The role of the antioxidant and longevity-promoting Nrf2 pathway in metabolic regulation

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Abstract

Purpose of Review—The vertebrate cap’n’collar family transcription factor Nrf2 and its invertebrate homologs SKN-1 (in worms) and CncC (in flies) function as master mediators of antioxidant and detoxification responses and regulators of the cellular redox state. Nrf2 controls gene expression programs that defend various tissues against diverse electrophilic stressors and oxidative insults, thus protecting the organism from pathologies that are caused or exacerbated by such stresses. Moreover, studies in model organisms implicate the Nrf2 pathway in the prevention of aging-related diseases, and suggest that SKN-1- and CncC-regulated gene expression can promote longevity. These facets of Nrf2 signaling have been thoroughly reviewed. This article discusses another aspect of the Nrf2 pathway’s function that has not yet received the same degree of attention but emerges as a topic of increasing interest and potential clinical impact: its role in metabolic regulation and its interaction with central signaling systems that respond to nutritional inputs.

Recent findings—Recent evidence identifies Nrf2 signaling as a mediator of the salutary effects of caloric restriction. Nrf2 signaling also cross-talks with metabolic signaling systems such as the insulin/Akt pathway as well as with the metabolism of lipids. Moreover, Nrf2 has a protective role in models of diabetic nephropathy.

Summary—The emerging role of Nrf2 as an effector of metabolic and longevity signals offers new therapeutic perspectives. The potential impact of pharmacological manipulation of Nrf2 signaling as a strategy for the prevention and treatment of metabolic disease can be envisioned.

Keywords

Nrf2; calorie restriction; aging; insulin signaling; obesity; diabetic nephropathy

Introduction

The appearance of oxygen on the planet about 2.5 billion years ago provided a universal source of metabolic energy that facilitated the emergence of aerobic life and made evolution, as we know it, possible. However, the reliance on oxygen also presented a serious challenge to the life forms evolving in this setting. The large majority of biological macromolecules, from proteins to lipids and nucleic acids, are vulnerable to oxidative attack. Biological systems are especially sensitive to reactive oxygen species (ROS), the reactive forms of oxygen which arise either as by-products of oxidative phosphorylation in the mitochondria,
or as the result of exposure to environmental chemicals and toxins. ROS can disturb the homeostasis of cells and tissues, which ultimately threatens the integrity of the organism. In response to this internal and environmental pressure, complex stress defense systems have evolved that protect the organisms against oxidative damage.

The multiplicity and complexity of biological defense and repair systems reflects the existence of different types of oxidative challenge. Acute episodes of stress may occur as a result of toxicant exposure, injury, inflammation, infection, or other insults. Such potentially fatal threats are typically met by a vigorous response involving the rapid and massive expression of a battery of antioxidant, detoxification, and repair proteins. Acute stress responses are characterized by the cessation of cell division, large-scale degradation of irreparably damaged proteins or organelles by proteasomal and autophagic mechanisms, and ultimately the removal of fatally injured cells by apoptosis.

In addition to the defense against intermittent and acutely threatening oxidant exposures, aerobic organisms have to cope with a continuous base-level oxidative challenge that results from a trickle of ROS that is a normal by-product of cell respiration and metabolism. One general mechanism that cells employ to protect themselves against this persistent assault is to maintain a reducing intracellular milieu. By keeping a significant concentration of reducing equivalents in the form of reduced glutathione, thioredoxin, and other redox buffers, ROS that appear in the cell can be rapidly neutralized.

**Nrf2 and homologous transcription factors as guardians of organism integrity**

The vertebrate transcription factor Nrf2 (NFE2-related factor 2) and its invertebrate homologs, including SKN-1 (*C. elegans*) and CncC (*D. melanogaster*) have emerged as master regulators of cellular detoxification responses and redox status [1–4]. These stress-sensing transcription factors are members of the cap’n’collar family. They function both in situations of acute challenge and as regulators of baseline antioxidant activity. In mice and in human cultured cells, many protective genes are induced in response to oxidative and electrophile chemical challenges in an Nrf2-dependent manner (reviewed in [5]). Among Nrf2-regulated gene products are phase II detoxification enzymes as well as a broad range of redox regulators that includes enzymes for glutathione synthesis, glutathione S-transferases, thioredoxin, peroxiredoxins, NAD(P)H quinone oxidase 1 (NQO1), heme oxygenase 1 (HO1), and many others. Upon exposure of cells to oxidative stress and electrophilic chemical insults, Nrf2 activity is markedly increased. The signal-dependent activation of Nrf2 function is a complex phenomenon, and the precise mechanisms involved remain a subject of intensive investigation (reviewed in [6–8]). One central and well-established mechanism of Nrf2 activation involves the post-translational stabilization of Nrf2 protein (Figure 1). Under non-stressed conditions, proteasomal degradation of Nrf2 is the default state. It is facilitated by interaction with of Nrf2 with a cognate substrate adaptor, Keap1, and its associated Cul3-based ubiquitinligase system [9,10]. The inhibitory effect of Keap1 on Nrf2 is dependent on the redox status of Keap1 cysteins (which function as redox sensors), such that Nrf2 ubiquitination and proteolysis are inhibited in oxidized conditions. It is clear, however, that additional mechanisms of Nrf2 activation exist, such as the phosphorylation of Nrf2 by stress-activated kinases, and the redox regulation of its shuttling between the nucleus and cytoplasm (reviewed in [4]).

An abundance of studies has documented a broad protective function of Nrf2 against a number of pathologies that are caused or aggravated by oxidative stress. This function is evident by the increased incidence of cancer, pulmonary disease, and inflammatory conditions in Nrf2 mutant mice as well as by their markedly increased sensitivity to...
environmental toxicants, inhaled irritants, and chemical carcinogens (reviewed in [2,4,5,11]). Interestingly, many of the pathologies against which Nrf2 plays a protective role, such as cancer and neurodegeneration, are largely regarded as age-related diseases.

**Aging, oxidative stress, and the Nrf2 pathway**

The oxidative stress theory of aging posits that oxidative damage to biological macromolecules is a key driver of aging, and that, conversely, mechanisms that delay the accumulation of oxidation products in the cells and tissues of an organism can promote longevity [12]. Consistent with this tenet and the established role of Nrf2 and its invertebrate homologs as master regulators of antioxidant gene expression, a number of studies support a function for the Nrf2 pathway in the regulation of lifespan. In both *C. elegans* and *D. melanogaster*, the genetic activation of the Nrf2 signaling can cause significant increases in longevity [13,14].

These findings raised the question of whether Nrf2 activation might be a part of broader biological systems previously known to regulate longevity. The best-studied examples for biological conditions that promote longevity are situations in which the organism is exposed to a real or apparent shortage of nutrients and/or metabolic energy. In this manner, calorie restriction (CR) (reviewed in [15,16]) and genetically constrained insulin/IGF signaling [17,18] act as universal signals for longevity. This response can somewhat simplistically be rationalized as follows: under unfavorable conditions where reproductive success is unlikely, organisms switch from a “reproduction mode” to a “longevity mode”, where resources are not invested into producing offspring but rather into the maintenance of fitness, viability and fertility.

The beneficial effects of CR and decreased insulin signaling may in part be explained by a decrease in oxidative stress sensitivity and a delay in the accumulation of oxidative damage with age. Recent evidence from a variety of model systems points to the Nrf2 pathway as an effector of longevity signaling. These experiments elucidated a new and in hindsight plausible regulatory connection between biological systems that sense and regulate both the activity of the Nrf2 system and the metabolic state. In the following we will summarize recent findings in these areas.

**The role of Nrf2 signaling in calorie restriction**

CR, i.e. restriction of food intake without malnutrition, is a regimen that has been shown to extend the lifespan of organisms across the evolutionary spectrum. In addition to its effect on longevity, CR can confer diverse health benefits, which include decreased risk of cancer, lower blood pressure, higher insulin sensitivity, and improved neuronal function (reviewed in [19]). These broad health effects might be perceived as a result of the switch from “reproduction mode” to “longevity mode”, discussed above. At least some of the benefits of CR correlate with increased resistance against oxidative stress and may involve a system like the Nrf2 pathway, which is known for its antioxidant, cancer preventive and lifespan-extending functions. For example, aging is associated with a decline in the abundance of antioxidant proteins; this has been shown to be reversible with pharmacological activation of Nrf2 [20,21].

Evidence for a function of Nrf2 as a CR effector has been provided by a number of studies in mice and worms. Pearson et al. [22] showed that CR induces antioxidant gene expression in mice and that this response is suppressed in nrf2−/− animals, arguing for an upregulation of Nrf2 function in response to CR. Importantly, the previously described cancer protective function of CR [23,24] was diminished in nrf2−/− mice. Thus, CR decreased the cancer incidence and tumor load elicited by a chemical carcinogenesis regimen in an Nrf2-
dependent manner. These experiments established that CR increased Nrf2-dependent gene expression, and that Nrf2 was a required factor for several of the health benefits afforded by CR.

Interestingly, not all benefits of CR were found to require Nrf2 function. For example, the increased insulin sensitivity of calorically-restricted animals was observed regardless of the nrf2 genotype. According to these data, Nrf2 does not affect insulin signaling in the CR state; conversely, however, insulin signaling influences the activity of the Nrf2 pathway (see below).

Another study that identified Nrf2 signaling as an essential effector of CR effects was conducted in C. elegans. Bishop and Guarente showed that the worm Nrf2 homolog SKN-1 is required for the lifespan-extending effect of CR in this system [25]. In contrast to their wild type counterparts, skn-1 mutant worms did not live longer when subjected to CR. Interestingly, SKN-1 function appeared to be required in two specific neuroendocrine cells, the so-called ASI neurons, for CR-induced longevity. This finding suggests that in this context Nrf2 signaling directs a non-cell-autonomous effect that involves a systemic longevity signal downstream of SKN-1. The nature of this signal and its precise relationship to the stress defense functions of SKN-1 remain to be elucidated.

It is worth noting that, in contrast to worms, in mice there was no requirement for Nrf2 in CR-induced lifespan extension. At present it is not clear whether this discrepancy is due to the experimental design of the mouse lifespan study or to biological differences between the worm and mouse systems, such as the increased redundancy among Nrf2-related transcription factors in mammals.

Some CR-mediated health benefits have also been reported in humans [26,27]. However, adhering to a strict CR regimen (as low as 50 % of ad libitum food intake) is not a realistic option for the vast majority of the population. Much effort has therefore been devoted to identify “CR mimetics”, drugs that induce metabolic and physiological changes akin to the shift to “longevity mode” induced by CR without the discomfort of a stringent diet [28]. Several such drugs are being evaluated, and two of the best known among them are resveratrol and metformin.

Resveratrol is a phytochemical that has been found to exert a number of health benefits and can extend lifespan in several model organisms in a manner that is comparable to CR [29,30]. Several studies have shown that resveratrol can induce Nrf2 function in cultured mammalian cells. For example, in endothelial cells resveratrol has anti-inflammatory effects that appear to be mediated by the induction of Nrf2 [31,32]. The antidiabetic drug metformin has also been suggested as a potential CR mimetic. Interestingly, supplementation of the diet with metformin can increase the C. elegans lifespan in a manner that is dependent on SKN-1 [33*].

Taken together, these studies identify Nrf2 as a plausible effector of some, though likely not all, beneficial effects of CR. Further investigations are required to determine whether Nrf2 is required for the lifespan extension or other salutary effects brought about by resveratrol. Similarly, it would be interesting to conduct systematic studies to determine whether well-described Nrf2-activating drugs such as oltipraz, curcumin, or sulforaphane have beneficial effects on lifespan. Finally, the doses of resveratrol or metformin required to confer Nrf2-dependent benefits are too high to encourage considering administration to humans for these indications. Thus, alternative drugs or drug combinations need to be identified or evaluated if the CR mimetics and/or Nrf2 inducers are to be considered for such uses in humans.
Crosstalk between Nrf2/SKN-1 and insulin/Akt signaling

In addition to their prominent functions in the control of energy metabolism and growth, insulin and IGF signaling pathways (IIS) are well-established as systems that can regulate longevity. Experiments in multiple model organisms have documented that conditions in which IIS activity is low, caused for example by loss-of-function mutations in genes encoding pathway components such as the insulin receptor or its downstream docking protein insulin receptor substrate 1 (IRS-1) can extend lifespan [34–37]. Interestingly, and consistent with our discussion above, decreasing IIS activity is associated with a gain in oxidative stress resistance.

Genetic dissection of these effects primarily in C. elegans and Drosophila revealed that the decreased levels of PI3-kinase signaling and Akt activity resulting from low IIS activity mediate the longevity and stress resistance effects. The transcription factor FoxO (DAF-16 in worms) is required for this lifespan extension [38,39]. Molecularly this effect is explained by an inhibitory phosphorylation of FoxO catalyzed by the IIS-responsive kinase Akt, which is relieved in conditions of low IIS [40,41]. Conceptually, the longevity effects of low IIS and of CR appear similar. In both cases conditions associated with nutrient or energy limitation confer longevity. However, the genetic requirements for lifespan extensions in the two conditions are not identical. For example, while FoxO/DAF-16 is necessary to mediate the effect of IIS loss-of-function, it seems mostly dispensable for the CR-induced longevity. Therefore, and despite the role of Nrf2 in CR-mediated longevity described above, it is not evident that Nrf2 would also be involved in the anti-aging effects of reduced insulin signaling. Data in support of such a function for Nrf2 in this context comes from studies in C. elegans [14]. Tullet et al. showed that, as in the case of FoxO/DAF-16, direct Akt-catalyzed phosphorylation of the worm Nrf2 homolog SKN-1 prevents the activation of its target genes. Thereby SKN-1 activity is de-repressed in long-lived IIS loss-of-function mutants. Interestingly, the functions of DAF-16 and SKN-1 as IIS effectors were independent, and the respective sets of target genes were overlapping but not identical [14]. These findings indicate that Nrf2 signaling is at the core of a longevity pathway that is triggered when insulin signaling is low and functions in parallel to the well-established FoxO pathway.

The role of Nrf2 in lipid metabolism and metabolic disease

The examples for the interplay between metabolic and Nrf2 signaling summarized above show that metabolic signals (CR, changes in IIS) can influence Nrf2 activity to modulate stress defenses and longevity mechanisms. In the next section we describe a setting in which this signal flow is inverted: the regulation of metabolic functions by Nrf2 signaling.

Gene expression profiling studies have shown that hundreds of genes are regulated in a manner that depends on Nrf2 (reviewed in [5]); most of these genes are likely direct transcriptional targets of Nrf2 [42,43]. Analysis of such data from diverse tissues revealed that Nrf2 has two broad classes of target genes: 1. A shared group of antioxidant and detoxification genes that are regulated by Nrf2 across different tissues and are ubiquitously required for defense against oxidants and electrophiles (such as glutathione S-transferases, NAD(P)H quinone oxidase 1, and heme oxygenase 1); and 2. diverse groups of tissue-specific genes that are required for the homeostasis and specialized function of each particular tissue [5]. Notably, in the liver Nrf2 regulates the expression of the same Phase II enzymes that mediate its antioxidant and cancer-preventive functions in other tissues, but it also has a group of liver-specific target genes. The majority of these genes encode proteins that are specific to the synthesis and metabolism of fatty acids and other lipids [44,45]. Interestingly, these genes are primarily repressed, rather then induced, by Nrf2. A recent
proteomic analysis of $nrf2^{-/-}$ mice further documented reduced protein abundance of factors important for lipid metabolism [46], including, for example, ATP-citrate lyase, the enzyme responsible for acetyl-CoA production, which is negatively regulated by Nrf2 [45,46]. The fact that Nrf2 regulates the expression of such genes suggests that it may have an important role in the control of lipid metabolism. Conversely, multiple studies have shown that Nrf2 activity can be induced by lipids, including prostaglandins, oxidized free-fatty acids and oxidized large-density lipoproteins (LDLs), which likely act as reactive species [47–49]. Thus, the Nrf2 pathway and the metabolism of lipids are intricately connected. Even though the underlying molecular circuitries remain to be fully elucidated, the obvious implication is that Nrf2 may have a role in the pathogenesis of metabolic diseases that involve deregulated lipid metabolism. Such a role has until recently been overlooked in studies of Nrf2, which have focused mostly on its antioxidant, anti-aging, and cancer preventive properties.

Consistent with the function of Nrf2 as a repressor of genes associated with lipid synthesis and metabolism, the genetic or pharmacological activation of Nrf2 in the liver resulted in reduction of liver lipid levels [50]. Conversely, $nrf2^{-/-}$ mice were highly prone to developing steatohepatitis/fatty liver when fed a special diet deficient in methionine and choline [51*,52*,53], whereas mice with increased Nrf2 due to decreased Keap1 were more resistant [53]. Moreover, $nrf2^{-/-}$ mice fed a high-fat diet for 4 weeks had higher expression levels of lipogenic and cholesterologenic transcription factors and their target genes in the liver, as well as higher liver concentrations of free fatty acids [54]. The role of Nrf2 as a regulator of lipid metabolism is not restricted to the liver, but likely extends also to the adipose tissue. In an in vitro adipocyte differentiation model employing mouse embryonic fibroblasts, the potent Nrf2 activator CDDO-Imidazolide (CDDO-Im) inhibited accumulation of lipid droplets in an Nrf2-dependent manner [55]. Consistently, administration of CDDO-Im to mice prevented the increase of adipose tissue mass, body weight, and serum triglyceride levels, as well as the fatty liver caused by a high-fat diet, and this protection against obesity was abolished in $nrf2^{-/-}$ animals [45]. However, in contrast to its inhibitory in vitro effects on adipocyte differentiation [55*], CDDO-Im was found to have no effect on the expression of genes related to lipid metabolism in adipocytes in vivo; rather, it appeared to exert its effects non-cell-autonomously by modulating Nrf2 activity in the liver [45]. Interestingly, the rate of weight gain following the high-fat diet was substantially slower in vehicle-treated $nrf2^{-/-}$ mice than in wild-type mice, and the weights of periuterine and mesenteric white adipose tissues as % of total body weight were smaller. Taken together, these results suggested that while activation of Nrf2 signaling by CDDO-Im negatively affects accumulation of fat, constitutive levels of Nrf2 may contribute to accumulation of fat following a high-fat diet [45]. Indeed, an independent study showed that $nrf2^{-/-}$ mice had decreased adipose tissue mass with smaller adipocytes, and were protected against weight gain and obesity otherwise induced by a high-fat diet [56]. These effects were attributed to a function of Nrf2 as a cell-autonomous regulator of adipocyte differentiation, leading to impaired adipogenesis in $nrf2^{-/-}$ mice. Nrf2 was further shown to directly control the expression and abundance of the major adipogenic transcription factor PPAR$\gamma$ by binding its cognate promoter, and thus affecting the expression of its downstream target genes [56**]. This is consistent with previous studies showing decreased PPAR$\gamma$ levels in the liver and lung of $nrf2^{-/-}$ mice [57], and decreased hepatic induction of PPAR$\gamma$ expression by a 4-week high-fat diet regimen in $nrf2^{-/-}$ mice [54]. Interestingly, this latter high-fat regimen was found to repress Nrf2 expression and activity [54]; in contrast, the ketogenic diet, a special high-fat low-carbohydrate regimen used to treat intractable seizures in children, was found to trigger systemic activation of Nrf2 [58]. Thus, the degree of Nrf2 activity may depend upon the particular composition (and potentially also duration) of the diet. Given the role of Nrf2 as a regulator of lipid homeostasis, the pharmacological manipulation of Nrf2 might be beneficial in treating metabolic disorders such as dyslipidemias and obesity. From a therapeutic perspective it is important to note that, statins,
which are used for treating dyslipidemias and the metabolic syndrome in humans, have been shown to activate Nrf2 in cell culture as well as in vivo [59,60], which may account for some of the antioxidant effects of these compounds [61*]. Beyond lipid metabolism, adipogenesis, and obesity, recent studies have also established a protective role for Nrf2 in normal glucose homeostasis, as well as in experimental models of type I diabetes based on administration of streptozotocin (STZ), which causes destruction of the insulin-producing cells of the pancreas [62–64**]. Compared with wild-type mice, nrf2−/− mice had lower basal serum insulin levels and also prolonged hyperglycemia in response to an intraperitoneal glucose challenge [62**]. After STZ administration, nrf2−/− mice had a more severe clinical phenotype with higher blood glucose levels, increased urine output, higher levels of serum β-hydroxybutyrate, triglycerides, and fatty acids, reduced hepatic glycogen, enhanced gluconeogenesis, and reduced glycolysis. Importantly, the Nrf2 activator oltipraz lowered blood glucose in wild-type but not nrf2−/− mice administered STZ [62**]. An independent study similarly showed that STZ-induced hyperglycemia increased oxidative and nitrosative stress and accelerated renal injury in nrf2−/− mice [64**]. The protective role of Nrf2 in diabetic nephropathy was confirmed in a third study that demonstrated higher ROS production and greater oxidative DNA damage and renal injury in nrf2−/− mice compared to wild-type animals [63**]. This latter study also documented that the glomeruli of patients with diabetic nephropathy were under oxidative stress and had elevated Nrf2 levels, thus suggesting that this protective function of Nrf2 extends to humans [63**]. Taken together, these recent investigations clearly established a protective role of Nrf2 in diabetic nephropathy, suggesting that dietary or pharmacological activation of Nrf2 could be used as a strategy to prevent or retard the progression of this debilitating complication of diabetes in humans.

Conclusion

Recent research has shown that Nrf2 has multiple functions beyond acute, transient stress responses to oxidative insults. These functions include long-term adaptations to metabolic conditions such as the response to CR, as well as the control of lipid metabolism under both normal and high-fat diet conditions (Fig. 2). These recent discoveries likely represent the onset of an entire new area of Nrf2-related research, with several outstanding scientific questions remaining to be elucidated (Table 1). It is important to delineate further the molecular and signaling connections between metabolic signals and the Nrf2 pathway, which may help identify drugable targets for metabolic diseases. Thus, future research in this area should address several outstanding questions such as: How do metabolic inputs into Nrf2 function differ from, or cross-talk to, stress inputs? Are the respective Nrf2 target genes complements different? - In other words, how much overlap is there between the Nrf2-dependently stress-inducible genes and the Nrf2-dependently CR-inducible genes? What are the exact cell-autonomous, paracrine, and/or endocrine mechanisms through which Nrf2 is implicated in lipid metabolism? How does the role of Nrf2 as a mediator of CR-associated benefits relate to its function as a regulator of lipid metabolism? Can Nrf2 activity be pharmacologically or nutritionally manipulated in a safe manner to help ameliorate obesity, diabetes, dyslipidemia, and other metabolic disorders in model organisms and ultimately in humans? An exciting perspective is offered by the availability of Nrf2 activating drugs, which have been identified as cancer chemopreventive agents. Based on the recent implication of Nrf2 in metabolic signaling and longevity regulation, it will be of interest to test these compounds as possible CR mimetics.

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Figure 1. Schematic model of Nrf2 activation

In basal conditions the protein levels of Nrf2 are kept low due to the rapid proteasome-mediated turn-over facilitated by the Kelch family protein Keap1 which acts as a Nrf2-specific E3 ligase adaptor protein. In response to various extracellular signals the inhibitory effect of Keap1 on Nrf2 is interrupted, such that Nrf2 is stabilized and accumulates in the nucleus. There it dimerizes with a small Maf protein, binds to AREs (Antioxidant Response Elements) in the regulatory regions of its target genes and modulates their transcription.
Figure 2. Model: Nrf2 as a convergence point for stress, metabolic, and longevity signals
Nrf2 can be activated by various types of oxidative and electrophile stress. Recent evidence reviewed in this article shows that Nrf2 function can also respond to Lipid Me metabolic and longevity signals such as CR, low levels of IIS, and lipid metabolites. We suggest that the transcriptome changes that occur in these conditions include different, but overlapping sets of Nrf2 target genes. The resulting Nrf2-induced genetic programs can contribute to stress protection, longevity, or changes in metabolism. A scientific challenge, or opportunity, arising from this model is to evaluate pharmacological options to evoke longevity programs, possibly by using Nrf2-activating drugs that have originally been identified as cancer chemopreventive agents.
### Table 1

**Scientific questions remaining to be elucidated**

- Is Nrf2 required for the lifespan extension or other salutary effects of resveratrol?
- Do Nrf2-activating drugs such as oltipraz, sulforaphane, or triterpenoids have similar effects as resveratrol?
- Are the signaling pathways that regulate Nrf2 function in response to stress, CR, or metabolic signals different?
- Are the Nrf2 target genes or their expression profiles different depending on the Nrf2-activating signal?
- Through which cell-autonomous, paracrine, and/or endocrine mechanisms is Nrf2 implicated in lipid metabolism?
- Does Nrf2 control of lipid metabolism contribute to CR-associated benefits?
- Can Nrf2-modulating compounds or dietary interventions help ameliorate metabolic disorders such as obesity, diabetes, and dyslipidemia?