The Nrf2 Antioxidant System and Multi-Drug Resistant Cancer

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Abstract

Many dietary antioxidants and ingredients in dietary supplements activate the endogenous nuclear erythroid-2 like factor-2 (Nrf2) transcription factor. It is a master regulator of endogenous cellular defense mechanisms. Nrf2 controls the expression of many antioxidant and detoxification genes, as well as antioxidant response elements (AREs) by binding to their promoter regions. This can help prevent many types of cancer, cardiovascular diseases and neurodegenerative diseases. However, if cancer does emerge, it can become multi-drug resistant and hijack the Nrf2 antioxidant system. Over-activated Nrf2 can cause (not prevent) cardiovascular diseases and multi-drug resistance cancers. This review article describes the Nrf2/ARE antioxidant system. When properly controlled, it relieves oxidative stress and prevents diseases caused by it. This control is necessary, because it’s important to maintain a healthy balance in the amount of reactive oxygen and nitrogen species (RONS) and the redox state of cells. That is, RONS are not always toxic. They are also part of a biological regulatory process that enables metabolism to adapt to changes and the needs of the entire organism. In addition, the Nrf2/ARE system communicates with other cellular signaling systems. In doing so, it increases the biosynthesis of mitochondria and protects them from oxidative damage. It also supports the activity of the endoplasmic reticulum (ER) and the unfolded protein response (UPR). Unfortunately, the Nrf2/ARE system also protects tumors and cancer stem cells, making them multidrug resistant. Fortunately, there are some dietary compounds and prescription drugs that inhibit the Nrf2/ARE system. This includes metformin (Gluophage®), which has many beneficial health effects.

Keywords: Nrf2, Cancer, Multi-drug resistance

Introduction

Inflammation is one of the most misunderstood aspects of human health [1]. Part of the misunderstanding is based on the assumption that data obtained from model organisms such as baker’s yeast (Saccharomyces cerevisiae), the fruit fly Drosophila melanogaster, mice, rats and dogs are relevant. That is, caloric restriction without starvation has extended the life spans of these organisms [2]. Lowering the consumption of calories in them reduced the total metabolic rate and the production of reactive oxygen and nitrogen species (RONS), as well as free radicals [3]. Note that many authors prefer the term reactive oxygen species, or ROS, over RONS. According to the free radical theory of aging [4], the accumulation of damage caused by free radicals cause’s problems in aging and eventually death. That is, oxidative metabolism of proteins, fats and carbohydrates in the mitochondria produce RONS and free radicals which can oxidize and damage DNA, lipids and proteins and lead to cancer as well as cardiovascular and neurodegenerative diseases [3,4]. However, there is an antioxidant paradox [5]. Dietary antioxidants have low bioavailabilities. Also, giving large doses of dietary antioxidant supplements to human subjects has seldom had any preventative or therapeutic effects. Moreover, most antioxidants (especially phenolic compounds) don’t work by reacting directly with RONS and free radicals [5].

Even though vitamins A and C as well as CoQ10 can react directly with RONS and destroy them, other antioxidants seldom do this. Instead, many other dietary antioxidants destroy RONS indirectly [1,6]. They activate the endogenous antioxidant response elements (AREs) that are present in cells [7]. The transcription of AREs is activated by the nuclear erythroid-2 like factor-2 (Nrf2), which is a transcription factor [8-10]. So, the Nrf2 signaling system is often called the Nrf2-ARE or Nrf2/ARE signaling system. It controls the expression of AREs by binding to their promoter regions [11].

However, many RONS act as biochemical messengers in normal, healthy metabolism [1,3,12,13]. RONS have nonlinear, hormetic effects. At low concentrations, they can have definite health effects. For example, exercise produces RONS and there is some temporary inflammation. Vigorous exercise increases blood flow, oxygen consumption and the production of RONS. They also modulate the growth factor signaling cascade and increase the availability and function of neurotransmitters. That is, exercise induces oxidative stress [1,12]. Regular exercise causes adaptive changes in cellular antioxidants. Redox balance regulates the generation of force in muscles. Maximum force is produced when the redox state is balanced. That is, there should be a proper balance between the concentrations and activities of pro-oxidants (RONS) and antioxidants [1,12].

The discovery that NO causes vasodilation led to a paradigm shift [1,12]. It is a RON that is harmful in some cases, but can also...
promote mitochondrial biogenesis [1,14]. It was once thought that the only role of mitochondria was to produce energy in the form of ATP. This is still recognized as a major role of mitochondria in healthy cells. However, it is now known that mitochondria also play important roles in apoptosis, autophagy, the production of RONS and free radicals, as well as handling Ca²⁺. Moreover, dysfunctional mitochondria that produce less ATP exist in aging skeletal muscle, the heart and adipose tissues [1,15]. This contributes to cardiovascular diseases and the age- and obesity-related decrease in muscle mass, as well as a decline in the function of skeletal muscles, which is known as sarcopenia. Even though there are fewer mitochondria in white adipocytes than brown adipocytes, they are still essential in their function. They are needed for the secretion of adipokines – the hormones that are produced by adipose tissue (AT). Moreover, mitochondrial dysfunction in AT can lead to insulin resistance and cardiac dysfunction. So, maintaining mitochondrial function in AT is important in increasing one’s lifespan and health span (the number of years that one remains healthy) [1,15]. On the other hand, obesity (especially excess abdominal fat) accelerates the physiological decline that occurs during aging [1,14].

Activating the Nrf2/ARE system can be healthy, but over-activating it can be deadly, since the concentrations of many RONS must be properly controlled [1,6]. When activated, the endogenous Nrf2 transcription factor can help prevent many diseases caused by smoldering inflammation. This includes cardiovascular diseases [6], neurodegenerative diseases [16], diabetes [17], lung diseases [17], kidney disease [17] and many types of cancer [17,18]. However, once cancer emerges, the Nrf2 antioxidant system can be hijacked to make tumors multi-drug resistant [19]. This is consistent with the new paradigm of systems-based thinking in medicine, in which context is one’s health and the degree to which cancer has developed and supplements on the Nrf2 antioxidant system depend on one’s health and the degree to which cancer has developed [7,20,21]. That is, the effects of dietary components and supplements on the Nrf2 antioxidant system depend on one’s health and the degree to which cancer has developed [7,20,21].

Several specific compounds exert their health effects by activating the endogenous Nrf2/ARE antioxidant system [6,7]. This includes epigallocatechin-3-gallate (EGCG), curcumin, sulforaphane, resveratrol, quercetin, cyanidin, catechin, epicatechin genistein luteolin, ellagic acid, chlorogenic acid, usrosic acid and possibly even ascorbic acid (vitamin C) [1,6,7,22]. However, some dietary compounds only activate the Nrf2 system after being metabolized by bacteria in the gut [23]. There is also at least one dietary supplement that appears to activate the Nrf2/ARE signaling system. It is called Protandim® and contains ashwagandha (Withania somnifera), Bacopa monnieri extract, green tea extract, silymarin, and curcumin [24]. They appear to act synergistically [24]. This supplement also increased the lifespan of male mice [25].

The Nrf2 transcription factor controls the expression of AREs by binding to their promoter regions [26]. However, one should be careful in controlling the doses of compounds and/or supplements that activate the Nrf2/ARE system. It has potentially deadly properties when over-activated in some forms of multidrug-resistant and radio-resistant cancer [1,19,20,27-36]. The Nrf2/ARE system helps reprogram the metabolism of cancer cells, which promotes their growth [33]. Cancer cell survival increases partly due to Nrf2 inducing proteasomal activity, thus ensuring that unfolded proteins are quickly and properly removed. Nrf2 also helps increase cell proliferation and increases the expression of anti-apoptotic genes. This can prevent apoptosis. When the Nrf2/ARE system is over-activated, tumors can metastasize. For example, the antitumor drugs asoxaliplatin and bortezomib may activate Nrf2/ARE system. Moreover, Japanese lung cancer patients with one particular single nucleotide polymorphism (SNP) in their human Nrf2 gene had a better prognosis after surgery [37]. This SNP is now recognized as a biomarker of cancer prognosis, and can be a crucial factor to consider in personalized cancer therapy [38]. So, it would be a good idea to measure the genetic polymorphisms in the Nrf2 gene in cancer patients before starting cancer treatment. To help this, a genotyping method has been developed to rapidly detect the SNPs in the Nrf2 gene in 30–45 min [38]. Thus, Nrf2 is a therapeutic target with great potential to prevent the progression of malignancy, and is also a useful biomarker that can be identified before beginning cancer therapy [33].

In addition, the Nrf2/ARE system keeps the concentrations of RONS in cancer stem cells (CSCs) low [39,40]. This maintains their resistance to stressful tumor environments [39]. Moreover, CSCs are thought to be the root cause of resistance to chemotherapy and radiation therapy, ultimately leading to treatment failure in patients being treated for advanced cancers [1,39]. These CSCs have low concentrations of RONS, as well as increased expression of drug transporters and a higher capacity to repair DNA [33,39,40]. These things help CSCs resist the stressful tumor environments caused by chemotherapy and radiation therapy. Moreover, the prognosis of cancer patients is negatively correlated with Nrf2 concentrations in tumors [39]. So, it’s important to note that there are some natural products and prescription drugs that can inhibit the Nrf2/ARE system [1,6,33,39]. In fact, at least three of them (EGCG, ascorbic acid and luteolin) have been shown to both activate the Nrf2/ARE system and inhibit it, depending on the biological context [6]. In contrast, when the Nrf2/ARE system is strongly inhibited, tumor cell migration and metastasis are also inhibited [6,33].

That is, several compounds have been shown to inhibit the Nrf2/ARE signaling system [6,41-49]. It should be noted that three of them, EGCG, ascorbic acid and luteolin, are both activators and inhibitors. This is because they were tested at different concentrations and different cells. For example, it took >200 μM EGCG to inhibit the Nrf2/ARE system in human lung adenocarcinoma A549 cells in vitro [44]. It is almost impossible for concentrations of EGCG to ever be so high in vivo. That is, the maximum concentration of EGCG that was found in the blood plasma of human subjects in a pharmacokinetic study was 77.9 ± 22.2 ng/ml, or about 0.17 μM [50]. Even when EGCG is inserted into nanoparticles, its maximum concentration in blood plasma was 704 ng/ml, or 1.5 μM [51]. Similarly, 0.83 μM luteolin activated the Nrf2/ARE system in hepatocytes that had been exposed to the carcinogenic dioxin, TCDD (2,3,7,8-tetrachlorodibenzodioxin), at a concentration of 0.2 nM (0.0002 μM) [52]. On the other hand, 1 μM luteolin inhibited the Nrf2/ARE system in hepatocytes in vitro [42]. So, the effect of luteolin depends on the type of cell to which it is administered. Similarly, the effect of ascorbic acid seems to depend on the type of cells to which it is administered. It activated the Nrf2/ARE pathway in rat RAW 264.7 macrophages when present at concentrations of 10 to 300 μM and increased the survival of endotoxemic mice at a dose of 300 mg/kg, i.p.
The Keap1-Nrf2-ARE signaling system

It is important to maintain a healthy balance between the concentration of RONS and the redox state of cells [56]. So, the activity of the Nrf2-ARE antioxidant system must be turned on only when it is needed. Its activity is limited by the binding of an inhibitor protein called Keap1 (Kelch-like enoyl-CoA hydratase-associated protein 1) [57]. So, it is often called the Keap1-Nrf2-ARE signaling system. When there is low oxidative stress, Nrf2 is bound to Keap1, which is anchored to actin in the cytoskeleton in the cytosol [8]. This complex makes the Nrf2 protein accessible to reaction with the ubiquitous protein called ubiquitin [58]. Ubiquination causes many transcription factors (including Nrf2) to be broken down (hydrolyzed) in subcellular organelles called proteasomes when DNA transcription should not be activated [58]. However, this hydrolysis of Nrf2 can be prevented by breaking the bonds between it and Keap1 [8,57,58]. That is, the Keap1 protein contains several cysteine residues with sulfhydryl groups that can react with RONS and electrophiles, thus breaking the bonds between Keap1 and Nrf2. Once the bonds are broken, Nrf2 moves to the cell nucleus, where it can bind to regulatory regions of DNA that turn on the transcription of genes coding for AREs. These natural antioxidant elements include the enzymes superoxide dismutase (SOD), thioredoxin (TXN), thioredoxin reductase (TXNRD), sulfiredoxin (SRXN), NAD(P)H:quinone oxidoreductase-1 (NQO1), HO-1, glutathione reductase (GR), glutaredoxin (Grx), glutamate cysteine ligase (GCL), glutathione S-transferase (GST), UDP-glucuronol transferase, thioredoxin reductase, peroxiredoxin sulfotransferase and γ-glutamylcysteine ligase catalytic subunit (GCLC) [17,59,60]. In addition, the expression of over 500 genes is modulated by the Nrf2/ARE pathway [30]. This includes phase I and II detoxification enzymes, transport proteins, proteasome subunits, chaperones, growth factors and their receptors, as well as some other transcription factors [30].

So, the Keap1-Nrf2-ARE signaling system is an example of a biological regulatory process that enables metabolism to adapt to changes and the needs of the entire organism [6,60]. These regulatory processes need a signal and a sensor to switch-on the adaptive process. They also need a transducer, a modulator of sensitivity, an effector, and a way to switch the signal off. It is also important that such processes communicate (or crosstalk) with other signaling systems. Keap1 is the redox sensor of the Keap1-Nrf2 system. The reactive sulfhydryls in the cysteine residues of Keap1 can sense oxidative stress. Once it is released from the cystolic complex with Keap1, Nrf2 becomes phosphorylated at Ser40, so it can enter the nucleus. Its activity can be decreased or enhanced by activating or inhibiting its export out of the nucleus. If Nrf2 becomes phosphorylated again (this time at Tyr568), it can be exported out of the nucleus. There are nuclear export signals in the leucine zipper domain and transactivation domain of Nrf2. They can be blocked by binding to the musculo-aponeurotic fibrosarcoma (Maf) protein [6,60].

So, oxidative stress and other primary signals are sensed by Keap1 [6,60]. They are transduced into the expression of AREs, modulated by phosphorylation of Ser40 and Tyr568 and turned off by nuclear export and subsequent destruction of Nrf2 in the proteasome. This is done by ubiquination. Several proteins are required, including a Cullin-3 based ligase (Cul3) that targets the Nrf2 protein in the Keap1-Nrf2 complex. The effectors of the primary signals are the target genes that code for AREs. The signals can be turned off by not just nuclear export, but also by other mechanisms. There are also Keap1 proteins in the nucleus. They can bind to Nrf2 and target it for degradation in nuclear proteasomes. The actin cytoskeleton must be polymerized for it to bind to the Keap1-Nrf2 complex. Cellular oxidants can activate the enzyme phosphatidylinositol 3-kinase (PI3K), which depolymerizes the actin. Re-polymerization allows Nrf2 to be exported out of the nucleus of the cell. Moreover, actin can be covalently modified by the attachment of glutathione. This leads to the de-polymerization of actin. This can be prevented by Grx, which is a small redox enzyme that uses glutathione as a cofactor. The Keap1-Nrf2 signaling system also activates the transcription of DNA coding for proteins like Cul3, Rbx and Keap1 that are cytosolic inhibitors of this system. Finally, there are many enzymes that can eliminate the system’s signals or prevent them from being formed in the first place [6,60].

The Keap1-Nrf2 signaling system is also affected by crosstalk with other signaling systems [6,60]. As mentioned previously, Nrf2 can be phosphorylated and dephosphorylated. This links it with protein kinases and phosphatases. In addition, it is affected by crosstalk with the mitogen-activated protein kinase (MAPK), casein kinase 2, the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), protein kinase C, PI3K and its partner, Akt [6,60]. That is, PI3K catalyzes the biosynthesis of phosphatidylinositol (3,4,5)-trisphosphate or PtdIns(3,4,5)-P<sub>3</sub>, which activates Akt, also known as protein kinase B [61,62]. It was named Akt because it was first found in a retrovirus calledAkt8 [61]. In addition, the tumor suppressor protein p53 has antioxidant functions that include activating the transcription of the gene coding for Nrf2 and the proper maintenance of mitochondria function, which limits the production of ROS [63]. However, p53 and Nrf2 have many different effects on different types of cells and under different physiological conditions. For example, p53 activates the form of regulated cell death called ferroptosis, which Nrf2 inhibits [64]. Ferroptosis is caused by an excess of poorly liganded iron and ROS, as well as activation of MAPKs, p53 and other signaling systems [64]. That is, some of the iron in our body is bound to hemoglobin, which is a tight ligand [65]. Free, unliganded iron (as Fe<sup>2+</sup>) is frequently consumed as a dietary supplement (FeSO<sub>4</sub>) by premenopausal.
The hydroxyl radical can oxidize lipids and lead to not just ferroptosis [6,64], but also atherosclerosis, cardiovascular diseases, neurodegenerative diseases and many forms of cancer [6,65,66]. When unfolded proteins are not completely destroyed, they can aggregate and accumulate in the endoplasmic reticulum (ER). This causes an unfolded protein response (UPR) that counteracts inflammation, and improves redox balance. Moreover, AMPK increases catabolism, improves endothelial function, reduces cellular energy homeostasis. It decreases anabolism and sustained cell proliferation by reprogramming glucose and glutamine metabolism. It does this by first targeting glycogen synthase kinase-3 (GSK-3). GSK-3 catalyzes the phosphorylation of the nuclear transcription factor, c-Jun N-terminal kinase (JNK), and c-Jun, which increases the expression of proteins involved in cell adhesion and cytoskeleton organization. Nrf2 also interacts and crosstalks with the Notch signaling pathway [6,69]. The Notch signaling pathway influences the cell cycle as well as cellular differentiation, survival, proliferation and apoptosis. It transduces primary signals at the cell membrane of target cells. It goes into the nucleus to activate the expression of several genes. The exact responses depend on the type of cells and their needs. The Notch pathway exerts pleiotropic effects in each tissue that expresses the Notch protein. Thus, Notch-signaling networks regulate various events in embryonic and postnatal development. Like the Notch-β-TrCP signaling system, Notch is conserved from worms (Caenorhabditis elegans) to humans. They can be regulated by reciprocal transcription. That is, Notch1 targets the expression of the gene coding for Nrf2, and Nrf2 targets Notch expression. Nrf2-Notch crosstalk protects against endogenous and exogenous stressors by activating the expression of defense systems. This leads to cytoprotection, while maintaining cellular homeostasis and tissue organization. These effects may vary between different tissues and within specific regions, such as the niche where adult tissue stem cells or progenitor cells reside [6,69].

Even though the Keap1-Nrf2-ARE signaling system exists in so many animals, the level of its activity is quite variable [6,70]. It is much more active in the relatively long-lived naked mole-rat (Heterocephalus glaber) than in other rodents with shorter lifespans. Moreover, species that live longer are more resistant to both chronic and unpredictable stressors. They are also more resistant to age-related diseases, including cardiovascular diseases. However, it is not the concentration of Nrf2 itself that controls its total cellular activity. Instead, it is the concentrations of Keap1 and the β-transducin repeat containing protein (βTrCP), both of which target cytosolic Nrf2 for proteolytic destruction. So, it was suggested that βTrCP could be a good therapeutic target. It is conserved in mice, mole-rats and humans. It could be a better target than Keap1, since low concentrations of it produce fewer harmful side effects than those caused by low levels of Keap1 [6,70].

However, the βTrCP protein does not act in isolation [6,71]. As mentioned previously, phosphorylation of serine residues in Nrf2 enable it to dissociate from the complex with Keap1 and enter the nucleus. There are several protein kinases that can catalyze this phosphorylation. They include protein kinase C, protein kinase RNA-like endoplasmic reticulum kinase (PERK), casein kinase 2, the SRC (sarcoma) family of protein kinases and glycogen synthase kinase-3 (GSK-3). In addition, the PI3K-Akt signaling system induces the expression of one of the genes coding for an ARE, HO-1. The PI3K-Akt signaling system also enables Nrf2 to sustain cell proliferation by reprogramming glucose and glutamine metabolism. It does this by first targeting glycogen synthase kinase-3 (GSK-3). GSK-3 catalyzes the phosphorylation of the SRC-related kinase, FYN. This tyrosine kinase is translocated to the nucleus, where it catalyzes the phosphorylation of Nrf2 at Tyr568. This targets the phosphorylated Nrf2 for nuclear export and degradation in the cytosol. The β-TrCP protein recognizes phosphorylated Nrf2 and targets it for ubiquination and proteolysis. So, Keap1 and β-TrCP have been described as limiter and regulator valves, respectively. They control the movement of Nrf2 in and out of the nucleus of the cell. Under normal redox homeostasis and the absence of stimulation by a growth factor, they both act to limit the flow of Nrf2 into the nucleus. Under normal redox homeostasis but in the presence
of signaling by a growth factor, the Keap1 “valve” stays closed while the β-TrCP opens to release a small percentage of the Nrf2 for entry into the nucleus. During both redox imbalance and receptor signaling, both the Keap1 and β-TrCP “valves” open the flow of Nrf2 into the nucleus. This combination is unlikely under normal physiological conditions, but could be caused by pharmaceutical intervention. That is, drugs might be developed that could reduce the concentration of Nrf2 by targeting the GSK-3/β-TrCP system [6,71].

Unanswered questions

It is well established that multidrug resistant cancer cells can hijack the Keap-Nrf2-ARE system [19,20]. Moreover, some compounds can activate it [1,6,7,22] while others inhibit it [1,6,33,39]. However, it is not known if continuous activation of the Keap-Nrf2-ARE system by dietary compounds and/or dietary supplements can help cancer cells hijack it. So, it has been suggested that the reason why consuming green tea did not prevent cancer [73] was because some of the subjects in the study may have over-consumed dietary supplements containing green tea extract or EGCG [74]. This may or not be true. Moreover, the effects of dietary supplements depend on the dosage taken and the entire diet that one consumes [1]. So, much more research is needed to determine if a person who starts chemotherapy or radiotherapy for cancer should stop taking any dietary supplement (like EGCG, resveratrol or curcumin) that contains large doses of compounds that activate the Nrf2/ARE system. That is, dietary supplements that are sold as being able to prevent cancer may be quite dangerous once a person is diagnosed with cancer and starts chemotherapy or radiotherapy. On the other hand, the much lower amounts of dietary antioxidants in green tea, Itadori tea, and turmeric and most fruits and vegetables are probably not a problem. Moreover, the dietary fiber in fresh fruits and vegetables help produce and maintain a healthy gut microbiome that can prevent cancer [1].

There has been another very interesting development that raises some important questions. Metformin (Glucophage®) has been shown to inhibit the Nrf2 system [33]. Metformin is one of the most important drugs that was originally approved to treat one disease (type-2 diabetes, T2D), but may be useful in treating other diseases, and possibly even slowing down aging [1,74-80]. It is the drug that is most frequently prescribed for lowering blood glucose in the first-line treatment of patients who have T2D, which affects one in nine adults in the USA. It can also help reduce the occurrence of the top five comorbidities of T2D: cardiovascular diseases, cancer, depression, dementia and frailty. However, the extent to which metformin does this depends on the phenotype of the patient [80]. Metformin is also quite useful in preventing T2D in people who are pre-diabetic [76]. It not only reduces the concentration of glucose in the blood, but also offers protection against cardiovascular diseases [74]. Metformin also decreases the concentration of insulin, decreases IGF-1 signaling and inhibits mTOR (mammalian target of rapamycin) [76]. It reduces the incidence of cancer and mortality and helps people retain proper cognitive function. It does this by inhibiting the mitochondrial complex 1 in the electron transport chain, thus reducing the endogenous production of reactive oxygen species (ROS) [76]. At the same time, it targets histone acetylation in cancer-prone epithelial cells [78]. It may also affect aging by activating AMP-activated protein kinase (AMPK) and reducing DNA damage. So, metformin favorably influences metabolic and cellular processes that are closely linked to the development of age-related problems, such as inflammation, autophagy, and cellular senescence [76]. Since it is so inexpensive and easy to obtain, metformin could be especially useful in countries where many people don’t have much money [74].

Perhaps the most exciting potential use of metformin (Glucophage®) is in extending the lifespan of not just people who have diabetes, but also in slowing down the aging process [1,74-80]. It does this by affecting several biochemical pathways that are important in how prediabetes affect [75]. This includes increasing the concentrations of the following proteins while activating genes that code for them: N-glycans (non-invasive surrogate markers of aging), SIRT1, p53 and p66Shc (an adaptor protein in the member of the SHC, or sarcoma homology family that functions as a redox enzyme that is linked to apoptosis). Metformin also increases the activity of SIRT1 and AMPK, while increasing the accessibility of the SIRT1 promoter, the length of telomeres and the accessibility of the SIRT1 promoter on chromatin [75]. It also decreases IGF-1 signaling inhibits mTOR and the mitochondrial complex 1 in the electron transport chain, while reducing the production of ROS and reducing DNA damage [76]. Metformin also favorably influences metabolic and cellular processes that are linked to the development of age-related conditions, such as inflammation, autophagy, and cellular senescence [76].

Partly due to these important health benefits of metformin, some scientists are rejecting the idea that we die of old age [79]. Instead, we die of cumulative failures that occur within our cells and tissues. These failures are not inevitable breakdowns, are reversible conditions of aging. One of the most important of these is AMPK. Its concentration is relatively high when we are young, but decreases with age. Recent studies have suggested that increasing the activity of AMPK can prevent and possibly reverse the life-shortening effects of aging [79]. Some scientists are beginning to refer to AMPK as a suppressor of aging itself [79,81].

It has been called a “metabolic master switch” [79,80]. It monitors the energy status of every human eukaryotic cell within us and triggers responses that maintain it within a healthy range [79]. That is, too little available energy starves the cell, while too much energy can produce too many ROS and disrupt cellular components. As AMPK decreases when we age, we become less energetic and more obese, while becoming increasingly vulnerable to cancer and diseases associated with impaired DNA and protein function. As a person accumulates abdominal fat, this leads to reduced insulin sensitivity, system wide smoldering inflammation and metabolic syndrome. This, in turn, can lead to many forms of cancer, as well as cardiovascular, neurodegenerative and autoimmune diseases (including T2D). The modern Western lifestyle with its overabundance of nutrients and low level of physical activity exacerbates this. When a person’s caloric intake is too high and/or physical activity too low, AMPK activation decreases. As a result, cells decrease their energy-releasing ATP-generating activities and shift to energy-storing processes that generate new fat deposits and make excess new glucose. Moreover, energy inefficiency eventually leads to the dysfunctions that are often described as inevitable diseases (or symptoms) of aging. So, restoring the activity of...
AMPK in the elderly may not only increase longevity, but also help to fight the symptoms of aging [79]. This hypothesis was supported by a clinical study in which subjects with T2D either received metformin (which activates AMPK) or placebo [81]. They were compared to subjects who did not have T2D. It was found that the subjects who were diabetic and received metformin lived a median of 15% longer than did matched controls without diabetes [81]. So, there is a clinical trial underway, called Metformin in Longevity Study (MILES) [82] and another that is being planned called Targeting Aging with Metformin, or TAME, that will look at the effects of metformin on longevity [77].

Even though metformin inhibits the Nrf2/ARE system, it can still extend the lifespan of type-2 diabetics and possibly many other people. So, one should avoid dogmatic thinking that can lead one to believe that anything that reduces the production of antioxidants must be unhealthy. Metformin has so many other health effects that overcome any possible problem that could be caused by it inhibiting the Nrf2/ARE in people who don’t have cancer. Moreover, consumption of dietary antioxidants, minerals, vitamins and fiber in fresh fruits and vegetables can help prevent many types of cancer. So, people should be encouraged to eat at least 800 g of fresh fruits and vegetables [83]. It has been estimated that “5.6 and 7.8 million premature deaths worldwide in 2013 may be attributable to a fruit and vegetable intake below 500 and 800 g/day, respectively” [83]. However, no food should be considered to be a “superfood” [1]. This term is a marketing gimmick that primarily targets women. It is essential that one considers how a food or spice is prepared as well as one’s entire diet. For example, cinnamon can potentially have many beneficial health effects [84]. However, if it is added to white (bleached) flour and sucrose in a cinnamon roll, it is no longer healthy – especially in people who are obese. If it is added to one’s oatmeal instead of adding sucrose, it can be quite healthy. Similarly, fresh fruits and vegetables are much healthier than canned fruits and vegetables that are packed in sugar water or salt water [1]. Moreover, extracts of fruits and vegetables that don’t contain dietary fiber are not nearly as healthy as fresh fruits and vegetables.

**Conclusions**

The Nrf2/ARE antioxidant system must be properly regulated to maintain good health. When it is activated by antioxidants in fresh fruits and vegetables, it can prevent many forms of cancer, as well as cardiovascular diseases, diabetes and neurodegenerative diseases. However, when it is over-activated in people who have started chemotherapy or radiotherapy it might cause their cancer to become multidrug resistant. Still, more research is needed to determine if continuous activation of the Nrf2/ARE system by dietary supplements can cause multidrug resistance to emerge or if this resistance is an effect of cancer and not a cause. Moreover, it is possible that metformin (Glucophage®) can prevent multidrug resistance from occurring because it inhibits the Nrf2/ARE system. However, it is possible that dietary supplements that activate the Nrf2/ARE system could counteract the ability of metformin to prevent the emergence of multidrug resistance. Finally, even though metformin does inhibit the Nrf2/ARE system, people who have type-2 diabetes and obtain prescriptions for metformin should take it. At the same time, they should decrease or cease their consumption of sucrose, except when they become temporarily hypoglycemic. They should also consume at least 500 g of fresh fruits and vegetables and avoid canned fruits and vegetables that are packed in sugar water or salty water.

**References**


