3-phosphate must be provided by anaerobic glucose metabolism or glycolysis and glyceroneogenesis, a truncated form of gluconeogenesis, from protein [63].



**Figure 1:** Triacylglycerol (TAG)-fatty acid cycle in adipocytes. Glycerol from lipolysis cannot be re-cycled because adipocytes do not express glycerol kinase. Glycerol-3-phosphate, the substrate for the fatty acyl-CoA transferase must be generated from glycolysis or glyceroneogenesis.

From an overall perspective, excess dietary CHO sets insulin into a state of overdrive to synthesize excess fat and to store the fat in adipocytes, thereby increasing and maintaining adiposity.

## EVOLUTION POINTS TO LIMITED CARBOHYDRATE CONSUMPTION

The evolution of the genus *Homo* in East Africa took place during a period characterized by high seasonal and longer-term climate variability, associated fluctuations between wood- and grasslands, arid and moisture climates, and food resources [64]. Unstable food resources and changing climates required the exploitation of new foods. Accordingly, the appearance of early *hominids* coincides with an increasing supplementation of a primarily frugivorous (plant-eating) diet with animal meat. Hints of butchering of animals and use of stone tools are currently dated back to more than 3 million years ago [65-66] preceding even the oldest known *Homo* fossils. About 1.9 million years ago [64], early *Homo* gave rise to the species *Homo erectus*, better adapted for walking longer distances and covering larger areas in search of food. The first human species leaving Africa and colonizing greater Eurasia, *Homo erectus* continued to incorporate meat into the diet as inferred from the production of bifacial hand axes used to butcher animal carcasses.

The emergence of modern humans, *Homo sapiens*, around 200,000 years ago saw a dramatic shift in dietary preference. Although the evidence is sparse, archaeological data indicate the

appearance, about 100,000 years ago, of a wide range of new food sources, including birds, fish, and shellfish [67]. It is estimated that modern humans left Africa and began colonizing Europe, Asia, and Australia approximately 50,000 years ago. And it was only 10,000 years ago that sedentary agriculture developed in some human groups. Over several millennia, this lifestyle diffused widely from centers in the Near East, China and Mesoamerica. According to this perspective, we were hunter-gatherers for 95% of our early evolution. Although the data are limited, the best evidence suggests CHO consumption in this period represented only 20-35% of total caloric intake [68]. There was no bread on the table, there were no cakes, puddings, pies, chips, or pasta. Wild fruits or tubers gathered were available only seasonally. In addition, it has been argued that gathered fruits did not measure up in either size or abundance to fruits now available year round. Such fruits may, in addition, have contained significantly less sugar and more fiber [69]. Looking at this pattern against a likely background of frequent periods of intermittent or longer-term fasting, one would have to conclude that low blood glucose and low insulin levels were characteristic features of humans for most of our evolutionary history.

## INSULIN AND KETONE BODY METABOLISM

Under modern conditions of CHO intake, in the well-fed state, the brain uses about 130 grams of glucose per day [70]. During the initial stages of dietary CHO restriction or during fasting, the brain continues to be supplied with glucose by hepatic glycogenolysis and gluconeogenesis, primarily from amino acids from protein breakdown, and to a lesser degree, by glycerol from lipolysis. Continued breakdown of endogenous proteins for gluconeogenesis, however, carries the risk of severe protein loss from muscle, causing ongoing and ultimately fatal damage to the heart and diaphragm. The evolution of ketone body (KB) metabolism promotes a selective survival advantage sparing body protein loss by providing an alternate energy substrate, initially for muscle and, in a few days, for the brain. The KBs,  $\beta$ -hydroxybutyrate, acetoacetate, and the non-enzymatic breakdown product, acetone, derive from acetyl-CoA, primarily from lipolysis of fat tissue. These KBs are synthesized primarily in the liver and are transported to peripheral cells where they are incorporated into the TCA-cycle via reactions catalyzed by a CoA transferase and thiolase:

succinyl-CoA + acetoacetate → succinate + acetoacetyl-CoA

acetoacetyl-CoA + CoA → 2 acetyl-CoA

In essence, KBs provide a method of transporting acetyl-CoA from the liver to other organs, including the brain. The availability of free fatty acid for ketogenesis depends on releasing insulin's inhibition of lipolysis which is accomplished by dietary CHO restriction. Feedback control is exerted by KBs which stimulate pancreatic insulin secretion, albeit to a much smaller extent than glucose, thus controlling fatty acid generation. Loss of this control due to the absence of insulin gives rise to the ketoacidosis of uncontrolled type 1 diabetes).

## BACKGROUND ON CANCER

Current theories on cancer describe an initial injurious event leading to a series of genetic mutations. Over-activation of oncogenes and/or loss of function in tumor suppressor genes must accumulate in order to transform a normal cell into one which displays rapid cell-division, growth, and immortality, the hallmarks of cancer. While we think of growth and proliferation as the major characteristics of life, the real demands on the living state come from keeping these processes under control. Hanahan & Weinberg's widely cited hallmarks of cancer [2,1], all describe a failure to control growth and replication (Figure 2).

### Classical hallmarks:

- Limitless replicative potential
- Sustained angiogenesis
- Evading apoptosis
- Self-sufficiency in growth signals
- Insensitivity to empty growth signals
- Tissue invasion & metastasis

**Figure 2:** Acquired Capabilities of Cancer as described by Hanahan & Weinberg [1] and Hanahan & Weinberg [2]

From an evolutionary perspective, the genetic mutations in pre-cancerous cells can be thought of as re-activating an ancient genetic program that has been switched off, regulated or silenced in contemporary animals. In this sense, tumors can be viewed as an atavism, an evolutionary back-transition towards the first simple multi-cellular organisms [71]. In the context of this paper, cancers adopt an abnormal metabolic reprogramming: reduced mitochondrial function and increased reliance on glycolytic metabolisms. Not included in the original hallmarks, the dependence of glycolytic mechanisms has emerged as a key feature of many cancers. Fermentation of glucose represents an ancient biochemical mechanism employed by prokaryotes at least 3.5 billion years ago [72] when oxygen concentrations in the atmosphere were low [73]. Some authors have argued that this feature alone explains most if not all, the other hallmarks, thereby classifying cancer as a metabolic disease [74]. The theory is supported by evidence that the metabolic environment drives the evolution from mutations within mitochondrial DNA toward a cancerous state [75].

In addition to avoidance of initiating events, repair of unavoidable random cellular damage and removal of potentially cancerous cells becomes critical in prevention. Adaptive mechanisms in humans include the various DNA damage repair methods and ultimately, the cellular suicide process known as apoptosis. Given an order of  $10^{13}$  cells in our body, however, errors in repair are inevitable and will be passed on to subsequent generations of cells, accumulating DNA mutations as we age. Indeed, careful organ autopsies of people who have died from non-cancer causes have revealed high rates of indolent tumors that remained quiescent, causing no symptoms in the patient's life [76,77]. It should be noted, however, that factors permitting and provoking tumors toward aggressive behavior, removing the “brakes” on cell growth, proliferation and metastasis, are hyperglycemia and the associated hyperinsulinemia [78–81].

Metabolic abnormalities appear to contribute to genomic mutation and instability. High blood glucose concentrations – especially those experienced during spikes in poorly-controlled type 2 diabetics – stimulate production of free radicals and especially reactive oxygen species (ROS)

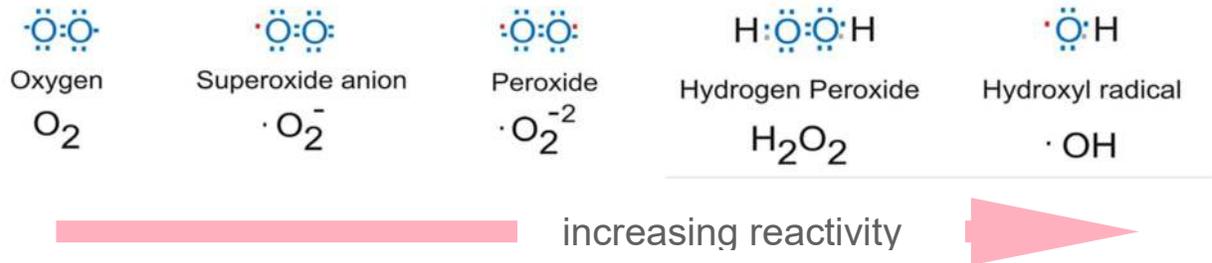
[18,19,82]: Figure 3). Hyperglycemia induces increased ROS in breast cancer cell lines [17,83] presumably contributing to genomic instability. Cancer cells appear to protect themselves from intrinsically high steady state levels of ROS by increasing the rate of glycolysis and activation of the pentose phosphate pathway, both resulting in production of high levels of the anti-oxidative pyruvate and lactate as well as NADPH, stabilizing reduced glutathione [84,85]. Starving such cells of glucose breaks down this defense mechanism and leads to cell death via ROS [86,87]. The overexpression of uncoupling protein 2 in cancer cells has also been proposed as a mechanism to mitigate ROS damage (see section on uncoupling proteins below).

## THE WARBURG EFFECT AND MITOCHONDRIAL FUNCTION

Warburg's original observations that rapidly growing cancers produced lactate even in the presence of oxygen led him to believe that this was a universal feature of all cancers [88–90]. While not true in all instances of cancer — most prostate cancers, for example represent an exception [91,92] — the Warburg effect has provided a crystallizing point in research into energy metabolism, mitochondrial function, reactive oxygen species, and the utility of PET scanning in cancer. While it is unlikely that the glycolytic profile that appears in different tumors has a single cause, several important hypotheses based on disruption in mitochondrial function and/or uncoupling of mitochondrial activity from energy production, have been proposed to explain the origin of the Warburg effect [16,93,94].

Mitochondrial DNA (mtDNA; 16,569 base pairs) codes for many of the components of oxidative phosphorylation including subunits of three proton translocases, complexes I, III and IV and complex V, the ATP synthase. Mutations, depletions or abnormalities in mtDNA have been identified in many cancers [75, 95–105]. Mitochondrial dysfunction further enhances production of the free radicals and ROS that are by-products of the redox reactions of electron transport (Figure 3)

Redox reactions can produce a variety of chemically reactive free radical compounds, the most abundant being ROS, which may be increased by mitochondrial dysfunction. See Figure 3.



**Figure 3:** Common forms of reactive oxygen species

### The role of uncoupling proteins

In addition to mitochondrial components that are directly involved in aerobic energy production, mechanisms of mitochondrial coupling and metabolic responses are candidates for explaining the tendency of rapidly growing cancers to by-pass aerobic oxidation even under aerobic conditions.

The free energy of aerobic oxidation is captured as a proton gradient across the inner mitochondrial membrane. Dissipation of the gradient is coupled to synthesis of ATP *via* the complex V, the ATP synthase. The process is not perfectly efficient and the loss of usable energy is described as proton leak or uncoupling, analogous to the action of small molecule uncouplers such as 2, 4-dinitrophenol or FCCP, which are protein ionophores. Uncoupling *in vivo* is mediated by several (currently 5) uncoupling proteins [106]. The wasted energy is dissipated as heat and the uncoupling protein 1 (UCP1) – the first discovered in mammals – functions as a mediator of non-shivering thermogenesis in brown adipose tissue. Brown adipose tissue is widely distributed in mammals and newborn humans [107].

The biological roles of UCP 3, 4 and 5 are quite speculative at present. UCP2, while ubiquitous in human cells, is measured at very low levels in most normal tissues, but is expressed at higher

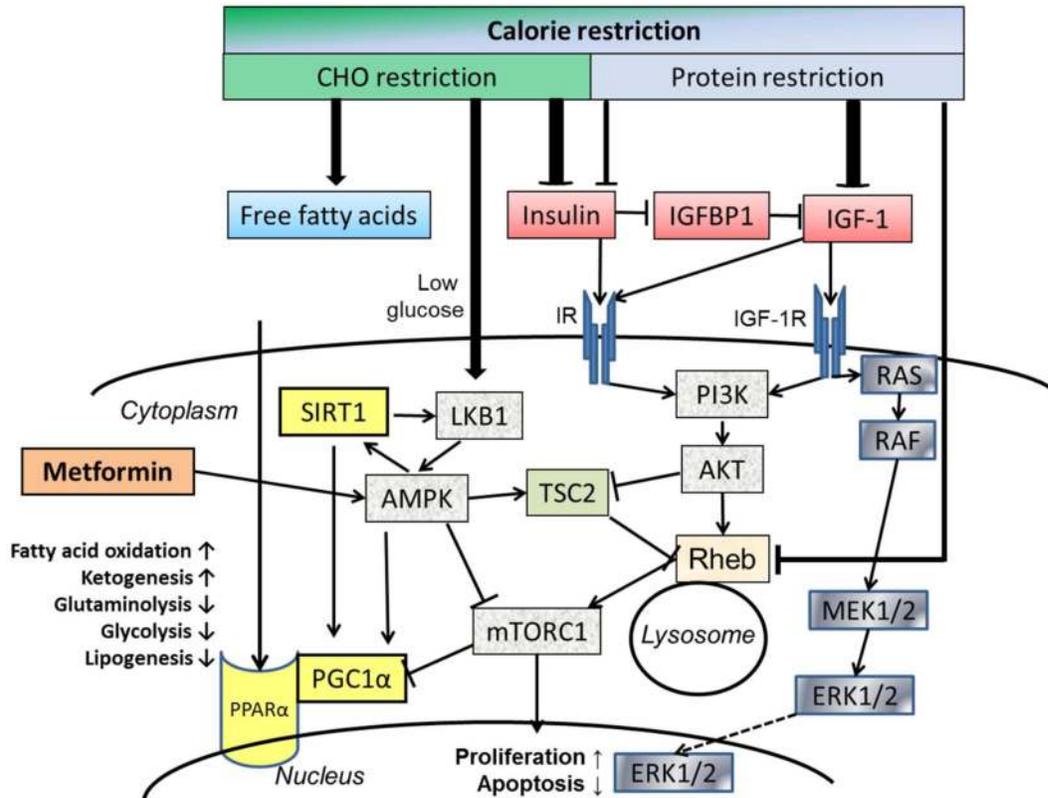
levels in regions of infection, inflammation [108,109] and malignant transformation [22,110–112]. It has been proposed that UCP2 acts in response to elevated levels of ROS, moderating and reducing their chemical reactivity [113,114]. Co-incubation of KBs with glucose-medium in cultured fibroblast lines (RFP3, MCH 064 and MCH065) induces a 30% decline in UCP2 expression compared with glucose medium alone [21]. ROS were not directly measured, but these data are consistent with KB-induced suppression of ROS in normal tissues and a reduced mutagenic microenvironment. It should be noted that cancer lines had much greater variability after the addition of KBs, with reduced UCP2 seen in MDA MB 231 and MCF 7 (40% and 15%, respectively), but increased UCP2 in some colon cancer lines (LoVo CaCO<sub>2</sub>), and reductions in others (RKO, SW48 and SW480). The significance of these findings is less clear. The most consistent description is that the response of UCP2 to ROS must be as complex as the ROS response itself. Since ROS function as normal signaling molecules as well as toxic free radical species, as modulators of both growth and death it should not be surprising that this will remain an interesting avenue of investigation moving forward.

### **Cell signaling pathways**

Recent studies have shown a direct role for elevated blood glucose levels in promoting oncogenic transformation of cells through the WNT/ $\beta$ -catenin pathway [78] or the EPAC/RAP1 and O-GlcNAc pathways [79]. High blood glucose also acts indirectly as a growth stimulus through its effect on insulin release that in turn increases the bioavailability of IGF-1 and IGF-2 through hepatic down-regulation of IGF binding proteins [20]. The direct effect of insulin/IGF signaling is activation of the insulin receptor, IGF-1 receptor and hybrid insulin/IGF-1 receptors. These in turn activate the PI3K–AKT–mTOR pathway as well as the Ras-Raf-MEK-ERK pathway (Figure 4), both of which stimulate cell growth, proliferation and cell survival [20,115]. Multiple modulators of these pathways exist, including calorie intake, CHO and protein restriction or drugs such as metformin, which down-regulates PI3K signaling through AMP kinase stimulation [116].

The regulatory effect of metformin has led to several current clinical trials testing its combination with chemotherapy and radiation therapy. Metformin has been quite safe in most clinical applications, but nonetheless poses a small risk of hypoglycemia and lactic acidosis.

However, from the perspective of the data presented here, the same effects could be achieved with a ketogenic diet without adverse side effects and without the need for a medication.



**Figure 4: Multiple tumor progression and survival pathways are activated via the Insulin/IGF axis.** Dietary restriction in the form of overall CR or specific restriction of CHO or protein has specific effects on the insulin/ IGF-1 system that transduces cellular signals through its insulin and IGF-1 tyrosine kinase receptors. This picture only provides a partial overview of the complexity of this signaling network. The classical action of activated ERK1 and ERK2 is their translocation into the nucleus where they activate mitogenic transcription factors. Similarly mTORC1 targets transcription factors that increase proliferation and counteract apoptosis. Activation of mTORC1 via IR/IGF-1R-PI3K-AKT converges with its activation by amino acids at the lysosomal membrane. There, the guanine nucleotide exchange factor (GEF) Rheb stimulates activity of mTOR which belongs to the mTORC1 complex. In contrast, a lack of growth signals activates the tumor suppressor tuberin (TSC2) which translocates to the lysosomal membrane and inhibits the Rheb-stimulated activation mTORC1. High insulin levels activate AKT which phosphorylates and inactivates TSC2, whereas CR or glucose withdrawal induce energy stress, decrease the intracellular ATP/AMP ratio

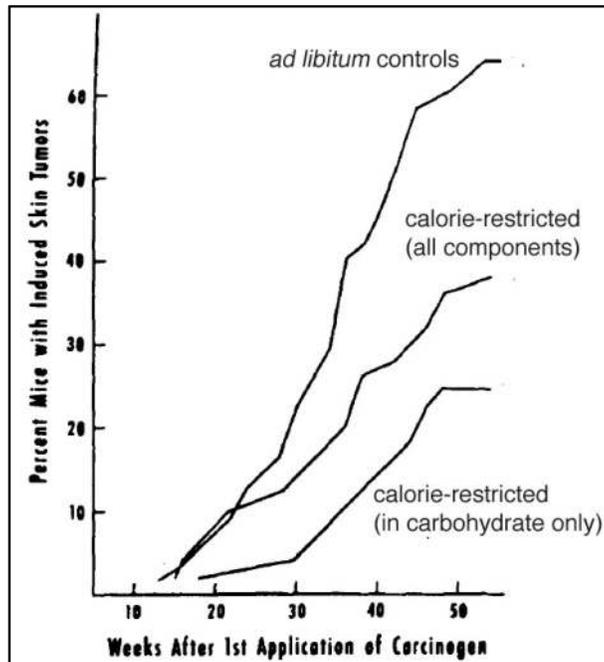
and activate TSC2 through liver kinase B1 (LKB1) – adenosine monophosphate-activated protein kinase (AMPK) signaling. AMPK can also directly inhibit mTORC1 by phosphorylating the regulatory-associated protein of mTOR (Raptor). AMPK has similar actions to the class III histone deacetylase SIRT1 which is a NAD<sup>+</sup> - dependent enzyme that is also activated under calorie or CHO restriction-induced energy stress through an increase in the NAD<sup>+</sup>/NADH ratio. AMPK and SIRT1 amplify each other and both activate the peroxisome proliferator activated receptor gamma 1 $\alpha$  co-activator (PGC-1 $\alpha$ ) protein that cooperates with peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ) to induce major metabolic shifts such as up-regulation of lipid oxidation and down-regulation of glycolysis. mTORC1, stimulated by insulin/IGF-1 signaling, inhibits these actions. Taken and modified from Ref. [20]

## CALORIC AND CARBOHYDRATE RESTRICTION IN CANCER PREVENTION

As early as 1909, it was shown that under-feeding could inhibit the growth of a transplanted tumor in mice [3]. In 1914, not long after he had discovered that an avian sarcoma was caused by a virus, Peyton Rous, using dietary calorie restriction (CR), was able to repress spontaneous tumor development in mice, along with reduced growth of transplanted tumors [117].

Over the next century, the effect of CR on cancer as a preventative means and potential treatment has gone in and out of fashion. In 1945 Tannenbaum found that the appearance of sarcoma induced in mice by addition of the carcinogen benzpyrene was repressed by CR of all macronutrients [5] (Figure 5). If the calories were reduced by restricting CHO alone, the effect was even more pronounced [4,118].

The impressive cancer preventive effects of CR in animal models have been confirmed through a meta-analysis [119] evaluating studies on spontaneous breast tumors in mice published between 1942 and 1995. CR led to an average decrease of 55% in tumor development in CR fed mice compared to *ad libitum* fed controls. A recent meta-analysis estimated a pooled odds ratio of 0.2 (95% confidence interval 0.12-0.34) for a lower tumor incidence after carcinogenic interventions in CR fed mice compared to *ad libitum* feeding [120]. In total, 40 out of 44 studies (90.9%) revealed the tumor-inhibitory effect of CR in laboratory animals with respect to tumor incidence, progression or metastasis. Interestingly, eight out of nine preclinical studies evaluated in this meta-analysis showed that a ketogenic diet was also able to slow down tumor growth, often even as monotherapy.



**Figure 5:** The formation of induced skin tumors and the effect of under-feeding. Modified from Tannenbaum (1945) [4].

Traditional experimental studies described as calorie-restriction (CR) or as, energy-restriction take the reduction in calories as the *cause* of the cancer inhibition. This is unfortunate and inherently misleading because CR can be accomplished in many ways, permitting manipulation of any or all of the *three* macronutrients – proteins, CHOs, and fat. Numerous studies describing cancer inhibition as due to energy or calorie restriction, achieved this goal by exclusively limiting CHO [121–124] or protein [125,126] consumption. The rationale of carbohydrate restriction, however, follows the idea that glucose, directly or indirectly, through the effect of insulin and other hormones, plays a dominant catalytic role biasing the disposition of other nutrients. In the case of energy utilization, the level of carbohydrate will determine how much of all the substrates are oxidized or stored. So, as described above, a hyperglycemic, hyperinsulinemic state is likely to exert substantial control over the emergence of the cancerous state. The impression that CR,

i.e. the reduction of calories themselves, “caused” the inhibition is therefore inaccurate and misleading.

More explicitly, calorie (or energy) restriction has been accomplished by proportional reduction of all macronutrients [97], or, in some cases, an absence of specification is usually taken to signify proportional reduction [127,128]. This experimental design has hazards, as proportional macronutrient reduction is an ambiguous concept whose effects cannot be assumed to be independent of the macronutrient *baseline constituents*. A diet composed of 55-70% CHO composition, recommended by the USDA until only recently, is quite different from one of 10-35% CHO, and even further removed from a ketogenic diet (of less than 10% CHO). Most of these proportional trials have been performed in mice and rats, where total CR of standard chow (55-70% CHO for rodents) represents *de facto* limitation of CHO, the predominant macronutrient.

Kalaany et. al. [97] reported that cancer proliferation was inhibited in wild-type mice after CR (proportional). Further they showed that this inhibition was reversed in genetically altered mice resulting in constitutive activation of the PI3K pathway. Since the PI3K pathway is directly downstream of the insulin receptor, the data strongly point to the dominant role of insulin inhibition by dietary CHO restriction in the wild-type cancer results. Unfortunately, this was not mentioned in the discussion of this article, and the semantics of nutrient and dietary restriction continue.

Ambiguities continue to propagate in the literature. As a result, ‘calorie restriction’ remains popular among approaches to cancer control [129]. We therefore re-emphasize that the insulin/IGF-1 pathway is activated principally by dietary consumption of CHO, and glucose is most often the preferred energy substrate for cancer cells due to metabolic dysfunction, as described above [90,130,10]. Protein consumption also stimulates this pathway albeit to a lesser extent than glucose in humans [20]. Mechanisms of protein activation of this pathway include the stimulation of pancreatic insulin and hepatic glucose production [131,132] as well as a general activation of mTORC1 by amino acids [133]. Intermittent fasting, a low-CHO or low-protein diet, or a ketogenic diet – all dietary elements which humans have often experienced over hundreds of thousands of years of evolution – all reduce insulin/IGF1 signaling. A reduction in blood glucose

levels via CHO restriction has been shown in cancer patients with *ad libitum* eating [134]. However, other studies failed to observe such an effect in cancer patients [135]. We point out that dietary CHO restriction results in normal range glucose concentrations (60-100 mg/dl) for the vast majority of people. Since most cancers over-express glucose 1 transporters, whose rate of glucose uptake is saturated at levels of 45 mg/dl, it is unlikely that blood glucose reduction has a direct effect on limiting cancer growth, so indirect effects via insulin/IGF1 signaling appear much more important.

For instance, a reciprocal association between blood glucose and KB levels has been shown in both mice [136] and human cancer patients [135]. The significance of high KBs lies in their ability to retard tumor growth [137–140]. For example, KBs inhibit glycolysis in both normal and cancer cells, but while normal tissues easily switch to KBs and fatty acids for fuel when glucose is limited, cancer cells in general cannot compensate for energy loss when glycolysis is impaired [21,22]. This is supported by a clinical trial showing that strict CHO restriction with ketosis can result in the down-regulation of glycolysis in some cancer patients with associated slower tumor growth as measured by imaging procedures [141].

$\beta$ -hydroxybutyrate, the principle KB manufactured by the liver – and to a lesser extent acetoacetate – has also recently been shown to be a histone deacetylase (HDAC1, 3 and 4) inhibitor [23]. Through this action,  $\beta$ -hydroxybutyrate promotes the hyperacetylation of histone proteins and induces a stress response which promotes autophagy and inhibits mTOR signaling [25].

Thus it becomes clear that methods to minimize circulating insulin and spikes of glucose as well as mediators of chronic inflammation may help to decrease the occurrence and progression of cancer. One such method emphasized in this paper is the reduction of dietary CHO intake to levels that are more in line with the likely human diet in the past.

## CONCLUSIONS: MOVING FORWARD BASED ON THE PAST

For over 95% of human evolution, the human diet was largely affected by periods of limited or no food consumption, intermixed with periods of plenty but limited CHO intake compared to

contemporary diets. As the evolutionary biologist Theodosius Dobzhansky stated in 1973: "Nothing in biology makes sense except in the light of evolution." Over a century of accumulated data appears to confirm that this statement applies to the effect of diet on cancer prevention.

CR – and more specifically CHO restriction – which were experienced throughout human evolution as normal periods without food and seasonal variation, appears to have been ingrained within human biology as a method to prevent cancer. Support for this hypothesis has been illustrated in ecologic data, epidemiologic data, preclinical and animal data, and more recently in human data.

Such evidence is encouraging both for treating and, more importantly, for preventing cancer. As described throughout this manuscript, a diet that more closely mimics the ancient human diet appears to result in metabolic changes and cellular pathway modulation favoring a physiologic state that is not conducive to cancer induction or growth. These dietary effects should be incorporated and tested in future clinical trials as they may stand as a potent weapon in the fight against cancer.

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