Nrf2 regulator of detoxification, antioxidant, antiinflammatory and cytoprotection is raised by health promoting factors

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Nrf2 master cytoprotection & detoxification regulator is raised by many health promoting factors

Abstract

The transcription factor Nrf2 activates the transcription of over 500 genes in the human genome, most of which have cytoprotective functions. Nrf2 produces cytoprotection by detoxification mechanisms leading to increased detoxification and excretion of both organic xenobiotics and toxic metals; it's action via over two dozen genes increases highly coordinated antioxidant activities; it produces major anti-inflammatory changes; it stimulates mitochondrial biogenesis and otherwise improves mitochondrial function; and it stimulates autophagy, removing toxic protein aggregates and dysfunctional organelles. Health-promoting nutrients and other factors act, at least in part by raising Nrf2 including: many phenolic antioxidants; gamma- and delta- tocopherols and tocotrienols; DHA and EPA long chain omega-3 fatty acids; many carotenoids of which lycopene may be the most active; isothiocyanates from cruciferous vegetables; sulfur compounds from allium vegetables; terpenoids. Other health promoting, Nrf2 raising factors include low level oxidative stress (hormesis), exercise and caloric restriction. Raising Nrf2 has been found to prevent and/or treat a large number of chronic inflammatory diseases in animal models and/or humans including various cardiovascular diseases, kidney diseases, lung diseases, diseases of toxic liver damage, cancer (prevention), diabetes/metabolic syndrome/obesity, sepsis, autoimmune diseases, inflammatory bowel disease, HIV/AIDS and epilepsy. Lesser evidence suggests that raising Nrf2 may lower 16 other diseases. Many of these diseases are probable NO/ONOO(-) cycle diseases and Nrf2 lowers and lowers effects of NO/ONOO(-) cycle elements.

The most healthful diets known, traditional Mediterranean and Okinawan, are rich in Nrf2 raising nutrients as apparently was the Paleolithic diet that our ancestors ate. Modern diets are deficient in such nutrients. Nrf2 is argued to be both lifespan and healthspan extending. Possible downsides to too much Nrf2 are also discussed. Nrf2 is not a magic bullet but is likely to be of great importance in health promotion, particularly in those regularly exposed to toxic chemicals.

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Introduction

Nrf2 (nuclear factor erythroid-2-related factor 2) has been known for over 10 years, to be an important transcriptional activator of antioxidant genes, producing important antioxidant protective responses. It has also been known for about the same time period, to be activated by many, but not all, phenolic antioxidants, such that much of the antioxidant effects of these compounds are produced through this regulatory response, rather than exclusively through direct chain breaking antioxidant chemistry.

However Nrf2 has been shown more recently to have many cytoprotective effects that go far beyond antioxidant effects. These include activation of over two dozen genes involved in detoxification of a wide variety of xenobiotic toxicants. Nrf2 and the system that regulates Nrf2 lowers inflammatory responses, it improves mitochondrial function and stimulates autophagy, a process by which both toxic protein aggregates and dysfunctional organelles can be degraded. Three of these effects, the lowering of oxidative stress, inflammatory biochemistry and improving mitochondrial function should lower the pathophysiology involved in dozens of chronic inflammatory diseases and so may be expected to be useful in the prevention or treatment of many common chronic diseases.

It has also been shown, in recent years, that many health promoting factors other than phenolic antioxidants act to raise Nrf2 activity. Most of these recent findings have been reviewed in a whole series of recent reviews[1-22] and it is the role of this paper to summarize the vast scope of these new findings, including the health-promoting and disease preventing effects of Nrf2.

The important detoxification roles of Nrf2 mean that raising Nrf2 activity is likely to be of particular importance to the hundreds of millions of people around the globe who are regularly exposed to toxic chemicals that cause diseases characterized by oxidative stress, inflammation and mitochondrial dysfunction, diseases which include most of the chronic diseases of 21st century life.

Diseases prevented and/or treated by raising Nrf2, at least in animal models

There are a very large number of chronic diseases, listed in Table 1 that have been shown to be prevented and/or treated by raising Nrf2. Conversely, lowering or knocking out Nrf2 function has often been shown to increase susceptibility to the same diseases. Most of these studies have been done in animal models although there are also an increasing number of human studies being reported.

Table 1. Diseases where raising Nrf2 is reported to be useful in prevention and/or treatment in animal models and/or humans

Citations	Diseases
2,4,9,16,22,23	Cardiovascular disease including atherosclerosis, ischemic cardiovascular disease, vascular endothelial dysfunction, heart failure
2,4,5,6,12,13, 19,23,24	Neurodegenerative diseases including Alzheimer's, Parkinson's, ALS, Huntington's disease
2,3,4,13,19	Cancer (prevention)
2,6,7,15,19,23	Chronic kidney diseases
2,8,10,20,23,25	Metabolic diseases: Type 2 diabetes; metabolic syndrome; obesity
2,8,19,20,23	Several types of toxic liver disease
2,6,16,21,23,28	Chronic lung diseases including emphysema, asthma, pulmonary fibrosis
4,14,27	Sepsis
2,4,16,23, 28-31	Autoimmune diseases
4,13,23,32	Inflammatory bowel disease
4,33	HIV/AIDS
11,12,34	Multiple sclerosis
17,18,35,36	Epilepsy

The finding that raising Nrf2 may be useful in prevention and/or treatment of this list of diseases seems almost too good to be true. However these diseases all have both oxidative stress and inflammatory aspects to them and many of them are also known to involve mitochondrial dysfunction. Protein aggregates have causal roles of several of them, aggregates that may be removed by Nrf2-dependent autophagy. A number of these diseases are caused by toxic exposure and may be lowered by Nrf2-dependent detoxification. The data on obesity are mixed, but with most data showing that Nrf2 acts to lower obesity. One of us (MLP) has argued that many of these diseases are caused by the NO/ONOO(-) cycle, a vicious cycle mechanism involving oxidative stress, inflammation and mitochondrial dysfunction, as well as other factors (discussed further below). It is therefore quite plausible that because of the common factors involved in these diseases, the Nrf2 regulatory response may prevent and/or treat each of them.

There are reports that still other diseases may be prevented or treated by raising Nrf2, although these other diseases have been less studied than those listed in Table 1. These include hemoglobinopathies including sickle cell disease and β-thalassemia [37], malaria[38], spinal cord injury[39], traumatic brain injury[40,41], altitude sickness[42,43], the three classic psychiatric diseases, major depression, schizophrenia and bipolar disorder[44-47], gastric ulcers[48,49], glaucoma[50], age-related macular degeneration[51], cataract[52,53], pathophysiological responses to herpes activation[54] and benign prostatic hyperplasia[55,56]. These diseases all involve oxidative stress and inflammatory aspects. Nrf2 is also shown to protect cells from effects of ionizing radiation[57,58]. Nrf2 was reported to lower skin sensitization produced by sensitizing chemicals[59,60]. Clearly we need much more research on these Nrf2 activities before any conclusion can be made, but these studies suggest that the disease spectrum for which Nrf2 may be protective may be larger than that covered in Table 1.

Gene Activation via Nrf2

Nrf2 is most known for its role in activation of genes having antioxidant effects. It acts by binding in the nucleus, along with some other proteins known as Raf to what are called antioxidant response elements (AREs) in the promoter regions of genes. However these AREs occur not only in promoter regions of antioxidant genes but also genes involved in other functions, particularly other cytoprotective functions. While over 500 genes are activated by Nrf2, there are also genes whose activity is lowered by Nrf2, some of which may be regulated by transcription factors regulated by Nrf2 and others may be regulated through AREs having repressive effects[4]. In summary, Nrf2 acts to activate numerous genes but it can also act via other transcription factors to increase or decrease transcription of various genes and may also be able to repress some genes through its direct effects on transcription.

Nrf2-dependent antioxidant effects

Among the antioxidant genes activated by Nrf2, one of the most commonly studied is the heme oxygenase 1 (HO-1) gene which converts free heme, which has pro-oxidant effects into iron, carbon monoxide (CO) and biliverdin, with the last being converted into the antioxidant bilirubin via an activity also raised by Nrf2, encoded by the two biliverdin reductase genes[1,2]. The iron released by heme oxygenase is sequestered by ferritin, since Nrf2 induces each of 4 ferritin genes, preventing iron-produced oxidative stress [1]. This coordinate control of multiple genes producing proteins that are functionally linked in producing an important biological response has been found repeatedly in Nrf2-mediated gene regulation. There are also antioxidant responses produced by CO from its regulatory role. Heme oxygenase appears to have a very important role in producing Nrf2 responses, based on studies using specific enzyme inhibitors or HO-1 gene knockout mice. A possible explanation for such an important role is discussed below.

A second commonly studied antioxidant gene activated by Nrf2 is the quinone oxidoreductase gene (NQO1), which produces an enzyme that prevents semiquinone redox cycling and consequent oxidative stress[2]. Two superoxide dismutase genes (SOD1 and SOD2) are activated by Nrf2, with each SOD lowering oxidative stress by lowering superoxide. The functionally linked catalase and two glutathione peroxidase genes are each induced by Nrf2, with each of these enzymes acting to lower H_2O_2, produced from superoxide by the SODs. So again, we see Nrf2 mediated coordinate regulation of multiple antioxidant genes[2].

Reduced glutathione (GSH) has often been described as the most important low molecular weight antioxidant produced in the human body. Each of the three genes encoding enzymes required for the de novo synthesis of GSH are activated by Nrf2, as is the gene for glutathione reductase [the enzyme that converts oxidized glutathione (GSSG) to GSH][1,2]. Genes encoding 8 enzymes that have roles in the synthesis of NADPH, the reductant needed by glutathione reductase are also activated by Nrf2. Other genes encoding enzymes that have roles in using GSH for antioxidant purposes, including two glutathione peroxidase genes (discussed in the previous paragraph), and the glutaredoxin 1 gene are each Nrf2 activated[2].

Five genes involved in thioredoxin-related antioxidant responses are activated by Nrf2, including peroxiredoxin-1 and -6 which destroy peroxides including peroxynitrite, an extremely reactive oxidant responsible for nitrosative stress[1]. The enzymes produced by these five genes and also glutaredoxin mentioned in the previous paragraph, represent a set of important and interacting antioxidant enzymes[61] each of which is coordinately regulated by Nrf2.

In summary, it can be seen from the above that there are 23 genes involved in antioxidant protection each of which is activated by Nrf2. There are in addition, still other genes activated by Nrf2. These include genes encoding products that act to remove toxic products of lipid peroxidation, others encoding enzymes that have roles in removing or repairing protein oxidation products and still others similarly regulated that help remove products of oxidative DNA damage in the process of DNA repair.

Detoxification genes activated by Nrf2

To the hundreds of millions of people around the world who are exposed daily to substantial levels of toxicants, detoxification may be the most important Nrf2-dependent cytoprotective mechanisms. Hayes and Dinkova-Kostova[1] list a total of 25 different genes that are activated by Nrf2, each of which encodes an enzyme that acts in detoxification of various toxic xenobiotics. Among those 25 genes[1] are 12 that have roles in metabolism of various carbon-containing xenobiotic toxicants leading up to but not including conjugation. They also list 5 genes activated by Nrf2 that increase glutathione conjugation, one that increases sulfate conjugation and two that lead to glucuronidation. Each of these eight genes have roles in increasing toxicant excretion which follows upon conjugation. There are also Nrf2-activated genes that increase transport of xenobiotic chemicals from the cell, thus increasing subsequent excretion from the body.

Two potentially important detoxification genes, not discussed in ref. [1] are the Mt1 and Mt2 genes for metallothionein, both of which are induced by Nrf2[62]. Metallothionein has roles in chelating, transporting and excreting both essential and toxic metals, including cadmium, mercury, lead and arsenic[63]. However it should be noted than when metallothionein was studied in a relatively short-term study of cadmium toxicity, it was concluded that Nrf2 effects on antioxidant responses were more important than were metallothionein effects in producing resistance to cadmium toxicity[62]. Metallothionein levels have been shown to have a role in determining lead toxicity[64]. Toyama et al[65] showed that Nrf2 stimulated mercury excretion with such excretion attributed by the authors to increased reduced glutathione. It should be noted that reduced glutathione is the most common low molecular weight thiol in the body and because mercury, lead, cadmium and arsenic all react with thiol groups, Nrf2-dependent raising reduced glutathione may be expected to increase detoxification of each of these toxic metals. The Nrf2 activating nutrient, curcumin has been shown to lower hepatotoxicity of arsenic, cadmium, chromium, copper, lead and mercury with such lowered toxicity attributed to both Nrf2 activation and direct chelation by curcumin[66]. This paragraph only reviews a fraction of the available information that relates to Nrf2 and toxic metal exposure. Nevertheless it suggests that Nrf2 probably has a substantial role in producing resistance to toxic metal exposure.
Nrf2 has a wide range of detoxification effects, producing increased resistance to toxic organic xenobiotics and toxic metals.

Anti-inflammatory effects of Nrf2

Nrf2 activation produces a wide variety of anti-inflammatory effects including lowered NF- B and lowered activity of a series of inflammatory mediators including cytokines, chemokines, adhesion molecules, COX-2, MMP-9 and iNOS[6,15,16]. The interaction between Nrf2 and NF- B is very complex, with each having effects that both increase and decrease the other. However it is clear that[6] "NF- B pathway is inhibited by several Nrf2 activators" but the specific mechanisms responsible for Nrf-2 mediated lowering of NF- B is still somewhat uncertain. However[6] "Conversely, recent experimental evidence indicates that NF- B may directly repress Nrf2 signaling at the transcriptional level." Two direct anti-inflammatory effects of Nrf2 are that it stimulates the transcription of the anti-inflammatory cytokine IL-10 gene[5] and it has also been shown to lower regulatory responses produced by TGF- .

In conclusion, Nrf2 produces a large number of anti-inflammatory effects, with many mediated by lowering NF- B activity and some others mediated through Nrf2-dependent increases in IL-10. NF- B acts in turn to lower Nrf2 activity. The mechanisms involved in Nrf-2 dependent lowering of NF- B activity are complex and not completely understood, although it seems likely that Nrf2-dependent lowering of oxidant levels has a role.

Mitochondrial biogenesis and autophagy

Most of the diseases listed in Table 1 are also characterized by energy metabolism and mitochondrial dysfunction. One of the mechanisms that may be included as cytoprotective may be increased mitochondrial biogenesis. Nrf2 produces such increased mitochondrial biogenesis acting in part by activating a related gene, Nrf1[20]. A large number of other genes involved in energy metabolism are also activated by Nrf2[1] and are thought to contribute to both mitochondrial biogenesis and improved mitochondrial function. There is a lot of crosstalk between Nrf2 and the AMPK protein kinase[67], which is stimulated by AMP and which therefore monitors energy levels. It is possible therefore that this may be an important interaction in controlling mitochondrial responses.

It is also the case that a number of health-promoting nutrients that stimulate Nrf2 also act to increase the process of autophagy by which damaged organelles and also damaging protein aggregates can be degraded proteolytically, with such autophagy occurring, in part, via a Nrf2-dependent process[68,69]. This stimulation of autophagy is useful in removing damaged mitochondria and other damaged organelles. It is also useful in removing protein aggregates that have roles in neurodegenerative and other diseases and autophagy has antioxidant roles are well. However it should be noted, that autophagy is inhibited by very high levels of Nrf2. In summary, Nrf2-dependent autophagy may be useful as a cytoprotective response in multiple ways, one of which has roles in improving mitochondrial function.

While most of the health promoting effects of Nrf2 can be understood in terms of its antioxidant, detoxification, anti-inflammatory and autophagy effects and its ability to stimulate mitochondrial biogenesis, still other health promoting effects may also occur. For example, in many of the chronic inflammatory diseases, there is substantial pathophysiological tissue remodeling involving fibrosis. Nrf2 has been reported to have antifibrotic effects in the lung, liver and kidney[70-73], acting by stimulating dedifferentiation of fibroblasts. Much of this antifibrotic effect is thought to be produced by an anti-inflammatory action of Nrf2 which lowers TGF-   signaling.

Nrf2 activity is raised by many health-promoting nutrients and other factors

The amazing list of health promoting factors that have been shown to act, at least in part, by raising Nrf2 are shown in Table 2.

Table 2. Health-promoting factors that raise Nrf2 activity

Citations	Health-promoting factors
2,3,4,5,8,15	Many but not all phenolic antioxidants
74,75	-tocopherols and tocotrienols (but -tocopherol has little activity)
2,3,4,5,7,8,15	Isothiocyanates from broccoli, cabbage and other cruciferous foods
2,4,5,8,15,19,20	Triterpenoids and other terpenes
2,23,76,77	Sulfur compounds including allyl sulfides in garlic/onion/allium foods
2,78,79	Many carotenoids with lycopene apparently the most active
3,80,81	Fish oil (long chain omega-3 fatty acids DHA and EPA)
3,82	Modest oxidative stress (hormesis)
4,22	Exercise, works in part via modest oxidative stress; may also work in the vasculature via laminar shear stress[9]

Each of the nine factors listed in Table 2 have an extensive literature on their health-promoting effects. Although all nine have been shown to raise Nrf2 activity, several of these can clearly act in other ways not involving Nrf2 to promote health.
For example, four of the nutritional factors are well establish to act independently of Nrf2 as follows:

- Phenolics, including tocopherols/tocotrienols, can act as chain breaking antioxidants.
- Carotenoids can act as scavengers of singlet oxygen and peroxynitrite.
- Fish oil has anti-inflammatory properties by acting as precursors of eicosanoids.
- Exercise can act in ways independent of Nrf2.

The phenolics act via three mechanisms to raise Nrf2, but some phenolics are completely inactive in this process. The ones that appear to act most directly, are ortho or para dihydroxyphenols which can get oxidized to quinones[2] which then act to raise Nrf2. The role of the phenol ring structures are also seen in the second type of chemical listed in Table 2, the different forms of vitamin E. These are also phenolic forms, but the phenol ring structures in the & forms are much more active than are -tocopherol in raising Nrf2[74,75]. -Tocopherol, the common form of vitamin E used in supplements, has modest activity in raising Nrf2; it may decrease Nrf2 activity *in vivo*, however, because it increases the degradation in the body of the other forms of vitamin E, including the & tocopherols and tocotrienols[83].

However each of these 9 factors, when tested in Nrf2-/- mouse knockout mutants have been shown to have lost most of their health-promoting properties as compared with their activity in Nrf2+/+ mice [see, for example, refs 84-91]. This shows, therefore that much of their health promotion requires the presence of a functional Nrf2 gene, at least in the mouse. Other cell culture studies on these nutritional factors have also supported an important role for Nrf2 elevation in response to these factors, as well.

Caloric restriction, another health promoting factor, acts in part by raising Nrf2[92-94]. There are traditional Chinese, Ayurvedic, European and Native American herbals that have been shown to act by raising Nrf2. Two of these were discussed earlier[95] but a full consideration of such herbals goes beyond the scope of this review.

There are still other phytochemical Nrf2 raising factors, some of which are harder to characterize than the categories listed in Table 2. For example, a number of plant-derived acetylenic compounds also raise Nrf2[2]. Dithiolethiones from cruciferous plants also act to raise Nrf2[2] as does -lipoic acid. It has also been reported that butyrate produced from dietary fiber fermentation in the colon, acts to raise Nrf2 in colonocytes[96]; this butyrate action may have implications regarding dietary fiber and Nrf2 control in the lower GI tract.

Three of these classes of chemicals act via their oxidation products to raise Nrf2 levels. The long chain omega-3 fatty acids DHA and EPA act via their oxidation product 4-hydroxyhexenal and other oxidation products to raise Nrf2[27,28,97]. The carotenoids act, primarily and possibly entirely, via their oxidation products to raise Nrf2[78,79]. Many of the phenolic antioxidants that raise Nrf2 are thought to act via their quinone oxidation products in raising Nrf2[1-6]. Sandberg et al[5] have argued that chronically inflamed tissues may become less susceptible to agents raising Nrf2. Similarly, Kumar et al[2] state that "Unfortunately, long-term inflammatory signaling can result in decreased Nrf2 activity and decreased antioxidant and defense capacity." It may be useful in therapy of diseases of chronic inflammation to use these three classes of Nrf2 raising nutrients, because the higher rates of oxidation of these nutrients in inflamed, oxidative stressed tissues may act to counteract otherwise lowered Nrf2 responses in such tissues.

The two most healthful known diets, the traditional Mediterranean diet and the traditional Okinawan diet, are both rich in Nrf2 activating nutrients

The traditional Mediterranean diet which is thought to be ideally the Cretan diet and perhaps the southern Greek and southern Italian diets of the 1960s and the traditional Okinawan diet of the same time period, are thought to be the most healthful human diets known, with high overall lifespans, large numbers of centenarians and low incidences of cancer and cardiovascular disease[98-105]. Diets in both of these locations are thought to have become much less healthful in recent decades, but studies of these two traditional diets are still important parts of our understanding of dietary factors that may influence human health. The question being raised here is whether it is likely that nutrients raising Nrf2 activity in these diets have an important role in producing the health promoting properties of these two diets.

The dietary factors listed in Table 2 which raise Nrf2 are all of plant origin except for the long chain omega-3 fatty acids which are best obtained from seafood. Consequently, it may be argued that the best diets for raising Nrf2 are diets with regular seafood consumption but otherwise containing large amounts of foods derived from plants, particular plants with low calorie densities which are likely to be consumed in larger quantities and therefore provide, in general more phytochemicals. Both the traditional Mediterranean and Okinawan diets clearly fit this description[98-105]. Furthermore several of the nutrient categories known to raise Nrf2 listed in Table 2 are thought to be high in each of these diets (see Table 3).

Table 3
Estimated Nrf2 raising nutritional components in the two most healthful diets known

Nutrient component	Traditional Mediterranean Diet	Traditional Okinawan Diet
Phenolic antioxidants	High consumption from olives and olive oil, herbs, legumes, eggplant, many leafy green vegetables	High consumption from soy, many green vegetables and herbs; also provided by purple sweet potato varieties; "Okinawan spinach" (Perilla, major source of rosmarinic acid)
Carotenoids	High consumption, especially from tomatoes and leafy green vegetables	Very high consumption from sweet potatoes and many leafy green vegetables
Long-chain omega-3 fatty acids	High consumption from fish; also purslane and walnuts provide fatty acid precursors to the human body	High consumption from fish; also leafy green vegetables provide some fatty acid precursors to the human body
Isothiocyanates	Probably average for European diets	High from cruciferous vegetables and daikon radish, but no higher than other East Asian diets
Terpenoids	High from Mediterranean herbs, olives, peel of fruits and eggplant	Uncertain; substantial levels in Perilla and some other herbs; may be high[99]
Allium-derived sulfur compounds	High consumption of garlic and onions	Relatively high (onions, other allium), probably similar to Chinese diet

Information derived from [98-105].

It can be seen from Table 3 that each of these health-promoting diets are very rich in nutritional components that raise Nrf2, including five of the six types of Nrf2 activating components listed in Table 3. The traditional Mediterranean diet is most characterized by high consumption of olives and olive oil, which are known to contain very high levels of phenolics and terpenoids; both olive-derived phenolics and terpenoids have been shown to raise Nrf2. The main caloric source in the traditional Okinawan diet is the sweet potato, often including purple sweet potatoes [98]. All sweet potatoes are very high in carotenoids and purple sweet potatoes are very high in anthocyanin phenolics which are potent Nrf2 activators. Murakami et al[99] showed that a large number of specific vegetables in the traditional Okinawan diet are potent agents that lower the production of both superoxide and nitric oxide in leukocytes, suggesting that these vegetables act in part by raising Nrf2. In some cases, they[99] implicated both phenolics and terpenoids in producing these responses, again suggesting a possible Nrf2 effect. While it is unlikely that all of the phytochemicals that may produce health-promoting effects in these two diets are acting mainly or solely via Nrf2, it is likely in our opinion, that Nrf2 has a major role in the health promotion in each of these two diets.

The Okinawan diet is thought to be very similar to what is often called the Paleolithic diet[105], the diet that our ancestors ate during much of human evolution. The only substantial difference is that in the Paleolithic diet, most of the omega-3 fatty acids came from wild terrestrial animals and plants, both of which are quite rich in omega-3 fatty acids[106], rather than primarily from fish. Specifically, the Okinawan diet is thought to closely resemble the Paleolithic diet in having very high levels of phenolic and carotenoid antioxidants as well as high omega-3 levels, probably also terpenoids and essentially no grain consumption[105], all of which may be relevant to Nrf2 control. It seems likely, therefore, that we evolved with much higher levels of Nrf2 raising nutrients in our diets and that almost all of us are currently in a dietary deficiency state for Nrf2 raising nutrients. This may be responsible for much of the extraordinary predominance of chronic diseases afflicting modern populations, characterized by oxidative stress, inflammation and mitochondrial dysfunction.

Is Nrf2 a master regulator of longevity and healthspan?

The concept that Nrf2 is a master regulator of not only longevity, but more importantly of healthspan was suggested by Lewis et al[107] in their paper entitled "Nrf2, a guardian of healthspan and gatekeeper of species longevity." They state[107] that "There is mounting evidence across evolutionarily distant species that Nrf2-ARE-dependent components are associated with both longevity and extension of healthspan." These studies include a number of genetic studies in the mouse and in several other species, showing that raising Nrf2 activity produces prolonged lifespans and healthspans and that lowering Nrf2 produces shorter lifespans and healthspans. The mouse studies are particularly important here because genetic manipulation in transgenic mice allows one to easily determine effects of both raised and lowered Nrf2 activity. One change that may contribute to determining lifespans and healthspans is replicative senescence of cells which has been reported to be delayed by Nrf2[108]. Conversely, a knockout of the Nrf2 gene leads to premature cellular senescence[109]. These roles of Nrf2 in determining cellular senescence rates should not be surprising, given the roles of oxidative stress in producing cellular senescence[110].

This general notion regarding Nrf2, longevity and healthspan is, of course, strongly supported by the many diseases including diseases of aging that are lowered, at least in animal studies by raising Nrf2 (Table 1). It is also strongly supported by the various health promoting nutritional and other factors that all raise Nrf2 and act, at least in part through the raising of Nrf2 (Table2). It is supported as well by the high levels of Nrf2-raising nutrients found in the two most healthful diets known, the traditional Mediterranean diet and the traditional Okinawan diet (Table 3).

How is Nrf2 regulated by the health promoting factors listed in Table 2?

Each of refs [1-22] has reviewed the mechanisms by which Nrf2 is regulated and each provides some information on how various factors raise Nrf2. Their discussions on mechanisms of Nrf2 regulation are, in general, much more detailed than is the discussion here. Consequently, the reader is encouraged to go to them and in particularly to [1-4] for more detailed information than is provided here.

Nrf2 protein under what have been called noninduced situations is mostly contained in an inactive complex with another protein known as Keap1. Keap1 has five reactive cysteine residues, in each of which reaction of inducing chemicals with the cysteine thiol, can start a process leading to release of Nrf2 from Keap1. Following release, Nrf2 can move into the nucleus, complex with other proteins called Maf, bind to ARE sequences on DNA and stimulate transcription of adjacent genes. The agents that react with these thiols are electrophilic and or oxidative and the reaction with these thiols is thought to be the most important mechanism of regulation of Nrf2. The five different cysteine thiols differ from one another in what compounds they react with.

However there are many other mechanisms that come into play, making the Nrf2 control system very complex. There are several protein kinases that have roles in regulating Nrf2, including the ERK/JNK pathway, PI3K/Akt/ GSK-3β pathway, protein kinase C, AMPK[67] and protein kinase G. In addition, when Nrf2 is bound to Keap1, Nrf2 tends to be targeted for proteasomal degradation, so that its levels are kept low. Release from Keap1 increases the stability of Nrf2 roughly 7-fold, leading to substantially increased levels. Furthermore, Nrf2 stimulates the transcription of its own gene and also the MafG gene, thus further stimulating Nrf2-dependent transcription. The P62 protein involved in autophagy that is activated by Nrf2 also is involved in a positive feedback loop, increasing Nrf2 activity[68]. While the mechanisms in the previous three sentences act to amplify Nrf2 activation, there is also two mechanisms that lower Nrf2 activation. Nrf2 also stimulates transcription of the Keap1 gene, lowering Nrf2 elevation. Furthermore Nrf2 also stimulates the transcription of a gene encoding INrf2, a protein that also lowers Nrf2 activity[111].

Nrf2 is also regulated by micro RNAs, including miR-200a, that lower the translation of the Nrf2 mRNA or mRNAs of Nrf2 related proteins[112,113]. Because levels of miR-200a are regulated by histone acetylation[113], such acetylation may bring in another level of control; this may explain part of the action of the histone deacetylase inhibitor butyrate, discussed above, in raising Nrf2[96]. Furthermore, the protein designated p300/CBP is an acetyltransferase that acetylates both histones and Nrf2 itself, with Nrf2 acetylation stimulating its activity in ARE-mediated gene transcription[114]. Consequently, the histone deacetylase inhibitor butyrate may also act by increasing Nrf2 acetylation to increase Nrf2 transcriptional activity.

Another regulatory linkage is that agents that stimulate the aryl hydrocarbon receptor (AhR) increase Nrf2 transcription, leading to increases in Nrf2 activity, a subject that has only fairly recently attracted much attention[115].

Protein kinase G has recently been shown to have a substantial role in activating Nrf2[116-119]. Its role may explain one of the long-standing puzzles about Nrf2, why does heme oxygenase-1 (HO-1) induction have such an important role in the action of Nrf2? This has been shown in a number of studies of Nrf2 action, where a specific inhibitor of heme oxygenase has been shown to greatly lower the biological effects of Nrf2 activation. Why then should HO-1 be so important in the action of Nrf2? One of the products of HO-1 enzymatic activity is CO, which acts, as does NO, to greatly stimulate the production of cGMP and therefore of protein kinase G stimulation of Nrf2[119,120]. It follows from this that HO-1 induction by Nrf2 may be an important positive feedback loop, producing a much more rapid increase in Nrf2 activity and therefore Nrf2-dependent responses over time following any initial steps raising Nrf2 activity, than will occur in the absence of increased HO-1 activity. It is our opinion, that substantial indirect effects of Nrf2 may be produced via increased cGMP/protein kinase G, effects that are distinct from this positive feedback loop.

How then do the agents listed in Table 2 stimulate Nrf2 activity? Isothiocyanates[2,7], H2O2 and other oxidants, phenolic antioxidants, long chain omega-3 fatty acids and carotenoids act by reaction with Keap1 reactive thiols with the last three of these acting through their oxidation products. Allium sulfur compounds, isothiocyanates and carotenoids act via ERK stimulation[121,122], with the latter two acting via two distinct mechanisms to raise Nrf2. Some flavonoids and other phenolics, including some that are inactive in the Keap1 reactions act as AhR agonists[123], some act via protein kinase signaling[124] and some act via their quinone oxidation products directly on Keap1 thiols[2]. Terpenoids are thought to act via three distinct mechanisms, directly on the Keap1/Nrf2 protein complex, through protein kinase regulation and also via miRNA regulation[2,19].

It follows from all this that phytochemicals and other agents can increase Nrf2 activity by reacting either directly or through their oxidation products with different cysteine residues on Keap1, by regulating the activity of a number of different protein kinases, by stimulating the AhR receptors or by acting via histone acetylation or other mechanisms to influence micro RNA synthesis and therefore Nrf2 activity. It follows from this that phytochemicals and other agents that act in different ways to raise Nrf2 may be expected to act synergistically together. An example of such synergism was reported by Saw et al[125] who showed that the carotenoid astaxanthin and the fish oil fatty acids DHA and EPA acted synergistically with each other in raising Nrf2. The components of Protandim were shown in cell culture to act synergistically in raising Nrf2 responses, probably due to the role of multiple signaling pathways in their actions in raising Nrf2[95].

Consequently phytochemically rich diets such as the traditional Mediterranean diet and the traditional Okinawan diet may be expected to be more active in Nrf2 activation than may be suggested from just looking at the activities of their individual Nrf2 raising nutrients, due to such synergisms.

Can too much Nrf2 over extensive time periods be toxic?

In general as indicated in ref.[107] raising Nrf2 produces prolonged lifespans and healthspans in animal studies. In addition, human diets rich in nutrients that raise Nrf2 including the traditional Mediterranean and Okinawan diets produce longer lifespans and lowered disease incidences. However, there are situations where chronic high-level Nrf2 stimulation produces pathophysiological responses in the body. Perhaps the clearest, well-documented example of this is where high level chronic raising of Nrf2 levels by TCDD (dioxin) leads to chloracne[126,127]. TCDD also has other, Nrf2 independent toxic effects but these acne-like changes skin properties are clearly caused by excessive, long-term levels of Nrf2, such that chloracne may serve as a marker for excessive Nrf2 stimulation. Arsenite and other arsenicals can also produce similar skin responses, acting via excessive Nrf2 activity[127], but again arsenite has other Nrf2 independent toxic effects. Both the TCDD and the arsenite effects act through AhR stimulation to produce elevated Nrf2 activity. These skin effects of excessive Nrf2 appear to be caused in part by the elevated sensitivity of keratinocytes to Nrf2.

This keratinocyte role also shows up in perhaps the most dramatic effect of excessive Nrf2. It was shown that Keap1 transgenic mouse knockout mutants developed hyperkeratosis in the esophagus and forestomach during gestation, which led to death from malnutrition after birth[128]. This was shown to be caused by excessive Nrf2 activity[128].

Months long, high level chronic elevation of Nrf2 is produced by certain conditions in the mouse, conditions that produce cardiac dysfunction[129,130]. While it is unclear how much of this dysfunction is caused by Nrf2, this may be another example where chronic, high level Nrf2 elevation may produce pathophysiological responses.

It is generally accepted that steady high level Nrf2 activity is much more likely to be damaging than is variable activation[2,131]. As stated earlier[131], "Pharmacologic induction of the pathway, however, allows for pulsed induction rather than permanent induction of the Keap1-Nrf2-signaling axis, which may reduce any untoward effects of constant pathway activation." The same reasoning applies to Nrf2 raising nutrients consumed at certain times of the day.

The possibility of what may appear to be paradoxical Nrf2 effects may occur where there is no Nrf2 at all, in Nrf2 knockout mutant cells. For example, it has been reported that such knockout cells are deficient in the activation of inflammasomes, showing that some Nrf2 activity may be required for some inflammatory responses[132].

In conclusion, it may be expected that levels of Nrf2 raising nutrients that occur in the Mediterranean or Okinawan diets will produce predominantly health-promoting effects. Nevertheless, very high chronic, long-term Nrf2 elevation can produce pathophysiological effects like almost any regulatory effect taken to extreme. Therefore, one needs to take care not to raise Nrf2 levels too high for too long. It is possible that some individuals may be much more susceptible to such pathophysiological effects, given the great amount of genetic heterogeneity in the human population. One way of minimizing any pathophysiological effects is to vary the levels of Nrf2-raising agents in the body at different times of the day. The acne-like skin responses to excessive, chronic long-term elevation of Nrf2 activity may serve as a visual indication of whether such excessive Nrf2 activity is occurring in humans in response to Nrf2 raising agents.

Summary

Gao et al[4] state that "Nrf2 activation or inhibition responding to oxidative or electrophilic stress , and designed to restore redox homeostasis, paves a new way to understand prevent or even cure complex diseases." The list of diseases in Table 1 where raising Nrf2 acts to prevent and/or treat the disease, at least in animal models is truly stunning. The regulation of Nrf2 and the regulatory responses produced by it are summarized in Fig. 1.

The action of 7 classes of health-promoting nutrients (box, inner left side, Fig.1) are each known to act to a great extent by raising Nrf2, as are 3 other health promoting factors. The two most healthful diets known, the traditional Mediterranean and Okinawan diets and the Paleolithic diet are all thought to be rich in Nrf2 raising nutrients, whereas modern diets are deficient in such nutrients (Fig.1, left). These findings strongly suggest that health-promotion by these diets acts, to a great extent via Nrf2 but that most of us are currently deficient in Nrf2 raising nutrients. Nrf2 acts, in turn via transcription of roughly 500 genes, to raise antioxidant responses, mitochondrial biogenesis and energy metabolism, detoxification of carbon-containing xenobiotics and toxic metals, autophagy of toxic protein aggregates and dysfunctional organelles and greatly lowering many inflammatory responses (Fig. 1, lower right). It is not surprising, therefore, that a large number of chronic diseases characterized by oxidative stress, inflammation and often mitochondrial function can be treated and/or prevented by raising Nrf2, at least in animal models (Fig.1, right). Nor should it be surprising that Nrf2 has been proposed to produce both lifespan and healthspan extension, given the many diseases of aging characterized by oxidative stress, inflammation and mitochondrial dysfunction (Fig1, upper right).

There are 16 other diseases that are reported to be prevented and/or treated by raising Nrf2[37-60], with each Nrf2-linked disease based on one or two studies. One would tend to ignore these, except for the fact that each of these diseases are diseases of oxidative stress and inflammation and therefore may plausibly be impacted by Nrf2; and also except for the fact that these are all based on recent and rapidly increasing numbers of studies (Table 4), suggesting that we are still in the early stages of findings about disease impacts of Nrf2.

Table 4: Nrf2 & Other Diseases
(Citations 37-60)

Year	#s of citations
2006	2
2007	1
2008	1
2009	1
2010	1
2011	4
2012	4
2013	6
2014 (5 months)	4

While no doubt it is too early to make a conclusion, it is difficult to escape the suggestion, from Tables 1 and 4, that we may be on the verge of a new literature on health effects of Nrf2 which may well become the most extraordinary therapeutic and most extraordinary preventive breakthrough in the history of medicine.

It is our opinion that raising Nrf2 is likely to be the most important health promoting approach into the foreseeable future. That is not to say that it is a magic bullet. More is not always better and other health promoting nutrients and other agents acting in other ways are likely to act along with Nrf2. Agents that lower NF- B via Nrf2-independent ways are likely to be useful. So are agents or diets that lower the production of advanced glycation end products with their RAGE receptor-mediated inflammatory responses. Nutrients that are health promoting in other ways, such as B vitamins and vitamin C, magnesium and some trace elements are likely to be useful, as are agents like high doses of the hydroxocobalamin form of B-12 which lowers peroxynitrite by lowering its two precursors. Other agents that act to improve mitochondrial function independent of Nrf2 are also likely to be useful, as well.

Many of the diseases that are thought to be prevented and/or treated by raising Nrf2 activity are also thought to be caused by what is called the NO/ONOO(-) cycle. These apparent NO/ONOO(-) cycle diseases that respond to Nrf2 include several cardiovascular and neurodegenerative diseases, asthma, multiple sclerosis, epilepsy, spinal cord injury and glaucoma[133-137]. Heart failure is now the best documented NO/ONOO(-) cycle disease[136]. The 23rd and most recent disease to be proposed to be caused by the local impact of the cycle is glaucoma[137]. Because the cycle involves oxidative stress including peroxynitrite elevation, inflammatory aspects and mitochondrial dysfunction, it should not be surprising that apparent NO/ONOO(-) cycle diseases may be prevented and/or treated by raised Nrf2. Because the NO/ONOO(-) cycle is primarily local, localized in different tissues in different individuals, it may cause a variety of different diseases, depending on where it is localized in the body[133-137]. One question that should be asked here is whether this notion that the Nrf2 regulatory system may be nature's way of preventing NO/ONOO(-) cycle diseases holds up in looking at other aspects of the cycle. While clearly increased Nrf2 activity may be expected to lower the oxidative/nitrosative stress, inflammatory and mitochondrial dysfunction parts of the cycle, and the majority of the cycle elements are part of these three aspects of the cycle[133-137], there are other cycle elements that are not involved with oxidative/nitrosative stress, inflammation or mitochondrial dysfunction. Does Nrf2 also lower pathophysiological consequences of these other parts of the cycle? Here, the data are limited, but what data we have are supportive of this prediction. Pathophysiological consequences of excessive NMDA activity[138-140] and excessive intracellular calcium levels[140,141] are both lowered by Nrf2. Tetrahydrobiopterin oxidation and depletion, another part of the cycle, have been shown to be elevated in a Nrf2 knockout mouse[142], suggesting but not proving that raising Nrf2 will lower this part of the cycle. Clearly we need more studies on these issues, but the data available to date support the view that Nrf2 may well be nature's way of preventing NO/ONOO(-) cycle diseases. It follows that our dietary deficiencies of Nrf2 raising nutrients may well be the central cause of the high incidence and prevalence of these diseases in the modern world.

The stunning apparent breadth of the effects of Nrf2 on diverse diseases produces a challenge for medicine. Medicine has historically focused mainly on the ways in which these various diseases differ from one another, as a way of understanding their differences. However it is possible that these diverse chronic inflammatory diseases all have a similar underlying mechanism and differ from one another primarily in their localization in the body, with the differences in localization being responsible for any differences in their etiologies. That does not necessarily mean that all these diseases are NO/ONOO(-) cycle diseases, but that may well be the most apparent available explanation.

It has become commonplace for some physicians and some other scientists to argue against the importance of oxidative stress in human disease. This despite the extensive and repeated evidence for the existence of and importance of protein changes produced by oxidative/nitrosative chemistry in dozens of chronic diseases. Watson, in his recent paper[143], shows that he knows about the existence of Nrf2, but is apparently completely unaware of its activation by oxidants and its role in producing complex and extraordinarily well coordinated enzymatic antioxidant responses. The complexity and coordination of these responses could not possibly have evolved without strong genetic selection based on the pathophysiological roles of oxidative/nitrosative stress in the etiology of many diseases. The Nrf2-controlled antioxidant mechanisms are telling us, therefore, that antioxidant mechanisms are among the most important mechanisms in metazoan evolution. While it is a major mistake to ignore the other Nrf2 cytoprotective mechanisms, it is also a major mistake to ignore the compelling evidence that the Nrf2 studies give us on the importance of enzymatic antioxidant mechanisms.

This paper started out emphasizing the special importance of Nrf2 to the hundreds of millions of people around the world who are exposed on a daily basis to toxic chemicals. The role of Nrf2 in producing complex and well coordinated detoxification mechanisms allows us to focus on raising Nrf2 as a way of substantially lowering the pathophysiological effects of such exposures by detoxification of the body, lowering levels of both organic, carbon-containing xenobiotic toxicants and also toxic metals.

Conflict of Interest:

One of us (MLP) declares that he is designing two nutritional supplement combinations aimed as raising the levels of Nrf2. That is our only possible conflict of interest.

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