Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors

Article *in* Sheng li xue bao: [Acta physiologica Sinica] · February 2015

Source: PubMed

CITATIONS
181

READS
9,628

2 authors:

Martin L Pall
Washington State University
121 PUBLICATIONS **4,903** CITATIONS
SEE PROFILE

Stephen Levine
Allergy Research Group Alameda California
6 PUBLICATIONS **213** CITATIONS
SEE PROFILE

Acta Physiologica Sinica, February 25, 2015, 67(1): 1–18
DOI: 10.13294/j.aps.2015.0001 http://www.actaps.com.cn

Review

Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors

Martin L Pall[1,*], Stephen Levine[2]

[1]*Washington State University, Portland, Oregon 97232-3312, USA;* [2]*Allergy Research Group, Alameda, California 94502, USA*

Abstract: The transcription factor Nrf2, nuclear factor erythroid-2-related factor 2, 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; its 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; long chain omega-3 fatty acids DHA and EPA; 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 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.

Key words: electrophiles and oxidants; Keap1; ERK; PI3K; GSK-3β; AMPK; protein kinases C and G; toxic xenobiotics and toxic metals; detoxification; chronic inflammatory diseases; oxidative and nitrosative stress

Nrf2是调控解毒、抗氧化、抗炎等细胞保护机制的重要转录因子——它的活性可被保健食物及其他因素增强

Martin L Pall[1,*], Stephen Levine[2]

[1]华盛顿州立大学，波特兰，俄勒冈州 97232-3312，美国；[2]Allergy Research Group，阿拉米达，加利福尼亚州 94502，美国

摘 要：转录因子Nrf2 (nuclear factor erythroid-2-related factor 2)可激活人类基因组中500多种基因的转录，这些基因大多数具有细胞保护功能。Nrf2通过解毒机制产生细胞保护作用，这些机制增强了有害异物和有毒金属的解毒和排泄。Nrf2经20多种基因的作用来增加高度协调的抗氧化活性；Nrf2也具有重要的抗炎作用；Nrf2促进线粒体的生物合成抑或提高线粒体功能；Nrf2增强细胞自噬以清除毒性蛋白的聚集体和功能异常的细胞器。有益健康的营养素和其他因素，包括酚类抗氧化剂、γ-和δ-生育酚和三烯生育酚、长链Ω-3脂肪酸DHA和EPA、类胡萝卜素(其中番茄红素可能活性最强)、十字花科蔬菜中的异硫氰酸酯、葱蒜类蔬菜中的硫化物、及萜类化合物，至少部分是通过增加Nrf2活性起作用的。其他一些有益健康并增加Nrf2活性

Received 2014-07-28 Accepted 2014-09-22

*Corresponding author. Tel: +1-503-232-3883; E-mail: martin_pall@wsu.edu

的因素包括低水平的氧化应激[毒物兴奋效应(hormesis)]、锻炼和热量限制。现已发现，增加Nrf2活性可预防和/或治疗模型动物和/或人类许多慢性炎症性疾病，包括各种心血管疾病、肾脏疾病、肺脏疾病、中毒性肝损伤疾病、癌症(预防)、糖尿病/代谢综合征/肥胖、败血症、自身免疫性疾病、炎性肠病、HIV/AIDS及癫痫。较少证据提示增加Nrf2活性可降低其他16种疾病的风险，这些疾病中的大多数可能是NO/$ONOO^-$环有关的疾病，而Nrf2可削弱NO/$ONOO^-$环元素的多种作用。已知最健康的饮食(地中海和冲绳地区的传统饮食)富含促进Nrf2活性的营养素，这就像我们的祖先在旧石器时代的饮食一样。Nrf2是否同时具有延长寿命和促进健康的作用是有争议的。Nrf2活性过度的可能负面作用也被讨论。Nrf2不是一个灵丹妙药，但可能对于促进健康非常重要，特别是对于那些日常暴露于有毒化学药品的人。

关键词：亲电体和氧化剂；Keap1；ERK；PI3K；GSK-3β；AMPK；蛋白激酶C和G；有害异物和有毒金属；解毒；慢性炎症性疾病；氧化和硝化应激
中图分类号：Q75；R151.4

1 Introduction

Nrf2 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 lower inflammatory responses, improve mitochondrial function and stimulate 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.

2 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.

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 (Martin L Pall) 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 pre-

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 diseases including atherosclerosis, ischemic cardiovascular disease, vascular endothelial dysfunction, and heart failure
2, 4, 5, 6, 12, 13, 19, 23, 24	Neurodegenerative diseases including Alzheimer's, Parkinson's, ALS, Huntington's diseases
2, 3, 4, 13, 19	Cancer (prevention)
2, 6, 7, 15, 19, 23	Chronic kidney diseases
2, 8, 10, 20, 23	Metabolic diseases: Type 2 diabetes; metabolic syndrome; obesity
2, 8, 19, 20, 23	Several types of toxic liver disease
2, 6, 16, 21, 23, 25, 27	Chronic lung diseases including emphysema, asthma, and pulmonary fibrosis
4, 14, 26	Sepsis
2, 4, 16, 23, 27–30	Autoimmune diseases
4, 13, 23, 31	Inflammatory bowel disease
4, 32	HIV/AIDS
11, 12	Multiple sclerosis
17, 18, 33, 34	Epilepsy

vented 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[35], malaria[36], spinal cord injury[37], traumatic brain injury[38,39], altitude sickness[40,41], the three classic psychiatric diseases, major depression, schizophrenia and bipolar disorder[42–45], gastric ulcers[46,47], glaucoma[48], age-related macular degeneration[49], cataract[50,51], pathophysiological responses to herpes activation[52] and benign prostatic hyperplasia[53,54]. These diseases all involve oxidative stress and inflammatory aspects. Nrf2 is also shown to protect cells from effects of ionizing radiation[55,56]. Nrf2 was reported to lower skin sensitization produced by sensitizing chemicals[57,58]. 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.

3 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.

4 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 mediates 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 is 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[59], 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.

5 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 8 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[60]. Metallothionein has roles in chelating, transporting and excreting both essential and toxic metals, including cadmium, mercury, lead and arsenic[61]. However it should be noted that 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[60]. Metallothionein levels have been shown to have a role in determining lead toxicity[62]. Toyama *et al.*[63] 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[64]. 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.

6 Anti-inflammatory effects of Nrf2

Nrf2 activation produces a wide variety of anti-inflammatory effects including lowered NF-κB 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-κB is very complex, with each having effects that both increase and decrease the other. However it is clear that[6] "NF-κB pathway is inhibited by several Nrf2 activators" but the specific mechanisms responsible for Nrf-2 mediated lowering of NF-κB is still somewhat uncertain. However[6] "Conversely, recent experimental evidence indicates that NF-κB 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-β[16].

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

7 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[65], 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[66,67]. 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 as 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[68–71], 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-β signaling.

8 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.

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 established 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 or-

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	γ, δ-tocopherols and tocotrienols (but α-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, 74, 75	Sulfur compounds including allyl sulfides in garlic/onion/allium foods
2, 76, 77	Many carotenoids with lycopene apparently the most active
3, 78, 79	Fish oil (long chain omega-3 fatty acids DHA and EPA)
3, 80	Modest oxidative stress (hormesis)
4, 9, 22	Exercise, works in part via modest oxidative stress; may also work in the vasculature via laminar shear stress

tho 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 γ & δ forms are much more active than that in α-tocopherol in raising Nrf2[72,73]. α-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 γ & δ tocopherols and tocotrienols[81].

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 [82–89]). 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[90–92]. 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[93] 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 α-lipoic acid. It has also been reported that butyrate produced from dietary fiber fermentation in the colon, acts to raise Nrf2 in colonocytes[94]; this butyrate action may have implications regarding dietary fiber and Nrf2 control in the lower gastrointestinal (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[26,27,95]. The carotenoids act, primarily and possibly entirely, via their oxidation products to raise Nrf2[76,77]. 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.

9 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[96–103]. 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[96–103]. 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).

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 [96]. 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.*[97] 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[97] 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[103], 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[104], rather than primarily from fish.

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[97]
Allium-derived sulfur compounds	High consumption of garlic and onions	Relatively high (onions, other allium), probably similar to Chinese diet

Information derived from[96–103].

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[103], 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.

10 Is Nrf2 a master regulator of longevity and healthspan?

The concept that Nrf2 is a master regulator of not only longevity, but also more importantly of healthspan was suggested by Lewis *et al*.[105] in their paper entitled "Nrf2, a guardian of healthspan and gatekeeper of species longevity." They state[105] 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[106]. Conversely, a knockout of the Nrf2 gene leads to premature cellular senescence[107]. These roles of Nrf2 in determining cellular senescence rates should not be surprising, given the roles of oxidative stress in producing cellular senescence[108].

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 (Table 2). 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).

11 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[65] 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[66]. 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[109].

Nrf2 is also regulated by microRNAs, including miR-200a, that lower the translation of the Nrf2 mRNA or mRNAs of Nrf2 related proteins[110,111]. Because levels of miR-200a are regulated by histone acetylation[111], 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[94]. 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[112]. 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[113].

Protein kinase G has recently been shown to have a substantial role in activating Nrf2[114–117]. Its role may explain one of the long-standing puzzles about Nrf2, why does 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[117,118]. 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], H_2O_2 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[119,120], 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[121], some act via protein kinase signaling[122] 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 microRNA 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.*[123] 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[93].

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.

11 Can too much Nrf2 over extensive time periods be toxic?

In general as indicated in ref.[105] 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[124,125]. TCDD also has other, Nrf2 independent

toxic effects but these acne-like changes in 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[125], 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[126]. This was shown to be caused by excessive Nrf2 activity[126].

Months long, high level chronic elevation of Nrf2 is produced by certain conditions in the mouse, conditions that produce cardiac dysfunction[127,128]. 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[2,129]. As stated earlier[129], "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[130].

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.

12 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 actions 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 (Fig. 1, upper right).

There are 16 other diseases that are reported to be

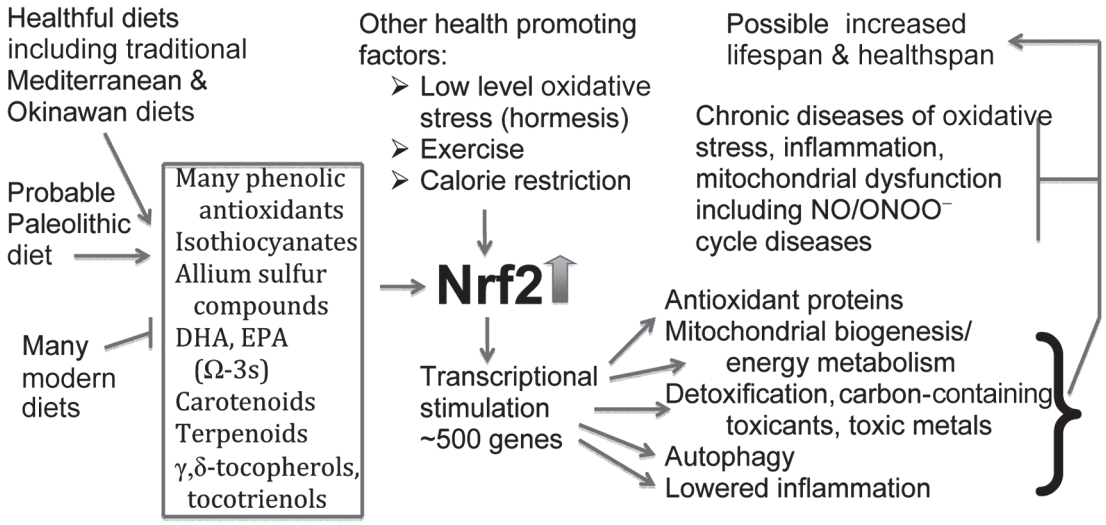

Fig. 1. Outline of the Nrf2 regulatory system.

prevented and/or treated by raising Nrf2[35–58], 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.

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-κB 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.

Table 4. Nrf2 & other diseases cited in citations [37–60]

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

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[131–135]. 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[135]. 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[131–135]. 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[131–135], 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[136–138] and excessive intracellular calcium levels[138,139] 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[140], 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 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[141], 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.

REFERENCES

1 Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides and interface between redox and intermediary metabolism. Trends Biochem Sci 2014; 39: 199–218.

2 Kumar H, Kim IS, More SV, Kim BW, Choi DK. Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic disease. Nat Prod Rep 2014; 31: 109–139.

3 Baird L, Dinkova-Kostova AT. The cytoprotective role of the Keap1-Nrf2 pathway. Arch Toxicol 2011; 85: 241–272.

4 Gao B, Doan A, Hybertson BM. The clinical potential of Nrf2 signaling in degenerative and immunological disorders. Clin Pharmacol 2014; 6: 19–34.

5 Sandberg M, Patil J, D'Angelo B, Weber SG, Mallard C. NRF2-regulation in brain health and disease: Implication of cerebral inflammation. Neuropharmacology 2014; 79: 298–306.

6 Buelna-Chontal M, Zazueta C. Redox activation of Nrf2 and NF-κB: A double end sword? Cell Signal 2013; 25: 2548–2557.

7 Saito H. Toxico-pharmacological perspective of the Nrf2-Keap1 defense system against oxidative stress in kidney diseases. Biochem Pharmacol 2013; 85: 865–872.

8 Seo HA, Lee IK. The role of Nrf2: Adipocyte differentiation, obesity, and insulin resistance. Oxid Med Cell Longev 2013; 2013: 184598

9 Mann GE, Niehueser-Saran J, Watson A, Gao L, Ishii T, de Winter P, Siow RC. Nrf2-ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: implications for atherosclerosis and preeclampsia. Acta Physiol Sin (生理学报) 2007; 59: 117–127.

10 Bocci V, Zanardi I. An integrated medical treatment for type-2 diabetes. Diabetes Metab Syndr 2014; 8: 57–61.

11 Arnold P, Mojumder D, Detoledo J, Lucius R, Wilms H. Pathophysiological processes in multiple sclerosis: focus on nuclear factor erythroid-2-related factor 2 and emerging pathways. Clin Pharmacol 2014; 6: 35–42.

12 Lee DH, Gold R, Linker RA. Mechanisms of oxidative damage in multiple sclerosis and neurodegenerative diseases: Therapeutic modulation via fumaric acid esters. Int J Mol Sci 2012; 13: 11783–11803.

13 Zhuang C, Miao Z, Sheng C, Zhang W. Updated research and applications of small molecule inhibitors of Keap1-Nrf2 protein-protein interaction: a review. Curr Med Chem 2014; 16. DOI: 10.2174/0929867321666140217104648.

14 Lee JW, Bae CJ, Choi YJ, Kim SI, Kwon YS, Lee HJ, Kim SS, Chun W. 3,4,5-Trihydroxycinnamic acid inhibits lipopolysaccharide (LPS)-induced inflammation by Nrf2 activation *in vitro* and improves survival of mice in LPS-induced endotoxemia model *in vivo*. Mol Cell Biochem 2014; 390: 143–153.

15 Pedruzzi LM, Stockler-Pinto MB, Leite M Jr, Mafra D. Nrf2-keap1 system versus NF-κB: the good and the evil in chronic kidney disease? Biochimie 2012; 94: 2461–2466.

16 Kim J, Cha YN, Surh YJ. A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. Mutat Res 2010; 690: 12–23.

17 Wang W, Wu Y, Fang H, Wang H, Zang H, Xie T, Wang W. Activation of Nrf2-ARE signal pathway protects the brain from damage induced by epileptic seizure. Brain Res 2014; 1544: 54–61.

18 Mazzuferi M, Kumar G, van Eyll J, Danis B, Foerch P, Kaminski RM. Nrf2 defense pathway: Experimental evidence for its protective role in epilepsy. Ann Neurol 2013; 74: 560–568.

19 Loboda A, Rojczyk-Golebiewska E, Bednarczyk-Cwynar B, Lucjusz Z, Jozkowicz A, Dulak J. Targeting Nrf2-mediated gene transcription by triterpenoids and their derivatives. Biomol Ther (Seoul) 2012; 20: 499–505.

20 Vomhof-Dekrey EE, Picklo MJ Sr. The Nrf2-antioxidant response element pathway: a target for regulating energy metabolism. J Nutr Biochem 2012; 23: 1201–1206.

21 Cho HY, Kleeberger SR. Nrf2 protects against airways disorders. Toxicol Appl Pharmacol 2010; 244: 43–56.

22 Reuland DJ, McCord JM, Hamilton KL. The role of Nrf2 in the attenuation of cardiovascular disease. Exerc Sport Sci Rev 2013; 41: 162–168.

23 Liby KT, Sporn MB. Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease. Pharmacol Rev 2012; k64: 972–1003.

24 Bergström P, von Otter M, Nilsson S, Nilsson AC, Nilsson M, Andersen PM, Hammarsten O, Zetterberg H. Association of NFE2L2 and KEAP1 haplotypes with amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2014; 15: 130–137.

25 Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, Yamamoto M, Kensler TW, Tuder RM, Georas SN, Biswal S. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J Exp Med 2005; 202: 47–59.

26 Kim JH, Choi YK, Lee KS, Cho DH, Baek YY, Lee DK, Ha KS, Choe J, Won MH, Jeoung D, Lee H, Kwon YG, Kim YM. Functional dissection of Nrf2-dependent phase II genes in vascular inflammation and endotoxic injury using Keap1 siRNA. Free Radic Biol Med 2012; 53: 629–640.

27 Jiang T, Tian F, Zheng H, Whitman SA, Lin Y, Zhang Z, Zhang N, Zhang DD. Nrf2 suppresses lupus nephritis through inhibition of oxidative injury and the NF-κB-mediated inflammatory response. Kidney Int 2014; 85: 333–343.

28 Fragoulis A, Laufs J, Müller S, Soppa U, Siegl S, Reiss LK, Tohidnezhad M, Rosen C, Tenbrock K, Varoga D, Lippross S, Pufe T, Wruck CJ. Sulforaphane has opposing effects on TNF-alpha stimulated and unstimulated synoviocytes. Arthritis Res Ther 2012; 27;14(5): R220.

29 Tsai PY, Ka SM, Chang JM, Lai JH, Dai MS, Jheng HL, Kuo MT, Chen P, Chen A. Antroquinonol differentially modulates T cell activity and reduces interleukin-18 production, but enhances Nrf2 activation, in murine accelerated severe lupus nephritis. Arthritis Rheum 2012; 64: 232–242.

30 Li B, Cui W, Liu J, Li R, Liu Q, Xie XH, Ge XL, Zhang J, Song XJ, Wang Y, Guo L. Sulforaphane ameliorates the development of experimental autoimmune encephalomyelitis by antagonizing oxidative stress and Th17-related inflammation in mice. Exp Neurol 2013; 250: 239–249.

31 Yalniz M, Demirel U, Orhan C, Bahcecioglu IH, Ozercan IH, Aygun C, Tuzcu M, Sahin K. Nadroparin sodium activates Nrf2/HO-1 pathway in acetic acid-induced colitis in rats. Inflammation 2012; 35: 1213–1221.

32 Scofield VL, Yan M, Kuang X, Kim SJ, Wong PK. The drug monosodium luminol (GVT) preserves crypt-villus epithelial organization and allows survival of intestinal T cells in mice infected with the ts1 retrovirus. Immunol Lett

2009; 122: 150–158.

33 Wang W, Wang WP, Zhang GL, Wu YF, Xie T, Kan MC, Fang HB, Wang HC. Activation of Nrf2-ARE signal pathway in hippocampus of amygdala kindling rats. Neurosci Lett 2013; 543: 58–63.

34 Milder JB, Liang LP, Patel M. Acute oxidative stress and systemic Nrf2 activation by the ketogenic diet. Neurobiol Dis 2010; 40: 238–244.

35 Macari ER, Lowrey CH. Induction of human fetal hemoglobin via the NRF2 antioxidant response signaling pathway. Blood 2011; 117: 5987–5997.

36 Olagnier D, Lavergne RA, Meunier E, Lefèvre L, Dardenne C, Aubouy A, Benoit-Vical F, Ryffel B, Coste A, Berry A, Pipy B. Nrf2, a PPARγ alternative pathway to promote CD36 expression on inflammatory macrophages: implication for malaria. PLoS Pathog 2011; 7(9): e1002254.

37 Jin W, Ming X, Hou X, Zhu T, Yuan B, Wang J, Ni H, Jiang J, Wang H, Liang W. Protective effects of erythropoietin in traumatic spinal cord injury by inducing the Nrf2 signaling pathway activation. J Trauma Acute Care Surg 2014; 76: 1228–1234.

38 Jin W, Wang H, Yan W, Zhu L, Hu Z, Ding Y, Tang K. Role of Nrf2 in protection against traumatic brain injury in mice. J Neurotrauma 2009; 26: 131–139.

39 Jin W, Zhu L, Guan Q, Chen G, Wang QF, Yin HX, Hang CH, Shi JX, Wang HD. Influence of Nrf2 genotype on pulmonary NF-kappaB activity and inflammatory response after traumatic brain injury. Ann Clin Lab Sci 2008; 38: 221–227.

40 Sharma NK, Sethy NK, Meena RN, Ilavazhagan G, Das M, Bhargava K. Activity-dependent neuroprotective protein (ADNP)-derived peptide (NAP) ameliorates hypobaric hypoxia induced oxidative stress in rat brain. Peptides 2011; 32: 1217–1224.

41 Lisk C, McCord J, Bose S, Sullivan T, Loomis Z, Nozik-Grayck E, Schroeder T, Hamilton K, Irwin DC. Nrf2 activation: a potential strategy for the prevention of acute mountain sickness. Free Radic Biol Med 2013; 63: 264–273.

42 Martín-de-Saavedra MD, Budni J, Cunha MP, Gómez-Rangel V, Lorrio S, Del Barrio L, Lastres-Becker I, Parada E, Tordera RM, Rodrigues AL, Cuadrado A, López MG. Nrf2 participates in depressive disorders through an anti-inflammatory mechanism. Psychoneuroendocrinology 2013; 38: 2010–2022.

43 Maes M, Fišar Z, Medina M, Scapagnini G, Nowak G, Berk M. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates--Nrf2 activators and GSK-3 inhibitors. Inflammopharmacology 2010; 2012; 20: 127–150.

44 Shirai Y, Fujita Y, Hashimoto K. Effects of the antioxidant sulforaphane on hyperlocomotion and prepulse inhibition deficits in mice after phencyclidine administration. Clin Psychopharmacol Neurosci 2012; 10: 94–98.

45 Rizak J, Tan H, Zhu H, Wang JF. Chronic treatment with the mood-stabilizing drug lithium up-regulates nuclear factor E2-related factor 2 in rat pheochromocytoma PC12 cells *in vitro*. Neuroscience 2014; 256: 223–229.

46 Shibuya A, Onda K, Kawahara H, Uchiyama Y, Nakayama H, Omi T, Nagaoka M, Matsui H, Hirano T. Sofalcone, a gastric mucosa protective agent, increases vascular endothelial growth factor via the Nrf2-heme-oxygenase-1 dependent pathway in gastric epithelial cells. Biochem Biophys Res Commun 2010; 398: 581–584.

47 Arisawa T, Tahara T, Shibata T, Nagasaka M, Nakamura M, Kamiya Y, Fujita H, Hasegawa S, Takagi T, Wang FY, Hirata I, Nakano H. The relationship between Helicobacter pylori infection and promoter polymorphism of the Nrf2 gene in chronic gastritis. Int J Mol Med 2007; 19: 143–148.

48 Himori N, Yamamoto K, Maruyama K, Ryu M, Taguchi K, Yamamoto M, Nakazawa T. Critical role of Nrf2 in oxidative stress-induced retinal ganglion cell death. J Neurochem 2013; 127: 669–680.

49 Wang L, Cano M, Handa JT. p62 provides dual cytoprotection against oxidative stress in the retinal pigment epithelium. Biochim Biophys Acta 2014; 1843: 1248–1258.

50 Varma SD, Chandrasekaran K, Kovtun S. Sulforaphane-induced transcription of thioredoxin reductase in lens: possible significance against cataract formation. Clin Ophthalmol 2013; 7: 2091–2098.

51 Liu H, Smith AJ, Lott MC, Bao Y, Bowater RP, Reddan JR, Wormstone IM. Sulforaphane can protect lens cells against oxidative stress: implications for cataract prevention. Invest Ophthalmol Vis Sci 2013; 54: 5236–5248.

52 Schachtele SJ, Hu S, Lokensgard JR. Modulation of experimental herpes encephalitis-associated neurotoxicity through sulforaphane treatment. PLoS One 2012; 7(4): e36216.

53 Clarke JD, Hsu A, Yu Z, Dashwood RH, Ho E. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. Mol Nutr Food Res 2011; 55: 999–1009.

54 Myzak MC, Hardin K, Wang R, Dashwood RH, Ho E. Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. Carcinogenesis 2006; 27: 811–819.

55 Mathew ST, Bergström P, Hammarsten O. Repeated Nrf2

stimulation using sulforaphane protects fibroblasts from ionizing radiation. Toxicol Appl Pharmacol 2014; 276: 188–194.

56 Reisman SA, Lee CY, Meyer CJ, Proksch JW, Sonis ST, Ward KW. Topical application of the synthetic triterpenoid RTA 408 protects mice from radiation-induced dermatitis. Radiat Res 2014; 181: 512–520.

57 El Ali Z, Gerbeix C, Hemon P, Esser PR, Martin SF, Pallardy M, Kerdine-Römer S. Allergic skin inflammation induced by chemical sensitizers is controlled by the transcription factor Nrf2. Toxicol Sci 2013; 134: 39–48.

58 van der Veen JW, Gremmer ER, Vermeulen JP, van Loveren H, Ezendam J. Induction of skin sensitization is augmented in Nrf2-deficient mice. Arch Toxicol 2013; 87: 763–766.

59 Lu J, Holmgren A. The thioredoxin antioxidant system. Free Radic Biol Med 2014; 66: 75–87.

60 Wu KC, Liu JJ, Klaassen CD. Nrf2 activation prevents cadmium-induced acute liver injury. Toxicol Appl Pharmacol 2012; 263: 14–20.

61 Sears ME. Chelation: harnessing and enhancing heavy metal detoxification--a review. ScientificWorldJournal 2013; 2013: 219840.

62 Yang CC, Chen HI, Chiu YW, Tsai CH, Chuang HY. Metallothionein 1A polymorphisms may influence urine uric acid and *N*-acetyl-beta-*D*-glucosaminidase (NAG) excretion in chronic lead-exposed workers. Toxicology 2013; 306: 68–73.

63 Toyama T, Shinkai Y, Yasutake A, Uchida K, Yamamoto M, Kumagai Y. Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury. Environ Health Perspect 2011; 119: 1117–1122.

64 García-Niño WR, Pedraza-Chaverrí J. Protective effect of curcumin against heavy metals-induced liver damage. Food Chem Toxicol 2014; 69: 182–201

65 Mo C, Wang L, Zhang J, Numazawa S, Tang H, Tang X, Han X, Li J, Yang M, Wang Z, Wei D, Xiao H. The crosstalk between Nrf2 and AMPK signal pathways is important for the anti-inflammatory effect of berberine in LPS-stimulated macrophages and endotoxin-shocked mice. Antioxid Redox Signal 2014; 20: 574–588.

66 Jain A, Lamark T, Sjøttem E, Larsen KB, Awuh JA, Øvervatn A, McMahon M, Hayes JD, Johansen T. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. J Biol Chem 2010; 285: 22576–22591.

67 Nezis IP, Stenmark H. p62 at the interface of autophagy, oxidative stress signaling, and cancer. Antioxid Redox Signal 2012; 17: 786–793.

68 Artaud-Macari E, Goven D, Brayer S, Hamimi A, Besnard V, Marchal-Somme J, Ali ZE, Crestani B, Kerdine-Römer S, Boutten A, Bonay M. Nuclear factor erythroid 2-related factor 2 nuclear translocation induces myofibroblastic dedifferentiation in idiopathic pulmonary fibrosis. Antioxid Redox Signal 2013; 18: 66–79.

69 Oh CJ, Kim JY, Min AK, Park KG, Harris RA, Kim HJ, Lee IK. Sulforaphane attenuates hepatic fibrosis via NF-E2-related factor 2012; 2-mediated inhibition of transforming growth factor-β/Smad signaling. Free Radic Biol Med 2012; 52: 671–682.

70 Ryoo IG, Ha H, Kwak MK. Inhibitory role of the KEAP1-NRF2 pathway in TGFβ1-stimulated renal epithelial transition to fibroblastic cells: a modulatory effect on SMAD signaling. PLoS One 2014; 9(4): e93265.

71 Hecker L, Logsdon NJ, Kurundkar D, Kurundkar A, Bernard K, Hock T, Meldrum E, Sanders YY, Thannickal VJ. Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. Sci Transl Med 2014; 6(231): 231ra47.

72 Hsieh TC, Elangovan S, Wu JM. Differential suppression of proliferation in MCF-7 and MDA-MB-231 breast cancer cells exposed to alpha-, gamma- and delta-tocotrienols is accompanied by altered expression of oxidative stress modulatory enzymes. Anticancer Res 2010; 30: 4169–4176.

73 Smolarek AK, So JY, Thomas PE, Lee HJ, Paul S, Dombrowski A, Wang CX, Saw CL, Khor TO, Kong AN, Reuhl K, Lee MJ, Yang CS, Suh N. Dietary tocopherols inhibit cell proliferation, regulate expression of ERα, PPARγ, and Nrf2, and decrease serum inflammatory markers during the development of mammary hyperplasia. Mol Carcinog 2013; 52: 514–525.

74 Ho CY, Cheng YT, Chau CF, Yen GC. Effect of diallyl sulfide on *in vitro* and *in vivo* Nrf2-mediated pulmonic antioxidant enzyme expression via activation ERK/p38 signaling pathway. J Agric Food Chem 2012; 60: 100–107.

75 Colín-González AL, Santana RA, Silva-Islas CA, Chánez-Cárdenas ME, Santamaría A, Maldonado PD. The antioxidant mechanisms underlying the aged garlic extract- and S-allylcysteine-induced protection. Oxid Med Cell Longev 2012; 2012: 907162

76 Yang CM, Huang SM, Liu CL, Hu ML. Apo-8'-lycopenal induces expression of HO-1 and NQO-1 via the ERK/p38-Nrf2-ARE pathway in human HepG2 cells. J Agric Food Chem 2012; 60: 1576–1585.

77 Linnewiel K, Ernst H, Caris-Veyrat C, Ben-Dor A, Kampf A, Salman H, Danilenko M, Levy J, Sharoni Y. Structure activity relationship of carotenoid derivatives in activation

of the electrophile/antioxidant response element transcription system. Free Radic Biol Med 2009; 47: 659–667.

78 Zhang M, Wang S, Mao L, Leak RK, Shi Y, Zhang W, Hu X, Sun B, Cao G, Gao Y, Xu Y, Chen J, Zhang F. Omega-3 fatty acids protect the brain against ischemic injury by activating Nrf2 and upregulating heme oxygenase 1. J Neurosci 2014; 34: 1903–1915.

79 Nakagawa F, Morino K, Ugi S, Ishikado A, Kondo K, Sato D, Konno S, Nemoto K, Kusunoki C, Sekine O, Sunagawa A, Kawamura M, Inoue N, Nishio Y, Maegawa H. 4-Hydroxy hexenal derived from dietary n-3 polyunsaturated fatty acids induces anti-oxidative enzyme heme oxygenase-1 in multiple organs. Biochem Biophys Res Commun 2014; 443: 991–996.

80 Maher J, Yamamoto M. The rise of antioxidant signaling—The evolution and hormetic actions of Nrf2. Toxicol Appl Pharmacol 2010; 244: 4–15.

81 Sontag TJ, Parker RS. Influence of major structural features of tocopherols and tocotrienols on their omega-oxidation by tocopherol-omega-hydroxylase. J Lipid Res 2007; 48: 1090–1098.

82 Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res 2002; 62: 5196–5203.

83 Kwak MK, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M, Kensler TW. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. J Biol Chem 2003; 278: 8135–8145.

84 McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, Wolf CR, Cavin C, Hayes JD. The Cap 'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. Cancer Res 2001; 61: 3299–3307.

85 Cho HY, Jedlicka AE, Reddy SP, Kensler TW, Yamamoto M, Zhang LY, Kleeberger SR. Role of NRF2 in protection against hyperoxic lung injury in mice. Am J Respir Cell Mol Biol 2002; 26: 175–182.

86 Itoh K, Tong K, Yamamoto M. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med 2004; 36: 1208–1213.

87 Nair S1, Xu C, Shen G, Hebbar V, Gopalakrishnan A, Hu R, Jain MR, Liew C, Chan JY, Kong AN. Pharmacogenomics of phenolic antioxidant butylated hydroxyanisole (BHA) in the small intestine and liver of Nrf2 knockout and C57BL/6J mice. Pharm Res 2006; 23: 2621–2637.

88 Wang H, Khor TO, Saw CL, Lin W, Wu T, Huang Y, Kong AN. Role of Nrf2 in suppressing LPS-induced inflammation in mouse peritoneal macrophages by polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid. Mol Pharm 2010; 7: 2185–2193.

89 Gerhäuser C, Klimo K, Hümmer W, Hölzer J, Petermann A, Garreta-Rufas A, Böhmer FD, Schreier P. Identification of 3-hydroxy-beta-damascone and related carotenoid-derived aroma compounds as novel potent inducers of Nrf2-mediated phase 2 response with concomitant anti-inflammatory activity. Mol Nutr Food Res 2009; 53: 1237–1244.

90 Martín-Montalvo A, Villalba JM, Navas P, de Cabo R. NRF2, cancer and calorie restriction. Oncogene 2011; 30: 505–520.

91 Pearson KJ, Lewis KN, Price NL, Chang JW, Perez E, Cascajo MV, Tamashiro KL, Poosala S, Csiszar A, Ungvari Z, Kensler TW, Yamamoto M, Egan JM, Longo DL, Ingram DK, Navas P, de Cabo R. Nrf2 mediates cancer protection but not prolongevity induced by caloric restriction. Proc Natl Acad Sci U S A 2008; 105: 2325–2330.

92 Ungvari Z, Parrado-Fernandez C, Csiszar A, de Cabo R. Mechanisms underlying caloric restriction and lifespan regulation: implications for vascular aging. Circ Res 2008; 102: 519–528.

93 Velmurugan K, Alam J, McCord JM, Pugazhenthi S. Synergistic induction of heme oxygenase-1 by the components of the antioxidant supplement Protandim. Free Radic Biol Med 2009; 46: 430–440.

94 Yaku K, Enami Y, Kurajyo C, Matsui-Yuasa I, Konishi Y, Kojima-Yuasa A. The enhancement of phase 2 enzyme activities by sodium butyrate in normal intestinal epithelial cells is associated with Nrf2 and p53. Mol Cell Biochem 2012; 370: 7–14.

95 Cipollina C, Salvatore SR, Muldoon MF, Freeman BA, Schopfer FJ. Generation and dietary modulation of anti-inflammatory electrophilic omega-3 Fatty Acid derivatives. PLoS One 2014; 9(4): e94836.

96 Willcox DC, Willcox BJ, Todoriki H, Suzuki M. The Okinawan diet: health implications of a low-calorie, nutrient-dense, antioxidant-rich dietary pattern low in glycemic load. J Am Coll Nutr 2009; 28 Suppl: 500S-516S.

97 Murakami A, Ishida H, Kobo K, Furukawa I, Ikeda Y, Yonaha M, Aniya Y, Ohigashi H. Suppressive effects of Okinawan food items on free radical generation from stimulated leukocytes and identification of some active constituents: implications for the prevention of inflammation-associated carcinogenesis. Asian Pac J Cancer Prev 2005; 6: 437–448.

98 Suzuki M, Wilcox BJ, Wilcox CD. Implications from and for food cultures for cardiovascular disease: longevity. Asia Pac J Clin Nutr 2001; 10: 165–171.

99 Kafatos A, Verhagen H, Moschandreas J, Apostolaki I, Van Westerop JJ. Mediterranean diet of Crete: foods and nutrient content. J Am Diet Assoc 2000; 100: 1487–1493.

100 Manios Y, Detopoulou V, Visioli F, Galli C. Mediterranean diet as a nutrition education and dietary guide: misconceptions and the neglected role of locally consumed foods and wild green plants. Forum Nutr 2006; 59: 154–170.

101 Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 1995; 1(6 Suppl): 1402S–1406S.

102 Simopoulos AP. 2001 The Mediterranean diets: What is so special about the diet of Greece? The scientific evidence. J Nutr 2001; 131(11 Suppl): 3065S-3073S.

103 Salen P, de Lorgeril M. The Okinawan diet: a modern view of an ancestral healthy lifestyle. World Rev Nutr Diet 2011; 102: 114–123.

104 Simopoulos AP. Omega-3 fatty acids and antioxidants in edible wild plants. Biol Res 2004; 37: 263–277.

105 Lewis KN, Mele J, Hayes JD, Buffenstein R. Nrf2, a guardian of healthspan and gatekeeper of species longevity. Integr Comp Biol 2010; 50: 829–843.

106 Kapeta S, Chondrogianni N, Gonos ES. Nuclear erythroid factor 2-mediated proteasome activation delays senescence in human fibroblasts. J Biol Chem 2010; 285: 8171–8184.

107 Jódar L, Mercken EM, Ariza J, Younts C, González-Reyes JA, Alcaín FJ, Burón I, de Cabo R, Villalba JM. Genetic deletion of Nrf2 promotes immortalization and decreases life span of murine embryonic fibroblasts. J Gerontol A Biol Sci Med Sci 2011; 66: 247–256.

108 Takahashi A, Ohtani N, Yamakoshi K, Iida S, Tahara H, Nakayama K, Nakayama KI, Ide T, Saya H, Hara E. Mitogenic signalling and the p16INK4a-Rb pathway cooperate to enforce irreversible cellular senescence. Nat Cell Biol 2006; 8: 1291–1297.

109 Niture SK, Khatri R, Jaiswal AK. Regulation of Nrf2-an update. Free Radic Biol Med 2014; 66: 36–44.

110 Narasimhan M, Patel D, Vedpathak D, Rathinam M, Henderson G, Mahimainathan L. Narasimhan M1, Patel D, Vedpathak D, Rathinam M, Henderson G, Mahimainathan L. PLoS One 2012; 7(12): e51111.

111 Eades G, Yang M, Yao Y, Zhang Y, Zhou Q. miR-200a regulates Nrf2 activation by targeting Keap1 mRNA in breast cancer cells. J Biol Chem 2011; 286: 40725–40733.

112 Sun Z, Chin YE, Zhang DD. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol Cell Biol 2009; 29: 2658–2672.

113 Seymour EM, Bennink MR, Bolling SF. Diet-relevant phytochemical intake affects the cardiac AhR and Nrf2 transcriptome and reduces heart failure in hypertensive rats. J Nutr Biochem 2013; 24: 1580–1586.

114 Astort F, Mercau M, Giordanino E, Degese MS, Caldareri L, Coso O, Cymeryng CB. Nitric oxide sets off an antioxidant response in adrenal cells: involvement of sGC and Nrf2 in HO-1 induction. Nitric Oxide 2014; 37: 1–10.

115 Kim SK, Joe Y, Zheng M, Kim HJ, Yu JK, Cho GJ, Chang KC, Kim HK, Han J, Ryter SW, Chung HT. Resveratrol induces hepatic mitochondrial biogenesis through the sequential activation of nitric oxide and carbon monoxide production. Antioxid Redox Signal 2014; 20: 2589–2605.

116 Liu XM, Peyton KJ, Wang X, Durante W. Sildenafil stimulates the expression of gaseous monoxide-generating enzymes in vascular smooth muscle cells via distinct signaling pathways. Biochem Pharmacol 2012; 84: 1045–1054.

117 Chung HT, Choi BM, Kwon YG, Kim YM. Interactive relations between nitric oxide (NO) and carbon monoxide (CO): heme oxygenase-1/CO pathway is a key modulator in NO-mediated antiapoptosis and anti-inflammation. Methods Enzymol 2008; 441: 329–338.

118 Rochette L, Cottin Y, Zeller M, Vergely C. Carbon monoxide: mechanisms of action and potential clinical implications. Pharmacol Ther 2013; 137: 133–152.

119 Gong P, Hu B, Cederbaum AI. Diallyl sulfide induces heme oxygenase-1 through MAPK pathway. Arch Biochem Biophys 2004; 432: 252–260.

120 Ho CY, Cheng YT, Chau CF, Yen GC. Effect of diallyl sulfide on *in vitro* and *in vivo* Nrf2-mediated pulmonic antioxidant enzyme expression via activation ERK/p38 signaling pathway. J Agric Food Chem 2012; 60: 100–107.

121 Amakura Y1, Tsutsumi T, Nakamura M, Kitagawa H, Fujino J, Sasaki K, Toyoda M, Yoshida T, Maitani T. Activation of the aryl hydrocarbon receptor by some vegetable constituents determined using *in vitro* reporter gene assay. Biol Pharm Bull 2003; 26: 532–539.

122 Mansuri ML, Parihar P, Solanki I, Parihar MS. Flavonoids in modulation of cell survival signalling pathways. Genes Nutr 2014; 9(3): 400.

123 Saw CL, Yang AY, Guo Y, Kong AN. Astaxanthin and omega-3 fatty acids individually and in combination protect against oxidative stress via the Nrf2-ARE pathway. Food Chem Toxicol 2013; 62: 869–875.

124 Schäfer M, Willrodt AH, Kurinna S, Link AS, Farwanah H, Geusau A, Gruber F, Sorg O, Huebner AJ, Roop DR, Sandhoff K, Saurat JH, Tschachler E, Schneider MR, Langbein L, Bloch W, Beer HD, Werner S. Activation of Nrf2 in keratinocytes causes chloracne (MADISH)-like skin disease

in mice. EMBO Mol Med 2014; 6: 442–457.

125 Tan NS, Wahli W. The emerging role of Nrf2 in dermatotoxicology. EMBO Mol Med 2014; 6: 431–433.

126 Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, Takahashi S, Imakado S, Kotsuji T, Otsuka F, Roop DR, Harada T, Engel JD, Yamamoto M. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nat Genet 2003; 35: 238–245.

127 Rajasekaran NS, Varadharaj S, Khanderao GD, Davidson CJ, Kannan S, Firpo MA, Zweier JL, Benjamin IJ. Sustained activation of nuclear erythroid 2-related factor 2/antioxidant response element signaling promotes reductive stress in the human mutant protein aggregation cardiomyopathy in mice. Antioxid Redox Signal 2011; 14: 957–971.

128 Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, Zhang XQ, Stevenson TJ, Peshock RM, Leopold JA, Barry WH, Loscalzo J, Odelberg SJ, Benjamin IJ. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. Cell 2007; 130: 427–439.

129 Slocum SL, Kensler TW. Nrf2: control of sensitivity to carcinogens. Arch Toxicol 2011; 85: 273–284.

130 Zhao C, Gillette DD, Li X, Zhang Z, Wen H. Nuclear factor E2-related factor-2 (Nrf2) is required for NLRP3 and AIM2 inflammasome activation. J Biol Chem 2014; 289(24): 17020–17009.

131 Pall ML. Explaining 'Unexplained Illness': Disease Paradigm for Chronic Fatigue Syndrome, Multiple Chemical Sensitivity, Fibromyalgia, Post-Traumatic Stress Disorder, Gulf War Syndrome and Others. New York: Harrington Park (Haworth) Press, 2007.

132 Pall ML. Teufelskreis NO/$ONOO^-$-Zyklus, oxidaver stress, mitochondriale, inflammatorische und neurologische dysfunktion. Umw Med Ges 2010; 23: 281–293.

133 Pall ML. Pulmonary hypertension is a probable NO/$ONOO^-$ cycle disease: A review. ISRN Hypertension 2013: Article ID 742418, 27 pages.

134 Pall ML. The NO/$ONOO^-$ cycle as the central cause of heart failure. Int J Mol Sci 2013; 14: 22274–22330.

135 Pall ML. Is open-angle glaucoma caused by the NO/$ONOO^-$ cycle acting at two locations in the eye? Med Hypothesis Discov Innov Ophthalmol 2014; 4 : 1–2.

136 Nada SE, Shah ZA. Preconditioning with *Ginkgo biloba* (EGb 761®) provides neuroprotection through HO1 and CRMP2. Neurobiol Dis 2012; 46: 180–189.

137 Shah ZA, Li RC, Ahmad AS, Kensler TW, Yamamoto M, Biswal S, Doré S. The flavanol (−)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway. J Cereb Blood Flow Metab 2010; 30: 1951–1961.

138 Nakamura T, Lipton SA. Preventing Ca^{2+}-mediated nitrosative stress in neurodegenerative diseases: possible pharmacological strategies. Cell Calcium 2010; 47: 190–197.

139 Lee JM, Shih AY, Murphy TH, Johnson JA. NF-E2-related factor-2 mediates neuroprotection against mitochondrial complex I inhibitors and increased concentrations of intracellular calcium in primary cortical neurons. J Biol Chem 2003; 278: 37948–37956.

140 Mukhopadhyay S, Sekhar KR, Hale AB, Channon KM, Farrugia G, Freeman ML, Gangula PR. Loss of NRF2 impairs gastric nitrergic stimulation and function. Free Radic Biol Med 2011; 51: 619–625.

141 Watson JD. Type 2 diabetes as a redox disease. Lancet 2014; 383: 841–843.