Nutrition And Food Science Journal
ISSN# 2833-440X

Review Article

Nutrition and Cancer: Vitamin E, Selenium and Poly-Unsaturated Fatty Acids as Dietary Modulators of Ferroptosis

Bornkamm GW*

Institute of Experimental Cancer Research, Ulm University, Germany

ARTICLE INFO

Received Date: April 01, 2024
Accepted Date: April 10, 2024
Published Date: April 12, 2024

KEYWORDS

Antioxidants; Ascorbate; Calorie restriction; Cancer cell metabolism; Fasting mimicking diet; Ferroptosis; Glutathione peroxidase 4; Iron; Ketogenic diet; Lipid peroxidation; Metabolic plasticity; Poly-unsaturated fatty acids; Selenium; Vitamin C; Vitamin E; Warburg effect

Citation for this article: Bornkamm GW. Nutrition and Cancer: Vitamin E, Selenium and Poly-Unsaturated Fatty Acids as Dietary Modulators of Ferroptosis. Nutrition And Food Science Journal. 2024; 7(1):143

Corresponding author:
Georg W. Bornkamm,
Institute of Experimental Cancer Research, Comprehensive Cancer Center Ulm, Ulm University, James-Franck-Ring (N27), 89081 Ulm, Germany, Tel: +49 89 8203688;
Email: georg.bornkamm@t-online.de

ABSTRACT

A number of studies over the last decade have firmly established three essential nutrients, vitamin E, selenium and poly-unsaturated fatty acids (PUFAs), as critically important modulators of ferroptosis *in vivo*. Ferroptosis is an iron-mediated mode of cell death brought about by detrimental membrane lipid peroxidation and kept in check by glutathione peroxidase 4 (GPX4). GPX4 and vitamin E complement each other in the prevention of lipid peroxidation, GPX4 by acting as a membrane repair enzyme with selenocysteine in its catalytic center that reduces membrane lipid peroxides into the respective alcohols using glutathione as its reducing substrate. Vitamin E acts as a lipophilic radical trapping agent in the membrane that is regenerated by Ferroptosis Suppressor Protein-1 (FSP1), a glutathione-independent ubiquinone oxido-reductase that catalyzes - besides vitamin E - the regeneration of the radical scavengers ubiquinone and vitamin K under consumption of NAD(P)H. The susceptibility versus resistance to ferroptosis inducers is strongly affected by the membrane composition, i.e. the content of PUFAs and the ratio of unsaturated and monosaturated fatty acids to PUFAs within membranes. Plant oils, algae and fish are rich in PUFAs as essential nutrients. Feeding a selenium-restricted diet along with PUFA supplements may render tumor cells more susceptible to inducers of ferroptosis and more vulnerable to chemotherapy *in vivo*. It may be anticipated that nutrition in the forthcoming decade is going to enter the stage of cancer therapy as an adjunct to chemo- or differentiation-inducing therapies *in vivo*. It is apparently time for a paradigm shift that has to acknowledge that antioxidants and selenium not only protect normal cells, but also incipient and advanced cancer cells from cell death, and render cancer cells more aggressive.

INTRODUCTION

Most patients confronted with the diagnosis of cancer are highly motivated to actively contribute to their physical recovery during and after the cancer treatment regimen and intensively search for opportunities for their personal input. An obvious recommendation of treating oncologists is supervised physical exercise which is generally accepted to impact favorably on quality-of-life-index, tolerance to treatment, and – although much less well documented - overall survival [1,2]. The other factor that patients can influence and control themselves is nutrition. This is a particularly difficult issue as patients are frequently exposed to conflicting information and advice by their physicians and different sources in the internet whose seriosity and quality of information the patients are usually unable to judge. It is indeed

extremely difficult to gain in depth information on the eventual benefits and harms of diets advertised in the internet. Such type of diets include for instance intermittent fasting, time-restricted eating, Fasting Mimicking Diets (FMD), Low Carbon (LCD), Ketogenic Diets (KD), and nutritional supplements.

THE WARBURG EFFECT AND THE METABOLIC PLASTICITY OF CANCER CELLS

The rationale behind all diets restricting carbohydrate intake is the Warburg effect, described by Otto Warburg 100 years ago, an energetically wasteful deviation of the cell metabolism denoted glycolysis towards decreased or halted TCA cycle activity in the mitochondria and increased glucose uptake and anaerobic glucose degradation to lactate in the cytoplasm, even under conditions when oxygen is not limiting [3] (Figure 1).

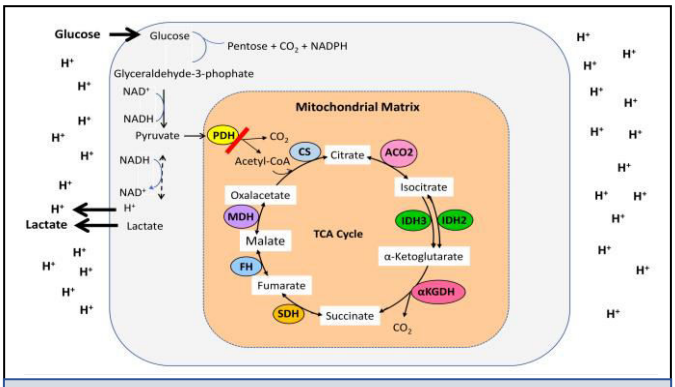

Figure 1: The Warburg effect.
Warburg described 100 years ago that cancer cells preferentially take up glucose and degrade it to lactate even under conditions when oxygen is not limiting, a phenomenon denoted after Otto Warburg. The Warburg effect is the basis af the various "cancer diets" that attempt to restrict the availability of glucose as the main fuel for cancer cell proliferation. We know today that this concept is much too simplistic and that cancer cells display a high degree of metabolic plasticity that allows tumor cells to adapt to various stress conditions including scarcity of many metabolites. Abbreviations: see legend to Figure 2.

As glycolysis is a highly inefficient way of ATP production as compared to oxidative phosphorylation in the mitochondrial electron transport chain, Warburg believed that this is a general hallmark of cancer and that restoration of oxidative phosphorylation and inhibition of glycolysis would eventually cure cancer. Based on the pivotal importance of glucose for the Warburg effect, the ketogenic diet attempts to substitute glucose as the main carbon source with lipids. At first sight, the concept of the ketogenic diet seems logically convincing, but metabolism is more complex and the assumption that glucose is the only fuel for cancer cells does not hold true. A closer look to cancer metabolism is urgently required. The ketogenic diet has the advantage to be generally accepted by the medical community as the standard therapy for therapy-resistant epilepsy in childhood [4], and for genetic GLUT1- [5] and pyruvate dehydrogenase deficiency [6].

With the re-awakened interest in metabolism and biochemistry, cancer metabolism has come into the focus of cancer research since about 20 years. It is becoming apparent that each type of cancer (and eventually even each pathway within one tumor entity that is activated by oncogenes or loss of tumor suppressor genes) has its own metabolic characteristics. The Warburg effect has been frequently misunderstood as a dogma rather than as a mode of metabolic adaptation that provides cancer cells with a well balanced mix of metabolic three-carbon-(C3)-intermediates as precursors for amino acid, protein, nucleotides and membrane synthesis as well as with NADPH generated in the pentose phosphate cycle providing the reducing equivalents for the anabolic processes of cell growth and proliferation [7]. Glycolysis also drives adaptation to hypoxic conditions and increases cell motility, migration and metastasis through upregulation of the transcription factor BACH1 [8]. Glycolysis, to be kept going, requires two essential steps: (i) NAD^+ has to be restored as hydrogen acceptor for glucose degradation by reduction of pyruvate to lactate, and (ii) to maintain the concentration gradient and the flux from pyruvate to lactate, lactate has to be exported from the cell through one of the monocarboxylate transporters (SLC16A family of genes), four of which (MCT1-4, preferably MCT1 and MCT4) expel protons and thus acidify the environment of the cell. Yet, if glycolysis is disabled by mutation of lactate dehydrogenase A, tumor growth in a murine B cell lymphoma models is not affected [9]. We have to envisage cancer metabolism not as a fixed system, but rather as a system of communicating pipes that is well adapted to environmental conditions and flexibly responds to stress conditions like fasting, hypoxia, overexposure to nutrients, or withdrawal of single metabolites. There are a number of essential building blocks like for instance essential amino acids, but there is a high degree of interchangeability amongst carbohydrates, lipids

and, to a lesser extent, amino acids and proteins [10,11]. If one entry into the pool of a given metabolite is closed, there is at least one other to maintain the flux of metabolites for anabolic processes. Furthermore, tumor cells have not to be regarded as a single metabolic system. There is metabolic heterogeneity due to differences in cellular composition, distance from blood vessels, metabolic gradients, and local differences in oxygen partial pressure and pH. One type of cells may secrete lactate due to active glycolysis, whereas other cells in the neighborhood may take up lactate, convert it to pyruvate by lactate dehydrogenase and NAD^+ and introduce it into the TCA cycle for degradation and ATP synthesis, provided that sufficient oxygen is available [12,13]. Acidic pH furthermore reprograms fatty acid metabolism by histone and mitochondrial acetylation towards increased fatty acid oxidation in mitochondria and concomitant fatty acid synthesis in the cytoplasm [14]. Hypoxia dampens oxidative phosphorylation and redirects glutamine into fatty acid synthesis via reductive carboxylation of α-ketoglutarate to citrate, cleavage of citrate to oxaloacetate and acetyl-CoA by ATP-dependent citrate lyase (ACLY), and carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase (ACC1) as the first step of fatty acid synthesis Figure 2).

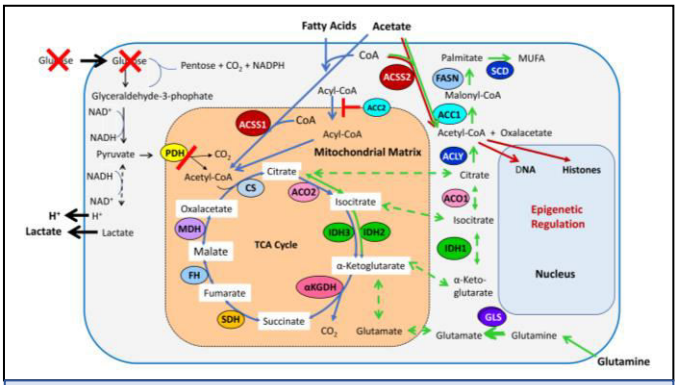

Figure 2: Adaptation of cancer cells to lipid metabolism under glucose restriction and moderate hypoxia.
Cancer cells take up fatty acids and/or acetate as carbon source and meet their energy demands by fatty acid oxidation (blue arrows). Glutamine drives anabolic lipid metabolism by reductive carboxylation of a-ketoglutarate to isocitrate and citrate, cleavage of citrate to oxaloacetate and acetyl-CoA by ATP-dependent citrate lyase (ACLY) in the cytoplasm and carboxylation of acetyl-CoA to malonyl-CoA as the first step of fatty acid synthesis (green arrows). Acetyl-CoA is the central metabolite that drives fatty acid oxidation in the mitochondria, fatty acid synthesis in the cytoplasm, and epigenetic reprogamming of gene expression by histone acetylation in the nucleus (red arrows) according to the metabolic requirements of the cells. Modified after Corbet et al. [14]. Seemingly opposing metabolic functions can be fulfilled simultaneously through compartmentalization of the cell.

Abbreviations: ACC = Acetyl-CoA Carboxylase; Aco = Aconitase; ACSS = Acetyl-CoA Snthetase; αKGDH = α-ketoglutarate dehydrogenase; CS = Citrate Synthase; FASN = Fatty Acid Synthetase; FH = Fumarate Hydratase; GLS = Glutaminase; IDH = Isocitrate Dehydrogenase; MDH = Malate Dehydrogenase; PDH = Pyruvate Dehydrogenase; SCD = Stearoyl-CoA Desaturase; SDH = Succinate Dehydrogenase.

KETOGENIC DIET, CALORIE RESTRICTION, FASTING AND FASTING MIMICKING DIET

With the metabolic plasticity of cancer cells in mind and their ability to adapt to metabolic stress conditions, it is obviously naïve to believe that cancer could be cured or halted by dietary restriction of glucose. In fact, many cancers including AML and glioblastoma rely on fatty oxidation at least when glucose becomes limiting [15-17]. Several types of solid tumors like for instance breast, ovary, prostate, colorectal cancer and also AML satisfy their demands on lipids by fatty acid synthesis [18,19]. The a priori rationale of the ketogenic diet vanishes with the switch from anaerobic glycolysis to fatty acid oxidation and lipid metabolism [17]. But it should be kept in mind that deviation from glucose to lipid catabolism is not the only consequence of the ketogenic diet. During starvation, fatty acids are degraded in the liver. Since oxaloacetate becomes limiting as an acceptor for acetyl-CoA for fatty acid oxidation during starvation, acetyl-CoA accumulates and is enzymatically converted into acetoacetyl-CoA and hydroxymethyl-glutaryl-CoA (HMG-CoA)(for details see Figure 3). HMG-CoA is cleaved into acetyl-CoA and acetoacetate. The majority of acetoacetate is reduced to β-hydroxybutyrate (BHB) and secreted into the blood stream. Peripheral organs (brain, heart, muscles, kidneys) take up the circulating acetoacetate and BHB, re-oxidize BHB to acetoacetate in the mitochondria, activate it by transferring CoA from succinyl-CoA to acetoacetate (a step the liver is unable to do), and cleave it into acetyl-CoA that is fueled into the TCA cycle. The brain preferentially uses glucose as fuel and is unable to break down fatty acids for ATP production. Therefore, ketone bodies play a pivotal role in supplying energy to the brain at periods of starvation, for instance at night during sleep or during exercise [Owen et al., 1967, Puchalska and Crawford, 2017)] [20,21]. Glucose, leptin and insulin levels are lower in individuals on a ketogenic diet with increased energy expenditure and insulin tolerance is improved [26]. Remarkably, β-hydroxybuyrate has recently

been reported to suppress colorectal cancer in a chemical carcinogen-induced as well as in a genetic murine colorectal cancer model through a receptor-mediated pathway altering

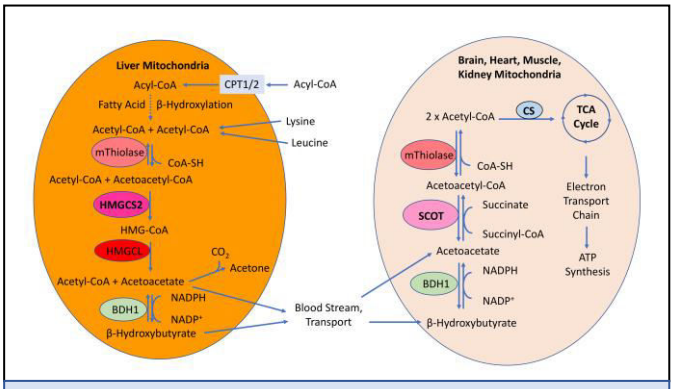

Figure 3: Ketone body metabolism during starvation.
Lipid breakdown during starvation leads to an increase of fatty acids in the liver that cross the mitochondrial membrane through the action of carnitine palmitoyl-transferase 1 and 2 and are degraded inside the mitochondria by the fatty acid β-hydroxylation pathway. Catabolism of lysine and leucine contributes to the formation of ketone bodies. As oxaloacetate as an acceptor for the endproduct acetyl-CoA in the liver is limiting, acetyl-CoA accumulates. Two or three molecules of acetyl-CoA condensate to actetoacetyl-CoA and hydroxymethyl-glutaryl-CoA (HMG-CoA) catalyzed by mitochondrial thiolase and hydroxymethyl-glutaryl-CoA synthase 2 (HMGCS2). HMG-CoA is cleaved to acetyl-CoA and acetoacetate by hydroxymethyl-glutaryl-CoA lyase (HMGCL). Acetoacetate (AA) is reduced by β-hydroxybutyrate dehydrogenase (BDH1) to β-hydroxybutyrate BHB) under NADPH consumption (left hand side). The liver is an organ that is able to build up, but not to degrade ketone bodies, whereas peripheral organs oppositely are able to degrade, but not to synthesize ketone bodies. A fraction of acetoacetate spontaneously decays into CO_2 and acetone, responsible for the particular smell of diabetic patients in hypoglycemic coma. AA and BHB leave the liver via monocarboxylic acid transporters, are released into the blood stream and are taken up by peripheral organs (brain, heart, muscles, kidneys) as readily oxidizable fuel for energy production (right hand side). In the mitochondria of peripheral organs β-hydroxybutyrate is re-oxidized to acetoacetate by BDH1, coupled to CoA by succinyl-CoA-transferase (SCOT=OXCT1), cleaved to acetyl-CoA by mthiolase and shunted into the TCA cycle for ATP production. mThiolase is a complex of several enzymes. Besides acting as the pivotally important fuel for energy production during starvation (in particular for the brain), acetoacetate and β-hydroxybutyrate act as ligands for G-protein-coupled receptors and as epigenetic modifiers of chromatin modulating transcription through acetylation and β-hydroxybutyrylation of histones. Cytoplasmic lipogenesis and cholesterol synthesis are non-oxidative branches of ketone body metabolism. Modified after Puchalska and Crawford (Puchalska and Crawford, 2017).

Furthermore, low energy status and ketone bodies have furthermore been shown to enhance anticancer immunity in murine cancer models by several mechanisms: (i) AMPK-mediated phosphorylation and degradation of PD-L1, the ligand of the T cell immune checkpoint receptor PD1, on antigen presenting cells, (ii) AMPK-mediated phosphorylation of the transcription factor EZH2 and inactivation of the polycomb repression complex 2 (PRC2) leading to increased expression of type I interferons and MHC class I genes [28], and (iii) by changing the gut microbiota which results in increased production of β-hydroxy-butyrate and blockade of checkpoint signaling through PD-L1 [29]. In a first in-human clinical trial (NCT03340935) cycling periods of five days on a plant-based, calorie-restricted, low carbohydrate, low protein fasting mimicking diet were well tolerated and reshaped the immune system towards contraction of peripheral blood immunosuppressive myeloid and regulatory T-cell compartments and towards enhanced intratumor cytotoxic Th1 responses and an enrichment in IFNγ activating signatures in tumor-infiltrating immune cells (Vernieri et al., 2022).

Finally, amino acids and acetyl-CoA are shunted into the synthesis of neurotransmitters with inhibitory neurotransmitters prevailing (γ-amino byturate, GABA) thus providing a rationale for the success of the ketogenic diet in the treatment of childhood epilepsy [30,31].Thus, even if substitution of glucose by lipids in the ketogenic diet does not expose the obvious vulnerability previously intended, there are other mechanisms that my impact favorably on the course of the disease.

Fasting and fasting mimicking diets are based on the concept that rapidly proliferating tumor cells must synthesize DNA, RNA, proteins and cellular membranes and therefore require more precursors as building blocks for the synthesis of macromolecules than resting cells. Thus, withdrawal of the fuel may stop the engine from running. Withdrawal of nutrients activates AMPK, downregulates the nutrient sensor mTORC1 and induces autophagy. By their higher demand, tumor cells become preferentially sensitized to chemotherapy or irradiation. The concept of introducing a fasting mimicking diet as an adjuvant to cancer therapy has been intensively pursued by Nencioni and Longo and their collaborators for more than a decade as an extension of the well documented observation that calorie restriction prolongs longevity, fights obesity and

diabetes type 2, and improves the quality of life [32-35]. At March 13, 2024, 16 clinical trials were registered at the NIH with different endpoints under the terms "fasting/fasting mimicking diet and cancer", 10 under "calorie restriction and cancer", and 54 under "ketogenic diet and cancer".

NUTRITIONAL RECOMMENDATIONS OF OFFICIAL CANCER INSTITUTIONS

There is a steadily growing interest in dietary regimens or factors affecting the growth of cancer cells *in vivo* as revealed by the dramatic increase in the number of publications over the last years. Yet, the optimism with which eventual cancer diets are advertised in publications stands in stark contrast to the recommendations of official institutions as for instance the World Cancer Research Fund WCRF), the American Institute for Cancer Research (AICR), the International Agency for Research against Cancer (IARC), and the German Cancer Research Center (DKFZ). They have formulated ten points for food intake and life style that are equally recommended for cancer prevention as well as for cancer patients: (i) maintain a normal body weight, (ii) stay physically active, (iii) eat mainly fiber-rich unprocessed whole grain products, vegetables, fruits and beans, (iv) avoid fast food rich in processed carbohydrates, fats and sugar, (v) restrict the consumption of red meat, (vi) avoid or restrict drinks with sugar and (vii) alcohol, (viii) do not use food supplements, (ix) and, addressed to mothers, breast feed your babies, and (x) after diagnosis of cancer: follow the recommendations of your oncologist and/or food consultant along the recommendations of the Cancer Societies. An exception to (viii) may be daily supplementation with vitamin D3 because many cancer patients exhibit vitamin D3 deficiency [36,37].

All official cancer institutions state that cancer cannot be cured by special diets. They deliberately discourage patients to rely on a specific type of type diet like calorie restriction, fasting and fasting mimicking diets or a ketogenic diet for one obvious reason: all these diets harbor the risk of malnutrition and unwanted weight loss which may unfavorably impact on the course of the disease and cancer treatment, and which may accelerate cachexia in the final stage of the disease. Clinical trials studying the impact of specific cancer diets have enrolled still a fairly low number of patients, have suffered from low patient compliance, low adherence of the patients to the diet, and are difficult to compare with regard to type of cancer, disease history, variables in the protocol like duration, patient's age, health status and body weight at the onset of the trial [38,39]. It is important to note that there is no other way than to study specific cancer diets in clinical trials with professional supervision of the metabolic state of the patients. Even if preclinical and first clinical trials appear promising, the side effects and risks of specific cancer diets may outpace the benefits as long as the number of patients is limited and the results of clinical trials are ambiguous.

FOOD SUPPLEMENTS: ANTIOXIDANTS PROTECT NOT ONLY NORMAL CELLS BUT ALSO INCIPIENT TUMOR CELLS

Cancer arises by accumulation of mutations or epigenetic changes of genes positively promoting or negatively controlling cell proliferation and/or survival (oncogenes and tumor suppressor genes) and/or differentiation. Mutations accumulate continuously during DNA replication and cell division as a consequence of oxidative DNA damage, but they are immediately repaired by a multitude of activated stress response pathways and DNA repair enzymes that steadily operate as tumor suppressor genes. Given the importance of oxidative DNA damage for the development of cancer, antioxidants are generally assumed to operate as cancer preventive agents. Therefore, daily consumption of vitamins and antioxidants as food supplements has gained quite widespread popularity.

It was thus surprising that supplementation of the food with Vitamin E and β-carotene (the most abundant precursor of vitamin A [retinol]) in male smokers had no favorable effect on cancer incidence and overall survival [40,41]. β-carotene supplementation increased rather than decreased the risk of lung cancer and reduced the overall survival. Lung cancer incidence was not altered in the vitamin E group, but Vitamin E supplementation increased the incidence of prostate cancer [42]. Bergo and collaborators were the first who systematically studied the role of antioxidant supplementation (N-acetyl-cysteine [NAC] and vitamin E) for cancer incidence and metastasis in genetically engineered murine models of B-Raf and Kras-driven lung cancer and found pronounced tumor promoting activity for both antioxidants. Antioxidant supplementation reduced the expression of endogenous

antioxidant genes, accelerated cell proliferation and reduced ROS production, DNA damage and p53 expression [43]. Likewise, NAC and the water-soluble vitamin E analogue Trolox suppressed expression of endogenous antioxidant genes, shifted the GSH/GSSG ratio towards GSH and strongly increased the frequency of metastases in a B-Raf-driven genetically engineered conditional murine melanoma model [44]. The simplest explanation for these findings is that antioxidants protect not only normal cells from DNA damage, they also protect incipient tumor cells which are likely to be present in the group of male smokers as well as in the genetically engineered murine oncogene-driven cancer models. Antioxidant supplementation apparently suppresses an important stress response checkpoint that controls cell cycle arrest and provides an opportunity for efficient DNA repair [43]. Mechanistically, antioxidants promote migration by activating RHOA [44] and stabilize the transcription factor BACH1. BACH1 downregulates antioxidant genes induced by NRF2 and upregulates genes involved in glycolysis like Hexokinase 2, GAPDH, and the lactate transporter MCT1 [8,45].

The double-edged sword of antioxidants is further corroborated by a large number of studies dealing with the oxidative stress-induced transcription factor NRF2. NRF2 is continuously targeted to degradation by binding to its negative regulator KEAP1. Oxidation of two critical cysteine residues in KEAP1 liberates NRF2 and allows NRF2 to migrate into the nucleus. In conjunction with its coactivator MAF, NRF2 binds to antioxidant response elements (ARE) (also denoted electrophile response element, EpRE) in the promoters and activates transcription of a large panel of antioxidant genes. NRF2 is a stress response system that protects normal cells and organism from oxidative damage, malignant transformation and cancer development [45-47]. Yet, when activated in tumor cells it confers its protective potential also to tumor cells and renders them more aggressive and resistant to chemotherapy [48,49].

As mentioned above, oncologists and professional nutritional consultants recommend their patients not to use food supplements, but rather to stick to unprocessed food rich in fibers, grains, vegetables, salads and fruits, and poor in red meat with the potential exception of Vitamin D3 [36]. This type of food contains sufficient amounts of antioxidants in the form of vitamins (vitamin A, C, E, K), trace elements like selenium, copper and molybdenium and plant-derived redox-active compounds which may act directly as antioxidants or may activate the transcription factor NRF2 which induces expression of a whole battery of antioxidant genes. More work is needed to understand what makes the difference between intake of antioxidants as a mix of supplements or as well balanced food. It seems plausible that vegetables contain not only plant antioxidants, but also NRF2 inducers in the form of polyphenols. Thus, under natural conditions antioxidants taken up with the food may not downregulate the endogenous antioxidant response. Another, although not exclusive possibility is that both conditions of antioxidant intake impact differently on the gut microbiota. It is interesting in this respect that antioxidant supplementation in healthy individuals precludes the health promoting effect of physical exercise in terms of glucose tolerance and induction of the antioxidant response in muscles [50].

THE CONTROVERSIAL CASE OF VITAMIN C AS A HIGH DOSE FOOD SUPPLEMENT AND ITS RECENT RE-EMERGENCE AS A SUPPORTIVE ANTICANCER DRUG

In the 1970-ies Cameron, Campbell and the double Nobel laureate Linus Pauling advertised high doses of vitamin C as palliative, and in rare cases even as curative treatment for patients with advanced cancer [51-53]. The reported beneficial action of high dose vitamin C has been challenged by Creagan, Moertel and collaborators and could not be confirmed in two randomized double blind clinical trials [54]. According to Chen et al.(Chen et al., 2007), the controversial results may have been caused by oral as well as intravenous administration of vitamin C in the original studies of Cameron, Campbell and Pauling, whereas in the randomized double blind clinical trials of Creagan & Moertel vitamin C was only given orally. The stage was set back to zero by Padayatty et al. who showed that it is impossible to achieve the desired high concentration of vitamin C in vivo when vitamin C is applied orally. Only the intravenous route established a sufficiently high concentration of vitamin C in vivo (Chen et al., 2007; Nauman et al., 2018; Padayatty et al., 2004). A number of recent studies have re-emphasized the notion that vitamin C in high

dose is selectively toxic to tumor cells (while sparing normal cells) and has elucidated the mechanism of action (Di Tano et al., 2020; Ferrada et al., 2023; Jankowski and Rabinowitz, 2022; Ma et al., 2014; Schoenfeld et al., 2017; Xia et al., 2017; Yun et al., 2015). Two not mutually exclusive mechanisms have been described to contribute to the selectivity of vitamin C for tumor cells: (i) the high demand of iron by tumor cells which leads to an increase in the labile iron pool [58,59], and (ii) the high expression of the glucose transporter GLUT1, one of the hallmarks of the Warburg effect, which is responsible for the uptake of dehydroascorbate (DHA), the oxidized form of vitamin C (ascorbate) [61]. High doses of vitamin C in the presence of transition metals exert pro-oxidant activity, reduce oxygen to hydrogen peroxide and oxidize ascorbate to DHA Chen et al. (2007) [62]. Hydrogen peroxide diffuses into the cells, whereas DHA is taken up by GLUT1 [61]. H_2O_2 is intracellularly reduced mainly by glutathione peroxidase 1 (GPX1) and not by GPX4, the gate keeper of ferroptosis under consumption of NADPH. When NADPH becomes limiting, the ratio of reduced to oxidized glutathione shifts towards the oxidized form. The dominant role of GPX1 as opposed to GPX4 was confirmed in cells in which either GPX1 or GPX4 had been deleted. The crucial importance of GPX1 appears plausible as ascorbate generates predominantly soluble intracellular H_2O_2 rather than membrane bound lipid hydroperoxides. Because many of the redox regulating enzymes including most glutathione peroxidases and thioredoxin reductases are selenoproteins, selenium depletion *in vitro* enhances the toxic action of ascorbate on tumor cells. Dietary selenium restriction in a xenograft murine tumor model in vivo thus enhances the curative action of ascorbate and prolongs survival (Jankowski and Rabinowitz, 2022). Likewise, a fasting mimicking diet synergizes with high dose ascorbate in KRAS-driven murine allo- and xenograft colon cancer models by affecting the labile iron pool (Di Tano et al., 2020). The role of DHA in ascorbate-induced tumor cell toxicity is debated. According to Yun et al., DHA taken up into the cell via GLUT1 is intracellularly reduced back to ascorbate by glutathione and oxidized glutathione accumulates. As a consequence, an exposed critical cysteine residue in the active center of Glyceraldehyde-Phosphate Dehydrogenase (GAPDH) is glutathionylated leading to GAPDH inactivation. Inhibition of GAPDH redirects the glycolytic flux towards the oxidative part of the pentose phosphate cycle, i.e. to Glucose-6-Phosphate Dehydrogenase (G6PD) and 6-Phosphogluconate Dehydrogenase (6PGD) and the production of NADPH as an attempt of the cells to counter NADPH depletion. PARP activation and the loss of NAD^+ accelerate energy crisis and cell death ensues [61]. Ascorbate-induced cell death shares with ferroptosis the iron dependency and the cell death opposing function of selenium, but neither GPX4 nor lipid peroxide-scavenging compounds like vitamin E, liproxstatin and ferrostatin were able to rescue tumor cells from ascorbate-induced cell death (Jankowski and Rabinowitz, 2022). Ascorbate-induced cell death and ferroptosis are thus related modes of cell death but they are not identical. As cysteine provides the sulfur for the formation of iron-sulfur clusters, it is not surprising that depletion of cystine/cysteine and ascorbate synergize in the induction of cell death [57]. Based on these detailed mechanistic findings we are presently encountering a revival of clinical trials using high dose intravenous vitamin C administration as an adjunct to chemotherapy (for review, see [55,63].

VITAMIN E: NUTRITIONAL MODULATOR OF FERROPTOSIS

Fibroblasts from $GPX4^{fl/fl}$ mice transfected with a tamoxifen-inducible Cre recombinase-estrogen receptor fusion gene die upon addition of Tamoxifen *in vitro* and cells may be rescued from GPX4-deletion-induced cell death by addition of vitamin E to the culture medium [64]. We stumbled across the crucial role of vitamin E in the regulation of ferroptosis *in vivo*, when we attempted to grow B cells from mice derived from B-cell-specific GPX4 knock-out mice *in vitro* and hematopoietic cells from lethally irradiated mice reconstituted with bone marrow of $GPX4^{fl/fl}$; $Rosa26^{CreERT2}$ mice that had been treated with tamoxifen. Even though the number of B cells was unaltered *in vivo* and the mice lacking Gpx4 in hematopoietic cells exhibited only a mild anemia, we were unable to grow B cells stimulated with CD40 ligand + IL-4 *in vitro* (Tobias Seibt and GWB, unpublished observation) nor hematopoietic colonies from bone marrow cells seeded into semisolid methyl-cellulose media and supplemented with hematopoietic growth factors [65]. We reasoned that the only difference between *in vivo* and *in vitro* conditions is vitamin E that is included in the chow *in vivo* and lacking in the culture medium *in vitro*. In fact,

supplementation of the culture medium with α-tocopherol, the water-soluble form of vitamin E, completely rescued B cell and hematopoietic colony-growth *in vitro*. To further characterize the role of vitamin E *in vivo*, mice were fed a vitamin E-depleted diet either concomitantly with induction of the Gpx4 knock-out by tamoxifen or 6 to 8 weeks after Gpx4 had been deleted. Remarkably, feeding a tamoxifen containing chow for three weeks induced a moderate anemia also in $Gpx4^{wt/wt;Rosa26\text{-}CreERT2}$ control mice. $Gpx4^{wt/wt;Rosa26\text{-}CreERT2}$ control mice completely recovered from this anemia within 6 to 8 weeks, whereas tamoxifen-treated$^{fl/fl}$; $Rosa26^{CreERT2}$ mice remained anemic. This indicates that Cre-mediated DNA damage induced a self-limiting anemia in normal mice [65]. When tamoxifen was administered simultaneously with initiation of the vitamin E deprived diet, mice developed a severe life-threatening anemia and the mice had to be sacrificed. We concluded that vitamin E deprivation potentiates the DNA damaging effect of Cre activation. In order to separate the DNA damaging effect of Cre activation from the effect of the gene knock-out as well as from the contribution of vitamin E deprivation to the phenotype, it is mandatory to let the mice recover from tamoxifen-induced Cre activation and DNA damage, before they are placed on a vitamin E-deprived diet [65].

Having established the importance of vitamin E for any eventual phenotype caused by Gpx4 deletion, we focused our attention onto those knock-out mice in which Gpx4 deletion had not caused any obvious phenotype. This was the case in mice with conditional deletion of Gpx4 in endothelial cells. In these mice vitamin E depletion prior to Cre activation unmasked multiorgan thrombus formation with variable pathologies and 80% lethality depending on the affected organs [66]. Liver-specific $Gpx4^{fl/fl}$; $Albumin^{Cre}$ died of liver failure in the first 48 hours after birth. However, survival could be completely rescued and the liver-specific phenotype could be masked by feeding the mice a vitamin E rich diet. This was reversible and subsequent dietary vitamin E deprivation unmasked the lethal Gpx4 knock-out phenotype of acute liver failure [67]. These findings establish vitamin E as a dietary switch with which the susceptibility versus resistance to ferroptosis can be fine tuned *in vivo* and *in vitro* (for the mechanism of vitamin E action, see Figure 4C). It is too early to state whether this dietary switch will gain practical importance for cancer therapy at some time in the future.

SELENIUM: NUTRITIONAL MODULATOR OF FERROPTOSIS

Another even more powerful modulator of resistance versus susceptibility to iron-mediated cell death is selenium in the diet. As mentioned, this includes the mode of high dose ascorbate-induced iron-dependent cell death related to ferroptosis that is neither rescuable by lipid peroxide scavenging agents nor by Gpx4 [58]. The discovery of selenium as an essential trace element is closely linked to the point discussed above: selenium and vitamin E have been discovered by Klaus Schwarz and collaborators as dietary factors essentially required for prevention of necrotic liver degeneration [68,69]. Selenium is taken up as sodium selenite and incorporated into selenocysteine, the 21st amino acid in a complicated multistep process. In an equally complicated process selenocysteine is incorporated into 25 proteins in humans many of which play essential roles in redox metabolism and in the synthesis of hormones in the thyroid gland. A so called "selenocysteine incorporation sequence" (denoted SECIS element) in the 3-untranslated region of selenoprotein mRNAs instructs the ribosome to incorporate selenocysteine at an opal UGA triplet codon of the messenger RNA instead of terminating translation. There is a strong hierarchy amongst selenoproteins that ensures that the selenoproteins most important for survival are still able to incorporate selenocysteine into the respective selenoproteins when selenium becomes scarce. GPX4 is at the top of the selenoprotein hierarchy to maintain cell survival as gate keeper of ferroptosis. It repairs damaged cell membranes by reducing phospholipid hydroperoxides to the respective alcohols (Figure 4B). The incorporation of selenocysteine instead of cysteine protects the active center of the enzyme from irreversible overoxidation by hydrogen peroxide [70].

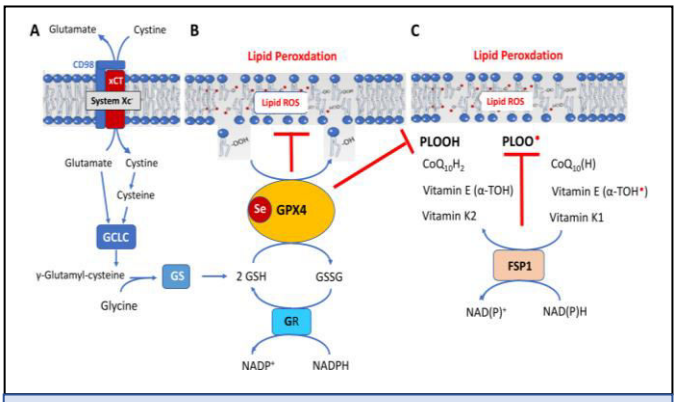

Figure 4: The central cellular axis protecting cells from ferroptotic cell death.
A) The cystine-glutamate antiporter (system Xc^-) imports cystine in exchange for glutamate that is intracellularly reduced to cysteine. Cystine uptake and coupling of cysteine to glutamate by γ-glutamyl-cysteine ligase are the rate-limiting steps for glutathione synthesis.
B) Glutathione is the reducing substrate of glutathione peroxidase 4, a selenoprotein and cell membrane repair enzyme with the unique ability to reduce lipid peroxides in biologic membranes to the respective alcohols in situ. Oxidized glutathione (GSSG) is reduced back to GSH by glutathione reductase at the expense of NADPH. In the absence of glutathione GPX4 accepts cysteine as reducing substrate (Banjac et al., 2008, Mandal et al., 2010).GPX4 stands out at the top of the hierarchy of selenoproteins, i.e. those to which selenium is redirected in vivo under selenium deficiency.
C) Due to their lipophilicity, vitamin E, vitamin K and ubiquinone act as lipid peroxide radical scavenging agents that may interrupt devastating chain reactions leading to membrane disruption and cell death. The NADPH-dependent ubiquinone oxido-reductase FSP1 (Ferroptosis Suppressor Protein-1, FSP1) regenerates the radical reaction chain breakers in a manner independent of glutathione. Depending on cell type and organ, the glutathione-dependent and the glutathione-independent system cooperate in vivo for full protection against ferroptosis. The levels of selenium and vitamin E are amenable to nutritional intervention. Abbreviations: GCLC = γ-glutamyl-cysteine ligase, catalytic subunit, GPX4 = glutathione peroxidase 4, GR = glutathione reductase, GS = glutathione synthetase, xCT = substrate determining subunit of the cystine-glutamate antiporter encoded by the SLC7A11 gene. Modified after Hassannia et al. [100].

Several gene products of the selenium metabolism have been identified as resistance factors and targetable vulnerabilities for the induction of ferroptosis. On top of the list is, of course, GPX4 itself, as master regulator of ferroptosis. Cancer stem cells, cancer persister cells and Acute Myeloid Leukemia cells (AML) are highly dependent on GPX4 [72-74]. LRP8, also known as ApoER2, is the receptor for selenoprotein P and its overexpression confers ferroptosis resistance to neuroblastoma cells with MYCN amplification [75] and to triple-negative breast carcinoma cells [76]. A particular dependency of AML cells for Selenophosphate Synthase 2 (SEPHS2) has been recently described for AML cells [77]. SEPHS2 catalyzes the conversion of selenide and ATP to selenophosphate and AMP, an early step in the biosynthesis of selenocysteine tRNA. Expression of the SEPHS2 gene in AML cells is driven by a super enhancer binding the oncogenic transcription factor MYB. High expression of SEPHS2 is strongly associated with poor prognosis of AML. Most remarkably, leukemic hematopoiesis is more dependent on SEPHS2 expression than normal hematopoiesis. Indeed, in a window for dietary intervention, selenium deprivation in the diet significantly delayed the onset of leukemia and synergized with chemotherapy in various oncogene-driven murine AML models [77]. Likewise, dietary selenium restriction in combination with pharmacological ascorbate treatment significantly prolonged survival of mice in the human U87MG-glioblastoma murine xenograft model [58]. Moreover, dietary selenium restriction prevented plasmacytoma development in mice upon i.p. injection of the mineral oil pristane as well as the pristane-induced inflammatory response that gives rise to plasmacytoma development [78].

There are only few reports on dietary selenium restriction and cancer. This reflects the paradigm shift in the history of selenium research [79]. From 1817, the year of the discovery of selenium by the Swedish chemist Jöns Jacob Berzelius, up to 1957 selenium has been regarded as a poison. This changed completely when Klaus Schwarz and coworkers provided evidence that selenium is an essential trace element that prevents development of necrotic liver degeneration in rodents in conjunction with vitamin E [68,69]. With the findings that glutathione peroxidase is a selenoenzyme [80] and that selenocysteine is the 21st amino acid [81,82] selenium research in biology and medicine gained momentum. The progress in selenium research was further accelerated by the description of the complete mammalian selenoproteome [83]. The essential function of selenoproteins in redox metabolism and thyroid hormone synthesis established selenoproteins as essential players in the homeostasis of the metabolism and the maintenance of life. Thus, selenium has acquired a firm place in biology and medicine as a "good guy". This is reflected by 56 ongoing clinical trials searched with the terms "selenium" and "cancer". All of these are based on the assumption that selenium is a "good guy", regardless whether the clinical trials deal with a role of selenium in cancer prevention, progression

of pre-malignant lesions or with a role in cancer therapy. Replenishing the assumedly deficient selenium pool is believed to decrease the cancer risk in healthy individuals and to improve the outcome of chemotherapy in patients with cancer. The notion that selenium is good not only for normal cells but also for cancer cells, has not yet entered the stage. It is therefore time for a second paradigm shift: Selenium should be regarded as a "good guy" for normal cels, an even "better guy" for cancer cells and a "bad guy" for the patient suffering from cancer.

It is apparently time to put more effort into the hypothesis that dietary selenium restriction may support cancer therapies for various types of cancer in animal models. As soon as a beneficial impact of selenium restriction as an adjunct to chemotherapy or induction of differentiation in AML is confirmed, it is mandatory to test the concept in clinical trials. It will be of crucial importance to see whether dietary selenium restriction is well tolerated or has severe side effects. Remarkably, *Mother Nature* has invented not only an intricate, energy consuming, costly selenium uptake and incorporation system during evolution, but also a highly elaborate biochemical system that redirects the remaining selenium under selenium deprivation into those organs (brain, testis) and selenoproteins (GPX4) that are most important for survival and have the highest demand for selenium [84]. Male fertility is strongly dependent on the mitochondrial and nuclear form of GPX4 [85-88]. In rats, male fertility is impaired from the second generation onwards, whereas it is maintained in Balb/c mice up to the third generation [77,89]. It appears possible (if not likely) that severe side effects will not occur within the time frame of therapeutic selenium restriction.

We may thus speculate that the so called hierarchy of selenoproteins under selenium deprivation may render dietary selenium restriction a feasible and realistic adjunct to other cancer therapies. It is, however, mandatory to exclude the possibility that under selenium demand cancer cells or some type of cancer cells may acquire the ability to hijack the physiologic mechanisms of selenium redistribution for their own sake at the expense of the organism.

MONO- AND POLYUNSATURATED FATTY ACIDS: THE SUBSTRATES OF LIPID PEROXIDATION AS MODULATORS OF FERROPTOSIS

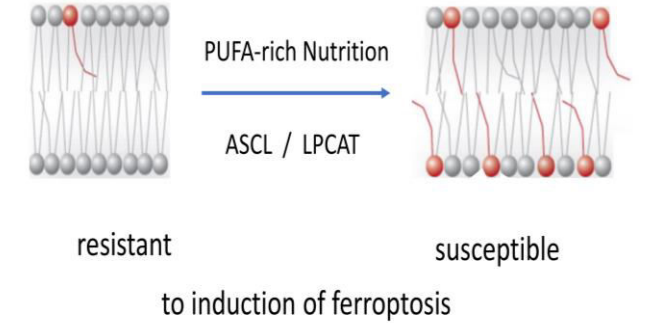

Figure 5: The cell membrane composition dictates the susceptibility versus resistance to ferroptosis.

Poly-unsaturated fatty acids are essential nutrients supplied mainly by plant oils, algae and fish. Membrane PUFAs are the substrates of lipid peroxidation. To be incorporated into cell membranes, PUFAs require two groups of enzymes: (i) fatty acid activating enzymes that couple the respective PUFA to co-enzyme A (ACSLs), and (ii) acyltransferases that transfer the fatty acid to phospholipids and incorporate them into membranes (acyl-transferases, e.g. lysophosphatidyl-choline-acyl-transferases, LPCATs). The PUFA content and composition determine the susceptibility versus resistance of cells to ferroptosis-inducing stimuli. Modified after Doll et al. [90].

A prerequisite of iron-mediated lipid peroxidation is the presence polyunsaturated fatty acids (PUFAs) within phospholipids membranes. PUFAs exhibit double bonds at position ω6 and ω3 (ω indicates that the carbon atoms are counted from the end of the molecule opposite to the carboxyl group). PUFAs cannot be synthesized by mammals and are taken up with the diet, plant oils being rich in ω6-, and algae and fish oils in ω3-fatty acids. The membrane fatty acid composition dictates important biological characteristics of membranes like for instance membrane fluidity and lateral diffusion of signaling molecules. Therefore, the content and composition of PUFAs is of critical importance for signal transduction, cell motility, migration, evasion and metastasis. The membrane fatty acid composition also determines the balance between susceptibility and resistance to inducers of ferroptosis: the higher the PUFA content, the more susceptible are cells to induction of ferroptosis (Figure 5). Enzymes that catalyze the preferential incorporation of PUFAs into membrane phospholipids like ASCL4 and LPCAT3 render several cell types more vulnerable to proferroptotic stimuli

including murine fibroblasts, human breast cancer cell lines and the near haploid human CML cell line KBM7) [90,91]. Enzymes with preference for MUFAs like for instance ACSL3 shift the balance towards resistance [92]. Generalizing statements have, however, to be taken with caution. Ferroptotic cell death induced by the linolenic acid isoform α-eleostearic acid (with three conjugated double bonds) was dependent on activation by ACSL1, an enzyme that couples coenzyme A to the respective fatty acids [93]. In two fatty acid auxotrophic human clear cell renal carcinoma cell lines ACSL3 was the only amongst five ACSL-isoforms that was able to activate fatty acids regardless whether they were saturated or unsaturated [94]. Because these cells do not exhibit de novo fatty acid synthesis, the composition of the fatty acids in the diet dictates the fatty acid composition in membrane phospholipids and hence, the susceptibility versus resistance of the cells to proferroptotic stimuli. Even though de novo fatty acid synthesis is physiologically restricted to special organs like adipose tissue, liver, kidney and the lactating breast, several types of human cancers are dependent on de novo fatty acid synthesis. These include breast, ovarian, prostate, colorectal cancer, glioblastoma multiforme, and AML. An important checkpoint in fatty acid biosynthesis is Stearoyl-CoA-Desaturase (SCD), an enzyme that introduces a double bond at position n9 (C atoms counted from the carboxyl group) into palmitate and stearate thus generating the monosaturated fatty acids palmitoleate and oleate, respectively. Pharmacological inhibition of SCD leads to an accumulation of saturated fatty acids (SUFAs), feedback inhibition of fatty acid biosynthesis via AMPK activation [95], and to pronounced cytotoxicity denoted lipotoxicity. The rise of saturated fatty acids activates PERK and the unfolded protein response pathway and induces caspase-dependent apoptosis [96,97]. Inhibition of SCD was thus shown to exert a dual role in human ovarian cancer: it induces apoptosis via the unfolded protein response, and simultaneously sensitizes the tumor cell to ferroptosis inducers by shifting the balance towards increased incorporation of PUFAs [98]. Exogenously added MUFAs shift the SUFA/MUFA ratio back towards MUFAs, activate AMPK, inhibit of de novo fatty acid synthesis and promote tumor cell survival by releasing the ER stress and rescuing the cells from apoptosis. Tumor entities that rely on de novo fatty acid synthesis, are intrinsically more resistant to ferroptosis inducers because de novo fatty acid synthesis drives SUFA and MUFA incorporation into phospholipid membranes at the expense of PUFAs thus rendering the cells less vulnerable [92]. An important role in the regulation of PUFA-mediated ferroptosis has recently been assigned to lipid droplets. Fatty acids are converted into triacylglycerols in the endoplasmatic reticulum catalyzed by diacylglycerol-acyl-CoA transferase (DGAT1) and incorporated into lipid droplets. Lipid droplets accumulate in many types of cancer cells. They serve as an energy store for cancer cells and by sequestering SUFAs away from the cytosol into organelles, they promote survival by preventing ER stress and lipotoxicity [99] On the other hand, lipid droplets may release PUFAs and sensitize tumor cells to inducers of ferroptosis especially under acidic conditions, a process that is enhanced by DGAT-inhibitors [100]. Most importantly, the PUFA content and composition of the diet dictates the susceptibility to ferroptosis induction, thus providing a novel complementary approach for treatment [93,100].

CONCLUSION

Nutrition as a supportive measure for cancer therapy is gaining general acceptance. It is mandatory that cancer cell metabolism be studied in animal models *in vivo* rather than in tissue culture because of the fundamental differences of growth conditions *in vitro and in vivo*. The finding that epigenetic regulation *in vivo* can be modulated by nutrition has important implications for cancer research. Likewise, the close link between metabolism and cell death research has opened new avenues for dietary support for (novel) cancer therapies. Regarding antioxidants and selenium, the wide spread belief "what is good for cancer prevention is also good for cancer therapy" has to be challenged and has eventually to be replaced by a new paradigm: "what is good for cancer prevention is even better for cancer cells and bad for the patient". It follows that the concept of dietary selenium restriction should be pursued in preclinical studies to pave the way for future translation into clinical trials. Specific diets like calorie restriction, fasting mimicking diet or the ketogenic diet are under intensive investigation in combination with other cancer therapies. More detailed, well performed clinical studies should evaluate the safety and potential side effects of the various forms of diets in order to meet or to dissipate the

concerns of all official institutions involved in cancer consulting and cancer therapy regarding malnutrition, weight loss and induction of cachexia. Our knowledge about the contribution of PUFAs should be incorporated into these nutritional diets that have to be evaluated in future clinical trials.

ACKNOWLEDGEMENT

I apologize to those authors whose work could not be cited in this review. I am grateful to Marlene Assmann who ignited my interest in the contribution of nutrition to cancer therapy.

REFERENCES

1. Otto SJ, Korfage IJ, Polinder S, van der Heide A, de Vries E, et al. (2015). Association of change in physical activity and body weight with quality of life and mortality in colorectal cancer: a systematic review and meta-analysis. Support Care Cancer. 23: 1237-1250.
2. Stout NL, Baima J, Swisher AK, Winters-Stone KM, Welsh J. (2017). A Systematic Review of Exercise Systematic Reviews in the Cancer Literature (2005-2017). PM R. 9: S347-S384.
3. Warburg O, Posener K, Negelein E. (1924). About the metabolism of the carcinoma cell. The Natural Sciences. 12: 1131-1137.
4. NG217, National Institute for Health and Care Excellence (NICE). (2022). Ketogenic diets for drug-resistant epilepsy: Epilepsies in children, young people and adults: diagnosis and management: Evidence review 12 (London). In Evidence review: Ketogenic diets for drug-resistant epilepsy: Epilepsies in children, young people and adults: diagnosis and management: Evidence review. 12: 1-44.
5. Kim YS, Kim W, Na JH, Lee YM. (2023). Nutritional Intervention Through Ketogenic Diet in GLUT1 Deficiency Syndrome. Clin Nutr Res. 12: 169-176.
6. Sofou K, Dahlin M, Hallbook T, Lindefeldt M, Viggedal G, et al. (2017). Ketogenic diet in pyruvate dehydrogenase complex deficiency: short- and long-term outcomes. J Inherit Metab Dis. 40: 237-245.
7. Vander Heiden MG, Cantley LC, Thompson CB. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 324: 1029-1033.
8. Wiel C, Le Gal K, Ibrahim MX, Jahangir CA, Kashif M, et al. (2019). BACH1 Stabilization by Antioxidants Stimulates Lung Cancer Metastasis. Cell. 178: 330-345.
9. Nilsson LM, Forshell TZ, Rimpi S, Kreutzer C, Pretsch W, et al. (2012). Mouse genetics suggests cell-context dependency for Myc-regulated metabolic enzymes during tumorigenesis. PLoS Genet. 8: e1002573.
10. Corbet C, Feron O. (2017). Cancer cell metabolism and mitochondria: Nutrient plasticity for TCA cycle fueling. Biochim Biophys Acta Rev Cancer. 1868: 7-15.
11. DeNicola GM, Cantley LC. (2015). Cancer's Fuel Choice: New Flavors for a Picky Eater. Mol Cell. 60: 514-523.
12. Faubert B, Li KY, Cai L, Hensley CT, Kim J, et al. (2017). Lactate Metabolism in Human Lung Tumors. Cell. 171: 358-371.
13. Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, et al. (2017). Glucose feeds the TCA cycle via circulating lactate. Nature. 551: 115-118.
14. Corbet C, Pinto A, Martherus R, Santiago de Jesus JP, Polet F et al. (2016). Acidosis Drives the Reprogramming of Fatty Acid Metabolism in Cancer Cells through Changes in Mitochondrial and Histone Acetylation. Cell Metab. 24: 311-323.
15. Carracedo A, Cantley LC, Pandolfi PP. (2013). Cancer metabolism: fatty acid oxidation in the limelight. Nat Rev Cancer. 13: 227-232.
16. German NJ, Yoon H, Yusuf RZ, Murphy JP, Finley LW, et al. (2016). PHD3 Loss in Cancer Enables Metabolic Reliance on Fatty Acid Oxidation via Deactivation of ACC2. Mol Cell. 63: 1006-1020.
17. Sperry J, Condro MC, Guo L, Braas D, Vanderveer-Harris N, et al. (2020). Glioblastoma Utilizes Fatty Acids and Ketone Bodies for Growth Allowing Progression during Ketogenic Diet Therapy. iScience. 23: 101453.
18. Peck B, Schulze A. (2016). Lipid desaturation - the next step in targeting lipogenesis in cancer? FEBS J. 283: 2767-2778.
19. Snaebjornsson MT, Janaki-Raman S, Schulze A. (2020). Greasing the Wheels of the Cancer Machine: The Role of Lipid Metabolism in Cancer. Cell Metab. 31: 62-76.
20. Cahill GF Jr. (2006). Fuel metabolism in starvation. Annu Rev Nutr. 26: 1-22.

21. Newman JC, Verdin E. (2017). beta-Hydroxybutyrate: A Signaling Metabolite. Annu Rev Nutr. 37: 51-76.
22. Bose S, Allen AE, Locasale JW. (2020). The Molecular Link from Diet to Cancer Cell Metabolism. Mol Cell. 80: 554.
23. Campbell SL, Wellen KE. (2018). Metabolic Signaling to the Nucleus in Cancer. Mol Cell. 71: 398-408.
24. Gao X, Lin SH, Ren F, Li JT, Chen JJ, et al. (2016). Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. Nat Commun. 7: 11960.
25. Lee JV, Shah SA, Wellen KE. (2013). Obesity, cancer, and acetyl-CoA metabolism. Drug Discov Today Dis Mech. 10: e55-e61.
26. Douris N, Melman T, Pecherer JM, Pissios P, Flier JS, et al. (2015). Adaptive changes in amino acid metabolism permit normal longevity in mice consuming a low-carbohydrate ketogenic diet. Biochim Biophys Acta. 1852: 2056-2065.
27. Dmitrieva-Posocco O, Wong AC, Lundgren P, Golos AM, Descamps HC, et al. (2022). beta-Hydroxybutyrate suppresses colorectal cancer. Nature. 605: 160-165.
28. Dai X, Bu X, Gao Y, Guo J, Hu J, et al. (2021). Energy status dictates PD-L1 protein abundance and anti-tumor immunity to enable checkpoint blockade. Mol Cell. 81: 2317-2331.
29. Ferrere G, Tidjani Alou M, Liu P, Goubet AG, Fidelle M, et al. (2021). Ketogenic diet and ketone bodies enhance the anticancer effects of PD-1 blockade. JCI Insight. 6: e145207.
30. Calderon N, Betancourt L, Hernandez L, Rada P. (2017). A ketogenic diet modifies glutamate, gamma-aminobutyric acid and agmatine levels in the hippocampus of rats: A microdialysis study. Neurosci Lett 642: 158-162.
31. Koppel SJ, Swerdlow RH. (2018). Neuroketotherapeutics: A modern review of a century-old therapy. Neurochem Int. 117: 114-125.
32. Brandhorst S, Longo VD. (2016). Fasting and Caloric Restriction in Cancer Prevention and Treatment. Recent Results Cancer Res. 207: 241-266.
33. Di Tano M, Longo VD. (2022). Fasting and cancer: from yeast to mammals. Int Rev Cell Mol Biol. 373: 81-106.
34. Nencioni A, Caffa I, Cortellino S, Longo VD. (2018). Fasting and cancer: molecular mechanisms and clinical application. Nat Rev Cancer. 18: 707-719.
35. Vernieri C, Fuca G, Ligorio F, Huber V, Vingiani A, et al. (2022). Fasting-Mimicking Diet Is Safe and Reshapes Metabolism and Antitumor Immunity in Patients with Cancer. Cancer Discov. 12: 90-107.
36. Kuznia S, Zhu A, Akutsu T, Buring JE, Camargo CA Jr, et al. (2023). Efficacy of vitamin D(3) supplementation on cancer mortality: Systematic review and individual patient data meta-analysis of randomised controlled trials. Ageing Res Rev. 87: 101923.
37. Schottker B, Kuznia S, Brenner H. (2021). Efficacy of vitamin D(3) supplementation on cancer mortality in the general population and the prognosis of patients with cancer: protocol of a systematic review and individual patient data meta-analysis of randomised controlled trials. BMJ Open. 11: e041607.
38. Romer M, Dorfler J, Huebner J. (2021). The use of ketogenic diets in cancer patients: a systematic review. Clin Exp Med. 21: 501-536.
39. Schmidt L, von Grundherr J, Rubin D, Mathies V, Hurbner J. (2022). Fasting during Chemotherapy. Ernaehrungs Umschau International. 11: 172-175.
40. Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 330: 1029-1035.
41. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, et al. (1996). Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med. 334: 1150-1155.
42. Klein EA, Thompson IM Jr, Tangen CM, Crowley JJ, Lucia MS, et al. (2011). Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA. 306: 1549-1556.
43. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, et al. (2014). Antioxidants accelerate lung cancer progression in mice. Sci Transl Med. 6: 221ra215.
44. Le Gal K, Ibrahim MX, Wiel C, Sayin VI, Akula MK, et al. (2015). Antioxidants can increase melanoma metastasis in mice. Sci Transl Med. 7: 308re308.

45. Kobayashi A, Ohta T, Yamamoto M. (2004). Unique function of the Nrf2-Keap1 pathway in the inducible expression of antioxidant and detoxifying enzymes. Methods Enzymol. 378: 273-286.
46. Motohashi H, Yamamoto M. (2004). Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med. 10: 549-557.
47. Rojo de la Vega M, Chapman E, Zhang DD. (2018). NRF2 and the Hallmarks of Cancer. Cancer Cell. 34: 21-43.
48. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, et al. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature. 475: 106-109.
49. Menegon S, Columbano A, Giordano S. (2016). The Dual Roles of NRF2 in Cancer. Trends Mol Med. 22: 578-593.
50. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, et al. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A. 106: 8665-8670.
51. Cameron E, Campbell A. (1974). The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. Chem Biol Interact. 9: 285-315.
52. Cameron E, Pauling L. (1976). Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. Proc Natl Acad Sci U S A. 73: 3685-3689.
53. Cameron E, Pauling L. (1978). Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. Proc Natl Acad Sci U S A. 75: 4538-4542.
54. Creagan ET, Moertel CG, O'Fallon JR, Schutt AJ, O'Connell MJ, et al. (1979). Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. N Engl J Med. 301: 687-690.
55. Nauman G, Gray JC, Parkinson R, Levine M, Paller CJ. (2018). Systematic Review of Intravenous Ascorbate in Cancer Clinical Trials. Antioxidants (Basel). 7: 89.
56. Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, et al. (2004). Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 140: 533-537.
57. Ferrada L, Barahona MJ, Vera M, Stockwell BR, Nualart F. (2023). Dehydroascorbic acid sensitizes cancer cells to system x(c)(-) inhibition-induced ferroptosis by promoting lipid droplet peroxidation. Cell Death Dis. 14: 637.
58. Jankowski CSR, Rabinowitz JD. (2022). Selenium Modulates Cancer Cell Response to Pharmacologic Ascorbate. Cancer Res. 82: 3486-3498.
59. Schoenfeld JD, Sibenaller ZA, Mapuskar KA, Wagner BA, Cramer-Morales KL, et al. (2017). O_2 - and H_2O_2 - Mediated Disruption of Fe Metabolism Causes the Differential Susceptibility of NSCLC and GBM Cancer Cells to Pharmacological Ascorbate. Cancer Cell. 31: 487-500.
60. Xia J, Xu H, Zhang X, Allamargot C, Coleman KL, et al. (2017). Multiple Myeloma Tumor Cells are Selectively Killed by Pharmacologically-dosed Ascorbic Acid. EBioMedicine. 18: 41-49.
61. Yun J, Mullarky E, Lu C, Bosch KN, Kavalier A, et al. (2015). Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science. 350: 1391-1396.
62. Chakraborty A, Jana NR. (2017). Vitamin C-Conjugated Nanoparticle Protects Cells from Oxidative Stress at Low Doses but Induces Oxidative Stress and Cell Death at High Doses. ACS Appl Mater Interfaces. 9: 41807-41817.
63. Ngo B, Van Riper JM, Cantley LC, Yun J. (2019). Targeting cancer vulnerabilities with high-dose vitamin C. Nat Rev Cancer. 19: 271-282.
64. Seiler A, Schneider M, Forster H, Roth S, Wirth EK, et al. (2008). Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. Cell Metab. 8: 237-248.
65. Altamura S, Vegi NM, Hoppe PS, Schroeder T, Aichler M, et al. (2020). Glutathione peroxidase 4 and vitamin E control reticulocyte maturation, stress erythropoiesis and iron homeostasis. Haematologica. 105: 937-950.
66. Wortmann M, Schneider M, Pircher J, Hellfritsch J, Aichler M, et al. (2013). Combined deficiency in glutathione peroxidase 4 and vitamin E causes multiorgan thrombus formation and early death in mice. Circ Res. 113: 408-417.

67. Carlson BA, Tobe R, Yefremova E, Tsuji PA, Hoffmann VJ, et al. (2016). Glutathione peroxidase 4 and vitamin E cooperatively prevent hepatocellular degeneration. Redox Biol. 9: 22-31.
68. Schwarz K, Bieri JG, Briggs GM, Scott ML. (1957). Prevention of exudative diathesis in chicks by factor 3 and selenium. Proc Soc Exp Biol Med. 95: 621-625.
69. Schwarz K, Foltz CM. (1957). Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J Proc Am Chem Soc. 79: 3292-3293.
70. Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, et al. (2018). Selenium Utilization by GPX4 Is Required to Prevent Hydroperoxide-Induced Ferroptosis. Cell. 172: 409-422.
71. Akiyama H, Zhao R, Ostermann LB, Li Z, Tcheng M, et al. (2023). Mitochondrial regulation of GPX4 inhibition-mediated ferroptosis in acute myeloid leukemia. Leukemia. 38: 729-740.
72. Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, et al. (2017). Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. Nature. 551: 247-250.
73. Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, et al. (2017). Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature. 547: 453-457.
74. Alborzinia H, Chen Z, Yildiz U, Freitas FP, Vogel FCE, et al. (2023). LRP8-mediated selenocysteine uptake is a targetable vulnerability in MYCN-amplified neuroblastoma. EMBO Mol Med. 15: e18014.
75. Li Z, Ferguson L, Deol KK, Roberts MA, Magtanong L, et al. (2022). Ribosome stalling during selenoprotein translation exposes a ferroptosis vulnerability. Nat Chem Biol. 18: 751-761.
76. Eagle K, Jiang Y, Shi X, Li M, Obholzer NP, et al. (2022). An oncogenic enhancer encodes selective selenium dependency in AML. Cell Stem Cell. 29: 386-399.
77. Felix K, Gerstmeier S, Kyriakopoulos A, Howard OM, Dong HF, et al. (2004). Selenium deficiency abrogates inflammation-dependent plasma cell tumors in mice. Cancer Res. 64: 2910-2917.
78. Tsuji PA, Santesmasses D, Lee BJ, Gladyshev VN, Hatfield DL. (2021). Historical Roles of Selenium and Selenoproteins in Health and Development: The Good, the Bad and the Ugly. Int J Mol Sci. 23: 5.
79. Flohe L, Gunzler WA, Schock HH. (1973). Glutathione peroxidase: a selenoenzyme. FEBS Lett 32: 132-134.
80. Cone JE, Del Rio RM, Davis JN, Stadtman TC. (1976). Chemical characterization of the selenoprotein component of clostridial glycine reductase: identification of selenocysteine as the organoselenium moiety. Proc Natl Acad Sci U S A. 73: 2659-2663.
81. Forstrom JW, Zakowski JJ, Tappel AL. (1978). Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry. 17: 2639-2644.
82. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, et al. (2003). Characterization of mammalian selenoproteomes. Science. 300: 1439-1443.
83. Burk RF, Hill KE. (2015). Regulation of Selenium Metabolism and Transport. Annu Rev Nutr. 35: 109-134.
84. Conrad M, Moreno SG, Sinowatz F, Ursini F, Kolle S, et al. (2005). The nuclear form of phospholipid hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. Mol Cell Biol. 25: 7637-7644.
85. Pfeifer H, Conrad M, Roethlein D, Kyriakopoulos A, Brielmeier M, et al. (2001). Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. FASEB J. 15: 1236-1238.
86. Schneider M, Forster H, Boersma A, Seiler A, Wehnes H, et al. (2009). Mitochondrial glutathione peroxidase 4 disruption causes male infertility. FASEB J. 23: 3233-3242.
87. Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, et al. (1999). Dual function of the selenoprotein PHGPx during sperm maturation. Science. 285: 1393-1396.
88. Behne D, Kyriakopoulos A, Scheid S, Gessner H. (1991). Effects of chemical form and dosage on the incorporation of selenium into tissue proteins in rats. The J Nutr. 121: 806-814.
89. Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, et al. (2015). Human Haploid Cell Genetics Reveals Roles for Lipid Metabolism Genes in Nonapoptotic Cell Death. ACS Chem Biol. 10: 1604-1609.

90. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, et al. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol. 13: 91-98.

91. Magtanong L, Ko PJ, To M, Cao JY, Forcina GC, et al. (2019). Exogenous Monounsaturated Fatty Acids Promote a Ferroptosis-Resistant Cell State. Cell Chem Biol. 26: 420-432.

92. Beatty A, Singh T, Tyurina YY, Tyurin VA, Samovich S, et al. (2021). Ferroptotic cell death triggered by conjugated linolenic acids is mediated by ACSL1. Nat Commun. 12: 2244.

93. Klasson TD, LaGory EL, Zhao H, Huynh SK, Papandreou I, et al. (2022). ACSL3 regulates lipid droplet biogenesis and ferroptosis sensitivity in clear cell renal cell carcinoma. Cancer Metab. 10: 14.

94. Scaglia N, Chisholm JW, Igal RA. (2009). Inhibition of stearoylCoA desaturase-1 inactivates acetyl-CoA carboxylase and impairs proliferation in cancer cells: role of AMPK. PLoS One. 4: e6812.

95. Piccolis M, Bond LM, Kampmann M, Pulimeno P, Chitraju C, et al. (2019). Probing the Global Cellular Responses to Lipotoxicity Caused by Saturated Fatty Acids. Mol Cell. 74: 32-44.

96. Young RM, Ackerman D, Quinn ZL, Mancuso A, Gruber M, et al. (2013). Dysregulated mTORC1 renders cells critically dependent on desaturated lipids for survival under tumor-like stress. Genes Dev. 27: 1115-1131.

97. Tesfay L, Paul BT, Konstorum A, Deng Z, Cox AO, et al. (2019). Stearoyl-CoA Desaturase 1 Protects Ovarian Cancer Cells from Ferroptotic Cell Death. Cancer Res. 79: 5355-5366.

98. Chitraju C, Mejhert N, Haas JT, Diaz-Ramirez LG, Grueter CA, et al. (2017). Triglyceride Synthesis by DGAT1 Protects Adipocytes from Lipid-Induced ER Stress during Lipolysis. Cell Metab. 26: 407-418.

99. Dierge E, Debock E, Guilbaud C, Corbet C, Mignolet E, et al. (2021). Peroxidation of n-3 and n-6 polyunsaturated fatty acids in the acidic tumor environment leads to ferroptosis-mediated anticancer effects. Cell Metab. 33: 1701-1715.

100. Hassannia B, Vandenabeele P, Vanden Berghe T. (2019). Targeting Ferroptosis to Iron Out Cancer. Cancer Cell. 35: 830-849.