NRF2 and the Phase II Response in Acute Stress Resistance Induced by Dietary Restriction

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Abstract

Dietary restriction (DR) as a means to increase longevity is well-established in a number of model organisms from yeast to primates. DR also improves metabolic fitness and increases resistance to acute oxidative, carcinogenic and toxicological stressors - benefits with more immediate potential for clinical translation than increased lifespan. While the detailed mechanism of DR action remains unclear, a conceptual framework involving an adaptive, or hormetic response to the stress of nutrient/energy deprivation has been proposed. A key prediction of the hormesis hypothesis of DR is that beneficial adaptations occur in response to an increase in reactive oxygen/nitrogen species (ROS). These ROS may be derived either from increased mitochondrial respiration or increased xenobiotic metabolism in the case of some DR mimetics. This review will focus on the potential role of the redox-sensing transcription factor NF-E2-related factor 2 (NRF2) and its control of the evolutionarily conserved antioxidant/redox cycling and detoxification systems, collectively known as the Phase II response, in the adaptive response to DR.

Introduction

The concept of immortality and the search for the fountain of youth is embedded in epic stories and fables going back thousands of years, and in all likelihood precedes recorded history. In the past century, we have seen dramatic increases in the expected mean lifespan of people throughout the world thanks to innovations in areas such as hygiene and medicine. In the United States in the past 30 years, death rates due to cancer and stroke - two leading causes of aging-related death - have declined 2.7% and 63%, respectively [1]. Despite these decreases, the absolute number of deaths from these conditions continues to increase [1].

Studies on the genetic and molecular basis of aging and longevity in lower organisms have given rise to the idea that a common process may drive both “normal” aging phenotypes experienced by most people (hair greying, reduced hormone levels, loss of muscle mass) as well as aging-related pathologies experienced only by some (cancer, stroke, heart disease and neurodegeneration). An important prediction of this concept is that interventions targeting the aging process itself will also delay or ameliorate a wide variety of aging-associated diseases.

The most potent intervention to retard the processes of aging is dietary restriction (DR) [2-4]. Generally defined as reduced food intake without malnutrition, DR describes a wide variety of nutritional interventions altering the composition and/or timing of food intake, including reduced daily calorie intake (also known as calorie restriction, or CR), reduced nutrient intake (e.g. protein, essential amino acids) or enforced periods of fasting between meals (e.g. every-other-day fasting, EOD). Modulating dietary intake as a means to defend against aging-related disease was established as a laboratory paradigm over 100 years ago [5]. It was first observed that restricting food intake of mice not only decreased the growth of implanted tumors, but also retarded angiogenesis and metastasis [5]. Life extension benefits of DR were first reported twenty years later [6]. In the ensuing decades, longevity benefits of DR have been reported in a wide variety of species, from single-celled yeast to non-human primates. The measured benefits of DR have also expanded to endpoints with arguably more immediate potential for clinical use, such as improved metabolic fitness and increased resistance to chemical [7] and radiation [8-10] stress or on both the cellular and organismal level [3,11-15].

Despite decades of research into the mechanistic (nutritional, physiological, molecular) basis of longevity extension by DR (as well as the aging process itself), a potentially unifying model of DR action has only recently emerged [16-18]. It draws on the concept of hormesis, or the beneficial adaptive response to the mild stress of nutrient/energy restriction. Here we focus on the potential hormetic role of reactive oxygen/nitrogen species (ROS) produced as a result of increased mitochondrial respiration or xenobiotic metabolism inactivation of Phase II antioxidant and detoxification systems [19,20] through stimulation of the NF-E2-related factor 2 (NRF2) transcription factor [21-23]. Although much of the experimental DR literature is focused on aging and longevity-related endpoints, in this review we will place special emphasis on DR-mediated benefits related to stress resistance with potential clinical relevance, such as resistance to surgical ischemia reperfusion injury, chemotherapeutic agents such as cisplatin can be cytotoxic/cytostatic, while low doses can actually increase cell proliferation. Whether or not this low-dose effect is “beneficial” is entirely context-dependent - good in the case of a regenerating liver after tumor resection, bad in the case of the resected tumor itself. Hormesis is the term most often associated with potential clinical relevance, such as resistance to surgical ischemia reperfusion injury, chemotherapeutic agents such as cisplatin can be cytotoxic/cytostatic, while low doses can actually increase cell proliferation. Whether or not this low-dose effect is “beneficial” is entirely context-dependent - good in the case of a regenerating liver after tumor resection, bad in the case of the resected tumor itself. Hormesis is the term most often associated

Hormesis Hypothesis of DR and DR Mimetics

Multiphasic Dose Response to Dietary Food Intake

It is commonly observed in biological systems that a given compound may have opposite biological effects at low and high doses [24,25]. This gives rise to a biphasic or multiphasic dose response when plotted over a range of concentrations, with a U or J shaped depending on the sign attached to the biological effect. For example, high doses of a chemotherapeutic agent such as cisplatin can be cytotoxic/cytostatic, while low doses can actually increase cell proliferation. Whether or not this low-dose effect is “beneficial” is entirely context-dependent - good in the case of a regenerating liver after tumor resection, bad in the case of the resected tumor itself. Hormesis is the term most often associated with potential clinical relevance, such as resistance to surgical ischemia reperfusion injury, chemotherapeutic agents such as cisplatin can be cytotoxic/cytostatic, while low doses can actually increase cell proliferation. Whether or not this low-dose effect is “beneficial” is entirely context-dependent - good in the case of a regenerating liver after tumor resection, bad in the case of the resected tumor itself. Hormesis is the term most often associated

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with “beneficial” actions in the low dose range of a compound that is toxic at higher doses. A biphasic or multiphasic dose-response itself implies nothing about underlying mechanism(s). Nonetheless, hormetic responses are thought to involve adaptive changes altering signal transduction, transcription and translation and leading to such “beneficial” biological outcomes as increased resistance to subsequent stress.

Dietary intake, similar to pharmaceuticals [26,27], radiation [28] and exercise [29], displays a multiphasic dose response [30] (Figure 1). Chronic underfeeding with malnutrition leads to reduced fecundity, cachexia, immunosuppression and even death [31-34] (Figure 1, points i to ii), while over nutrition leads to increased insulin resistance, inflammation and hypertension as well as increased morbidity and mortality due to diabetes, cardio-pulmonary diseases and cancer [35-38] (Figure 1, points iii to iv). Opposite biological effects are observed for these endpoints upon DR, defined here as the level of food intake between malnutrition (point ii) and that required for maintenance of body weight significantly below the set point achieved by ad libitum feeding (point iii).

Critics of the hormesis hypothesis of DR have questioned its validity on at least two levels. First at issue is whether the benefits of DR reflect an experimental artifact of laboratory life - unlimited access to nutrient/energy dense food. In Figure 1, this would imply that point iii, which we consider as optimal nutrition for rapid growth to adulthood and maintenance of adult body weight, actually represents a state of over nutrition, and that DR simply returns the animals to a less artificial level of food intake [39]. While we agree that the placement of point iii is somewhat arbitrary, the fact that the benefits of reduced food intake can be observed in prospective studies in relatively lean people [40] and in a dose-dependent fashion near the point of starvation in rodents [41,42], put to rest fears that DR is an experimental artifact related to over-feeding of control subjects. A second criticism of the hormesis hypothesis is that neither the hormetic compound nor the underlying mechanism is well defined [42-44]. Below we address this concern by considering the ability of DR and DR mimetics to generate bona fide hormetic agents - reactive oxygen and electrophilic species - and the potential for the adaptive response to these stressors to underlie at least some of the benefits of DR.

**DR, ROS and Mitohormesis**

A common link among hormetic stimuli is their ability to generate reactive oxygen/nitrogen species (ROS) and other reactive metabolites either directly or indirectly [25,28,45]. At the hormetic dose, a transient increase in ROS may stimulate upregulation of antioxidant, detoxification and survival mechanisms on the cellular and organismal levels, thus providing a robust defense against larger and potentially more dangerous oxidative or toxicological stressors [46].

If ROS is the hormetic agent essential for the beneficial hormetic response, what is its source? Although not known for certain, emerging evidence points to an increase in mitochondrial respiration as a potential source of hormetic ROS. In the case of DR, evidence in yeast [47-49], C. elegans [46], flies [50] and rodents [51] points to increased rates of oxidative phosphorylation often accompanied by increased mitochondrial biogenesis. Altered energy metabolism, including a shift in energy substrate utilization from carbohydrate to fat [29,45,47,52-61], may also play a role in DR and DR mimetics like exercise. Hormesis driven by oxidative stress derived specifically from mitochondrial respiration has been termed “mitohormesis” [55,58]. First proposed as a medical hypothesis, supporting experimental evidence has since been provided in a number of model systems. In worms, the DR mimetic 2-deoxyglucose inhibits glycolysis, increasing fatty acid oxidation and mitochondrial ROS while at the same time extending lifespan [46]. In humans, blocking oxidative stress by the addition of antioxidants vitamin C, vitamin E and/or n-acetyl-cysteine not only attenuates the beneficial results of acute bouts of exercise on insulin sensitivity [29], but also increases long-term oxidative damage and impaired healing of overworked muscles [62]. In dogs, where...
treadmill running preconditions against cardiac ischemic injury, inhibition of fatty acid oxidation with the acyl-CoA dehydrogenase inhibitor 3-hydroxydecanoate [63,64] prevents the benefits of exercise preconditioning without itself causing any damage [53]. Acyl-CoA dehydrogenase activity and fatty acid oxidation [65] are increased upon short-term starvation by Sirt3-mediated deacetylation in the mitochondria [66]. Sirt3 expression and activity are both increased upon exercise, fasting and caloric restriction but decreased on high fat diets [67,68]. This stimulation of Sirt3 activity not only promotes oxidation of fatty acids, but is also necessary for the phosphorylation/activation of AMPK and CREB [68] as well as the mitochondrial superoxide dismutase SOD2 [69].

Can different energy substrates generate different amounts of ROS even in the absence of increased mitochondrial oxidative respiration? Long-chain fatty acids are broken down first in peroxisomes and then oxidized to completion at the inner mitochondrial membrane and in the matrix. β-oxidation of lipids is a complex pathway comprised by over 16 proteins and is controlled at several steps dependent on substrate and metabolite availability [70]. The production of reactive metabolites - superoxides and peroxides- is possible during β-oxidation of lipids in peroxisomes and in the mitochondria if it is not efficiently or directly coupled to ATP production. ROS production at complex II, QH₂, and complex III from long-chain fatty acid oxidation derived FADH₂ is greater than what is formed from NADH-linked substrates channeled first through complex I [71,72], although this is a still unresolved and controversial issue [73]. Dietary intake of moderately oxidized fats also results in increased antioxidant and detoxification systems in the livers of pigs [74].

It is still not well understood as to the relative and/or differential effects that increased respiration, increased fatty acid oxidation, differential substrate utilization, uncoupling, or increased mitochondrial biogenesis have on effective hormetic ROS production due to DR. Nonetheless, mitochondrial metabolism and activity is central and essential for the benefits of dietary restriction in eukaryotes.

**DR Mimetics, ROS and Xenohormesis**

“DR mimetic” is a term used loosely to define a compound or stimulus that imparts functional benefits of DR, but in the absence of reduced food intake [75]. Such functional benefits range from longevity extension in model organisms, as with rapamycin and metformin [48,76], to improved metabolic fitness in the face of over nutrition, as with resveratrol [77]. Aerobic exercise can also be considered a DR mimetic based on its ability to improve metabolic fitness and stress resistance, although itdoes not necessarily extend lifespan [78].

As with DR, most DR mimetics have multiple targets and pleotropic modes of action, and so their underlying mechanisms remain unproven or unknown. In principle, DR mimetics may work by creating a state of actual or perceived nutrient/energy deficiency, for example inhibition of glycolysis by 2-deoxyglucose, activation of AMP-Activated Protein Kinase (AMPK) by metformin [79], or inhibition of the nutrient/energy sensor mTOR by rapamycin. In this way, DR mimetics may function like DR by transiently increasing ROS to hermetic levels as a result of increased fatty acid oxidation and mitochondrial respiration.

However, mitochondria are not the only potential source of ROS induced by DR mimetics. Many pharmacological DR mimetics, such as metformin, resveratrol and curcumin, are xenobiotics that activate Phase I detoxifying enzymes such as the cytochrome P-450 superfamily, also resulting in potential increases in oxidative stress [80,81]. These Phase I enzymes initiate detoxification and export of xenobiotics from the cell by oxidizing these molecules and making them more suitable substrates for Phase II enzymes. In doing so, they also create highly reactive metabolites with the potential to overwhelm the Phase II response and create oxidative stress. Many DR mimetics are natural substances used by their hosts in stress resistance. For example, rapamycin is a macrolide antibiotic made by soil bacteria; plant polyphenols such as resveratrol can defend against microorganisms, insects, herbivores, and even sunlight. The idea that such xenobiotics trigger stress responses in other organisms when ingested has been coined “xenohormesis” [82,83]. Thus, hormesis-inducing ROS produced as a result of mitochondrial respiration (mitohormesis) or Phase I oxidation reactions (xenohormesis) may be a common mechanistic link among different DR regimens and mimetics.

Table 1 summarizes DR regimens and mimetics reported to induce stress resistance together with the underlying pathways GENES implicated in that study. Three major conclusions are evident: 1) DR and DR mimetics are powerful tools to prevent damage from a variety of acute stressors including ischemia/ischemic reperfusion injury [84-87], toxic levels of chemicals and drugs [88-93], trauma [94], infection [95], inflammation [96] and radiological damage [9,2] 2) different DR regimens (type, duration, dose) and mimetics can provide protection against the same stressors across a variety of different species; and 3) maintenance of cellular antioxidant, detoxification and redox cycling programs is a common theme in protection against acute stress. These same pathways are also intricately involved in the Phase II response to toxicological and electrophilic stress, as described below.

**Phase II Response**

The Phase II response is an evolutionarily conserved adaptation to a wide variety of stressors, including ROS, xenobiotics and electrophiles, comprising the induction of over 200 genes and the repression of several dozen others [21,97-99]. The induced genes are largely responsible for the synthesis, use and/or recycling of cellular anti-oxidant and detoxification reducing agents, including glutathione (GSH), thioredoxin (Trx) and nicotinamide adenine dinucleotide phosphate (NADPH) [98] (Figure 2). The induction of the Phase II response is predominately facilitated by the transcription factor NRF2 binding to the antioxidant response element (ARE) in these gene promoters and stimulating their expression [21,98,100]. The major effector components of the Phase II Response induced by DR and DR mimetics are shown in Figure 2.

**Glutathione Metabolism**

Glutathione, a tripeptide composed of γ-L-glutamyl-L-cysteinyl-glycine, exists ubiquitously in tissues and cells in its reduced or oxidized form (GSH or GSGS, respectively). The de novo synthesis of GSH occurs in the cytosol in a two-step process. The first and rate-limiting step, catalyzed by the heterodimer glutamate cysteine ligase (GCL) - composed of catalytic (GCLC) and modifier (GCLM) subunits-conjugates the amino acids l-glutamate and l-cysteine to form γ-glutamyl-l-cysteine [101]. Activity of GCL is negatively regulated by low l-cysteine concentrations and/or high GSH levels [101]. In the second step, catalyzed by glutathione synthase (GS), γ-glutamyl-l-cysteine is combined with l-glycine to form reduced glutathione (GSH). Expression of GS and both GCL subunits is under control of the Phase II response [102] due to the presence of antioxidant response elements (AREs) in each gene promoter [103,104]. Activation of GCL and GS gene expression increases intracellular GSH concentrations [101,105].

Once produced, GSH can be utilized for cellular functions
<table>
<thead>
<tr>
<th>Type of DR &amp; DR Mimetic</th>
<th>Duration/Dose of DR/ DR Mimetic</th>
<th>Organism and/or Cell Type</th>
<th>Pathways/Genes/Activity Altered (+/-)</th>
<th>Resistance Against Acute Stressor</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein/Amino Acid (Trp) Deficient</td>
<td>6-14 days</td>
<td>Mice</td>
<td>(+) GGN2, (-) eIF2α</td>
<td>Renal and hepatic ischemia/reperfusion injury</td>
<td>84</td>
</tr>
<tr>
<td>Glucose Deficient</td>
<td>0.5g/L prior to insult</td>
<td>Rat and Human (Gial cells)</td>
<td>N/A</td>
<td>H₂O₂, and menadione (oxidative stress) Cyclophosphamide (chemotherapeutic)</td>
<td>11</td>
</tr>
<tr>
<td>Methionine Deficient</td>
<td>~8.5-months</td>
<td>Mice</td>
<td>(-) in serum IGF-1, Insulin, and Glucose</td>
<td>Injection of toxic doses of acetaminophen</td>
<td>88</td>
</tr>
<tr>
<td>25% Restriction</td>
<td>140 days</td>
<td>N/A</td>
<td></td>
<td>Methylxazoxymethanol (MAM) induced tumorgenesis</td>
<td>7</td>
</tr>
<tr>
<td>30% Restriction</td>
<td>2-4 weeks</td>
<td>Mice</td>
<td>(-) GH, (-) IGF-1, (+) HO-1, (+) GR, (+) Angiopoietin, (+)GSTs</td>
<td>Renal and hepatic ischemia/reperfusion injury</td>
<td>14</td>
</tr>
<tr>
<td>20-40% Restriction</td>
<td>&gt;5 weeks to 42 weeks</td>
<td>Mice</td>
<td>(+) NRF2, (+) NQO1, (+) HO-1, (+) GCLC, (+) GST, (+) Gpx</td>
<td>Induced Mutagenesis/Tumorgenesis by DMB/A/TPA</td>
<td>129</td>
</tr>
<tr>
<td>30-40% Restriction</td>
<td>N/A</td>
<td>Humans</td>
<td>N/A</td>
<td>Critically ill patients in a medical-surgical ICU</td>
<td>252</td>
</tr>
<tr>
<td>40% Restriction</td>
<td>adult lifetime</td>
<td>Mice</td>
<td>(-) IGF-1, (-) Insulin</td>
<td>Excitoxotin kainic acid induced damage to the dorsal hippocampus</td>
<td>89</td>
</tr>
<tr>
<td>40% restriction</td>
<td>6 months</td>
<td>Rats</td>
<td>(+) deacetylation, (+) Sirtuins</td>
<td>Cardiac ischemia/ischemic reperfusion injury</td>
<td>57</td>
</tr>
<tr>
<td>60 % Restriction (approximate)</td>
<td>800 kcal/day for 2 weeks</td>
<td>Humans</td>
<td>No sig. diff. in weight/BMI between the two groups</td>
<td>Laparoscopic gastric bypass surgery</td>
<td>251</td>
</tr>
<tr>
<td>Every 2-Day Intermittent Fasting</td>
<td>N/A</td>
<td>C. elegans</td>
<td>(+) RHEB-1, (+) TOR</td>
<td>Heat and oxidative stress</td>
<td>12</td>
</tr>
<tr>
<td>EOD Intermittent Fasting</td>
<td>adult lifetime</td>
<td>Mice</td>
<td>(+) IGF-1, (-) Insulin</td>
<td>Excitotoxin kainic acid induced damage to the dorsal hippocampus</td>
<td>89</td>
</tr>
<tr>
<td>EOD Intermittent Fasting</td>
<td>2-3 weeks</td>
<td>Mice</td>
<td>N/A</td>
<td>Lethal (8.7 Gy) and sub-lethal (5.26 Gy) total body ionization radiation</td>
<td>8</td>
</tr>
<tr>
<td>EOD Intermittent Fasting</td>
<td>3 weeks</td>
<td>Mice</td>
<td>(+) metabolic rate, (+) lipogenesis</td>
<td>Sub-lethal whole body gamma irradiation</td>
<td>9 &amp; 10</td>
</tr>
<tr>
<td>Fasting</td>
<td>48-180 hours before and 5-56 hours post-treatment</td>
<td>Stage I-IV cancer patients (44-76 yrs)</td>
<td>N/A</td>
<td>Chemotherapy for breast, esophageal, prostate and lung cancer</td>
<td>253</td>
</tr>
<tr>
<td>Fasting</td>
<td>4 days</td>
<td>Rats</td>
<td>N/A</td>
<td>Hypoxic/Ischemic injury to the brain</td>
<td>85</td>
</tr>
<tr>
<td>Fasting</td>
<td>1-4 days</td>
<td>Liver donor Rats</td>
<td>(+) GSH, (+) HO-1, (+) HSP-60, (+) HSP-70</td>
<td>Liver ischemia and transplantation</td>
<td>187</td>
</tr>
<tr>
<td>Fasting</td>
<td>3 days</td>
<td>Mice</td>
<td>(+) HO-1, (+) SOD, (+) GPx, (+) GSR, (-) IL-6</td>
<td>Hepatic ischemia/reperfusion injury</td>
<td>186</td>
</tr>
<tr>
<td>Fasting</td>
<td>3 days</td>
<td>Mice</td>
<td>(-) IGF-1, (+) IGFβPs</td>
<td>Doxorubicin (oxidative stress)</td>
<td>90</td>
</tr>
<tr>
<td>Fasting</td>
<td>2-3 days</td>
<td>Mice</td>
<td>N/A</td>
<td>Etoposide</td>
<td>11</td>
</tr>
<tr>
<td>Fasting</td>
<td>1-3 days</td>
<td>Mice</td>
<td>(-) GH, (-) IGF-1, (+) HO-1, (+) GR, (+) Angiopoietin, (+)GSTs</td>
<td>Renal and hepatic ischemia/reperfusion injury</td>
<td>14</td>
</tr>
<tr>
<td>Fasting</td>
<td>2 days</td>
<td>D. melanogaster</td>
<td>Independent of Hif-1</td>
<td>Anoxia and reoxygenation injury</td>
<td>13</td>
</tr>
<tr>
<td>Water only media</td>
<td>1-2 days</td>
<td>Yeast</td>
<td>(-) RAS2</td>
<td>H₂O₂, menadione, methylmethanen sulfonate and cyclophosphamide</td>
<td>11</td>
</tr>
<tr>
<td>Fasting</td>
<td>24 hours post-brain injury</td>
<td>Yeast</td>
<td>Insulin independent, Ketone dependent</td>
<td>Traumatic brain injury</td>
<td>94</td>
</tr>
<tr>
<td>Exercise (running)</td>
<td>10 weeks of treadmill or voluntary wheel running</td>
<td>Rats</td>
<td>N/A</td>
<td>Doxorubicin induced cardiotoxicity</td>
<td>91</td>
</tr>
<tr>
<td>Exercise (running)</td>
<td>5 x 5 min run/rest 10 min or 24 hr prior to insult</td>
<td>Dogs</td>
<td>Opening of mitochondrial Kᵥᵥ channels</td>
<td>Myocardial ischemic/ichernic reperfusion injury</td>
<td>53</td>
</tr>
<tr>
<td>AICAR</td>
<td>100 mM for 48 hours</td>
<td>D. melanogaster</td>
<td>(+) AMPK</td>
<td>Anoxia and reoxgenation injury</td>
<td>13</td>
</tr>
<tr>
<td>Curcumin</td>
<td>200 mg/kg per day for two weeks</td>
<td>Mice</td>
<td>(-) IFN-γ, (-) IL-17</td>
<td>Interleukin (IL)-10 deficient mouse model of pro-inflammation</td>
<td>96</td>
</tr>
<tr>
<td>Curcumin</td>
<td>200mg/kg a day for 4 days</td>
<td>Rats</td>
<td>(+) NRF2, (+) HO-1</td>
<td>Dimethylnitrosamine (DMN)-induced hepatic injury</td>
<td>189</td>
</tr>
<tr>
<td>Impaired glucose metabolism by DOG</td>
<td>5 mM for 6 days</td>
<td>C. elegans</td>
<td>(+)aak-2/AMPK, (+) CAT</td>
<td>Paraquall and sodium azide (inducers of toxic ROS levels)</td>
<td>46</td>
</tr>
<tr>
<td>Methylenic Blue</td>
<td>4 x 50 mg/day prior to chemotherapy</td>
<td>Humans with solid tumors</td>
<td>N/A</td>
<td>Ifosfamide (chemotherapeutic)-induced encephalopathy</td>
<td>92</td>
</tr>
<tr>
<td>Olitpraz</td>
<td>2 days supplemented in food</td>
<td>D. melanogaster</td>
<td>(+) NRF2/CncC, (+) GST</td>
<td>Paraquall (inducers of toxic ROS levels)</td>
<td>130</td>
</tr>
<tr>
<td>Olitpraz</td>
<td>10-100 µM prior to insult</td>
<td>Human (H9 cells)</td>
<td>(+) GSH, (+) NQO1</td>
<td>Human Immunodeficiency Virus (HIV) infection</td>
<td>95</td>
</tr>
<tr>
<td>3H-1,2-dithiole-3-thione (D3T)/Olitpraz</td>
<td>25-100 µM prior to insult</td>
<td>Mouse (Cardiac cells)</td>
<td>(+) NRF2, (+) NQO1, (+) CAT, (+) GSH, (+)GR, (+)GST</td>
<td>XO/Xanthine and SIN-1 cytotoxicity</td>
<td>98</td>
</tr>
</tbody>
</table>

throughout the cytoplasm, mitochondria, endoplasmic reticulum and even extracellular space. In the context of stress resistance, these functions include detoxification of reactive electrophiles, xenobiotics and free radicals including ROS; and the solubilization of reactive metabolites to facilitate their export from the cell [101]. Being just a tripeptide with no inherent catalytic activity, GSH action requires a collection of accessory enzymes, including glutathione S-transferases (GSTs), glutathione peroxidases (GPxs) and glutathione reductase (GR).

GSTs include over twenty mammalian genes in eight different classes, referred to as alpha, kappa, mu, omega, pi, theta, zeta, and microsomal to signify the location and substrate of that particular GST [106,107]. Enzymatically, GSTs catalyze the conjugation of GSH to the electrophilic group on a variety of reactive endogenous and exogenous substrates toxic to the cell. These substrates can include xenobiotics, reactive metabolites as well as oxidized lipids and proteins [108]. By doing so, GSTs detoxify the potentially harmful substrate and allow for its breakdown and/or transport out of the cell by way of multidrug resistance protein (MRP), which subsequently pumps the toxic molecule out of the cell.

### Table 1: DR and DR Mimetic-Mediated Pathway Modulations and Induced Resistance to Acute Stress

<table>
<thead>
<tr>
<th>DR/Mimetic</th>
<th>Duration/Dose</th>
<th>Organism</th>
<th>Pathways/Genes Modulated</th>
<th>Stressor Provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resveratrol</td>
<td>5-40µM for 12 hours</td>
<td>Rat (Adrenal glad cells)</td>
<td>(+) NRF2, (+) NQO1, (+) GCLM, (+) Trx, (+) HO-1</td>
<td>Ethanol induced cytotoxicity and death</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>0.25 mg/kg</td>
<td>Mice</td>
<td>(+) NRF2, (+) HO-1</td>
<td>Cerebral focal ischemic stroke model</td>
</tr>
<tr>
<td>Sulforaphane</td>
<td>3 µM prior to insult</td>
<td>Mouse (Embryonic Fibroblasts)</td>
<td>(+) NRF2, (+) GSTs, (+) GSH, (+) NQO1, (+) GR</td>
<td>Cigarette Smoke</td>
</tr>
</tbody>
</table>

### Figure 2: Phase II Antioxidant and Detoxification Response Stimulated by DR

Model of the Phase II Response in which GSH and NADPH act as co-factors to maintain a reduced cellular environment and remove toxic xenobiotics and metabolites. Phase II Response effectors upregulated by DR and/or DR mimetics are shown in green. Pro-oxidant and toxic molecules are shown in red. GSH= glutathione, GR= glutathione reductase, GPxs= glutathione peroxidases, GSTs= glutathione transferases, GCL= glutamate cysteine ligase, NQO1= NAD(P)H dehydrogenase (quione 1), Trxs= thioredoxin, TrxR= thioredoxin reductase, CAT= catalase, HO-1= heme oxygenase 1, SOD= super oxide dismutase, DOG= 2-Deoxy-D-glucose.
and to maintain the homeostatic GSH: GSSG ratio [111]. A decrease in this ratio above the normal 9.5:0.5 [112] can increase susceptibility to oxidative stress, protein dysfunction via glutathionylation, and cell death [113,114]. GR uses the reducing power of NADPH to reduce GSSG, producing two molecules of GSH and one molecule of NADP+ [108]. Due to the importance of GSSG recycling, both GR and NADPH production via the pentose phosphate pathway enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are under control of the Phase II response [115-117].

A number of studies have demonstrated age-related changes in glutathione homeostasis and their modulation by DR. For example, age-related decline in GSH and reduced GSH:GSSG ratios attributable to reduced GCL expression [118] have been reported in ad libitum fed rodents [19] and humans [119], most notably those with diabetes [120], and may underlie vulnerability to cancer, Parkinson’s disease, Alzheimer’s disease [121] and heart disease [119]. In rodents, DR can blunt age-related GSH decreases [19], while in human epithelial lung cells the DR mimetic resveratrol can increase GSH levels and protect against cigarette smoke toxicity [122]. Deficiencies in GSTs, including an age-related decline in GST activity [123], are documented risk factors in several human pathologies. These include second hand smoke-induced childhood asthma [124], epoxide-induced cellular damage [125], pulmonary asbestosis [126], and cancer of the larynx, lung and bladder in smokers [127,128]. GSTs can be increased in abundance and activity by a variety of DR regimens, including fasting [14,102] and 30-40% DR [14,123,129], as well as DR mimetics Oleipraz [98,130], procyanidin B2 [131], and sulphoraphane [132]. Age-related declines in Gpx activity, most notably in the intestinal mucosa, hepatocytes and kidney [20], can also be counteracted by DR and DR mimetics in rats [19,23]. Recent data from the CALERIE Trial of Human Caloric Restriction indicate that plasma Gpx activity increases and plasma protein carbonyl levels decrease over a six month course of 10-30% DR in moderately overweight adults [133], indicative of a reduction in oxidative damage [134]. Exercise can also enhance Gpx expression. In rats, 20 weeks of voluntary wheel running in rats increases cardiac and skeletal muscle Gpx activities over 400% and 300%, respectively [135]. Stimulation of Gpx can also be accomplished using 20-40% DR [129] or the DR mimetic sulphoraphane [136]. This stimulation of Gpx via these preconditioning interventions correlates with and is thought to contribute to their protection against DMBA/TPA-induced skin carcinogenesis or azoxymethane/dextran sulfate sodium-induced colon carcinogenesis. Congenic deficiency in GR expression or activity in humans is rare [137], possibly signifying an essential role for GR function. However, GR expression can be increased by DR, possibly contributing to surgical stress resistance in mouse kidney and liver [14]. The DR mimetics Oleipraz [98] and sulphoraphane [132] are also capable of inducing GR expression and providing protection against xenobiotic carcinogens and toxic compounds.

**Thioredoxin and Thioredoxin Reductase**

Thioredoxin (Trx) is a 12-KD protein enzyme with a conserved disulfide/dithiol sequence of -Trp-Cys-Gly-Pro-Cys- [138,139] that can directly reduce oxidized proteins, nucleic acids, lipids as well as small signaling molecules by itself becoming oxidized [139,140]. Thioredoxin reductase (TrxR) recycles oxidized Trx back to the reduced form [138] using NADPH as a reducing molecule, similar to GR. Expression levels and activity of Trx and TrxR are induced during the Phase II response [141,142]. Enhanced expression of Trx in transgenic mice has been linked to increased longevity, telomerase activity and resistance to stressors such as UV radiation [143,144]. Genetic overexpression of Trx and TrxR protects against mitomycin C and diepoxybutane-associated DNA damage and chromosomal instability in human fibroblasts obtained from donors with the accelerated aging/cancer predisposition syndrome Fanconi anemia [145,146]. An age-associated decline in Trx may be linked to the pathogenesis of Alzheimer’s disease (AD), as over expressing Trx in AD cells inhibits Ab amyloid toxicity [147]. Adult lifetime DR of 40% blunts Trx and TrxR decline in rats [19]. Similarly in C. elegans, Trx is upregulated upon DR and essential for lifespan extension upon DR as well as in a genetic model of DR [148]. The DR mimetic plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) protects human neuronal cells against tert-butyl-hydroperoxide (tBHP) mediated oxidative stress and cell death by the activation of several Phase II enzymes, including Trx [149]. Plumbagin treatment for up to 6 hours prior to focal cerebral ischemia also protects against neurologic damage and reduces infarct size in mice [149].

**NAD (P) H-quinone oxidoreductase 1**

An additional member of the Phase II Response that depends on the reducing power of NADPH to provide cellular protection against oxidative stressors is the NAD (P) H-quinone oxidoreductase (NQO1) [150]. NQO1 localizes to the cytoplasmic surface of the plasma membrane [151] and acts as a two-electron reductase on a number of substrates, mainly quinones. By transferring reducing potentials from NADPH onto quinones, the resulting hydroquinones [150,152], such as coenzyme Q (CoQ), keep the plasma membrane in a reduced state by maintaining exogenous antioxidants such as vitamin C (ascorbate) [153], inhibiting lipid peroxidation and activation of sphingomyelinase [154,155] and preventing subsequent ceramide release and ceramide-dependent caspase activation [155].

NQO1 deficiency is a risk factor for therapy-related acute myeloid leukemia, basal cell carcinomas, pediatric leukemia and benzene-induced liver toxicity [150]. Reduced NQO1 expression with age is associated with reduced CoQ and other plasma redox molecules and increased oxidative stress [156,157].

Evidence for a potential role of NQO1 in cellular protection induced by hormeric doses of carcinogens dates back to the 1960s [150]. Small amounts of azo dyes and aromatic olefins can stimulate NQO1 activity and protect mouse tissues against lethal subsequent doses of the carcinogen 7,12-dimethylbenzen (a) anthracene (DMBA) [158,159]. Long-term DR can also stimulate NQO1 expression, resulting in enhanced plasma membrane antioxidant capacity and decreased levels of 8-isoprostane and protein carbonyls in liver [156] and brain [4] of aged rats, and increased resistance to DMBA/TPA-induced carcinogenesis in mice [129]. Short-term treatment with DR mimetics Oleipraz [98], plumbagin [149], and sulphoraphane [132] also stimulate NQO1 expression and increase protection against oxidative stress and chemical toxins.

**Heme Oxygenase-1**

Iron, including heme-bound iron can catalyze the formation of toxic hydroxyl radicals (OH) from hydrogen peroxide (H₂O₂) through the Fenton reaction [160]. Under normal physiological conditions, this poses at most a minor threat to cells and tissues due to the matched rate of synthesis of heme and hemoproteins [160] including myoglobin [161], hemoglobin [162], neuroglobin [163] and heme scavenging protein hemopexin [164] in red blood cells, as well as intracellular proteins such as those of the cytochrome P-450 superfamily, peroxidases and catalases [160,165]. Increases in oxidative stress due to tissue injury, radiation and chemical toxins [166] can lead to disassociation of heme from hemoproteins [160], leading to hydroxyl...
radical generation through Fenton chemistry. Infectious or congenital diseases such as malaria [167], sickle cell anemia, thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency and lupus can result in red blood cell lysis and further saturate the body’s heme scavenging defense systems [168].

Cellular defenses against free heme overload are centered on the inducible enzyme heme oxygenase-1 (HO-1) [169]. HO-1 can be induced directly by heme or indirectly by oxidative stress, which can increase heme levels by damaging heme-containing intracellular proteins. Oxidative stress can also activate the Phase II transcription factor NRF2 [170,171] via modulation of its redox-sensitive binding partner, KEAP1 (see below).

Increased HO-1 expression protects cells from both extracellular and intracellular free heme by catalyzing the transfer of electrons from NADPH to heme, keeping iron in the reduced state and allowing for molecular oxygen to induce the degradation of heme into free iron, biliverdin and CO [165]. Because free iron remains a threat, it is picked up and stored by ferritin [160]. Biliverdin is further processed by the enzyme biliverdin reductase using NADPH as the reducing agent to form bilirubin, an antioxidant that can itself lend protection against ischemia reperfusion injury and damage caused by sepsis [160]. CO mediates many of HO-1’s cytoprotective effects, for example by suppressing apoptosis [172,173], rejection of transplanted organs [174] and cardiac ischemic reperfusion injury [175,160].

How CO protects cells from oxidative damage is not entirely known, but may involve mitohormesis. Upon release from heme, CO binds to iron in other hemeoproteins, including mitochondrial cytochrome c oxidase, resulting in a small, quick burst of mitochondrial ROS [60]. This potentially mitohormetic dose [55,176] can then lend protection by activating the p38 beta MAPK cell survival signal cascade [177] and transcription factors including PPARy [178], HIF1-a [179] and NRF2 [180]. Activation of these cell survival and protection pathways, notably through NRF2, can further increase the expression of HO-1 as well as the other Phase II antioxidant and detoxification genes, enabling cells and tissues to fight off further free heme and oxidative stress-induced damage. Interestingly, this system of small amounts of free heme initiating a feed-forward cytoprotective response centered on the Phase II Response has been reported for the protection against atherosclerotic lesions and endothelial stress in a mouse model of sickle cell disease [181]. This brings into question whether the increases in hemolysis and free heme due to foot strike during running [182] can be seen not as detrimental, but positive and attributing to the benefits of moderate running. Additionally, future research on barefoot/ minimalist shoe- versus traditional shoe- running may shed light on the potential beneficial effects of fasting can also be transferred from organ donors to recipients. In rats, one to four days of water-only fasting by the donor increases HO-1 expression and maintenance of GSH concentrations in the donated livers, correlating with increased short-term and long-term survival of the recipients [187,188].

Preconditioning with 200mg/kg a day for 4 days of the DR mimetic curcumin stimulates HO-1 expression in rats and conveys protection against acute stressors such as dimethylnitrosamine (DMN) -induced hepatic injury [189]. Another DR mimetic, plumbagin, stimulates HO-1 expression in human neuroblastoma cells and protects them against tBHP-mediated oxidative stress and death [149]. Preconditioning with plumbagin for 6 to 24 hours before the onset of a cerebral focal ischemic stroke also increases HO-1 expression and reduces total infarct size as well as general neurologic damage [149].

**NRF2: The “On Switch” for The Phase II Response**

The major evolutionarily conserved transcription factor required for induction of the suite of detoxification and antioxidant proteins constituting the Phase II response is NRF2. Known as SKN-1 [76,190] in worms and CncC [130] in flies, NRF2 is a member of the Cap’n’Collar transcription factor family with a conserved basic region-leucine zipper domain that binds to the antioxidant response element (ARE) [(T/C) TGCTGA (C/G) TCA (T/C)] [191] of gene promoters and has the ability to induce or repress expression of these genes. There are over 230 genes induced by NRF2, either directly or indirectly, and approximately 30-40 genes that are inhibited in an NRF2 dependent manner [142,192].

Activation of NRF2 is central to the induction of potent cellular antioxidant and detoxification systems. Many diseases, including the aging-related diseases of cancer, Alzheimer’s, Parkinson’s and diabetes, are correlated with decreased activity of NRF2 and show improvements of symptoms upon NRF2 activation through gene therapy or DR in experimental models [193].

NRF2/SKN1 was first connected to DR in worms, where it was shown to be required for DR-mediated lifespan increase [190]. Interestingly, in rodents, Nrf2 mediates the cancer protection induced by DR, but is not required for DR’s longevity characteristics [129]. Maintenance and stability of NRF2 levels and its activation are induced by oxidative and electrophilic stress [194-196]. In the scope of this review, we hypothesize that activation of NRF2 by DR regimens and mimetics depends on their ability to stimulate ROS production, either through increased mitochondrial fatty acid oxidation or activation of Phase I oxidases. The gene expression program engaged by NRF2 activation can then suppress the ROS prompted by mitohormesis or xenobiotic metabolism, and at the same time mount a total cellular defense against a multitude of stressors.

**Genes Regulated by NRF2**

The majority of genes induced by NRF2 are those of the Phase II antioxidant and detoxification response, including glutathione synthesis genes (GCLC, GCLM), glutathione reductase (GR), glutathione transferases (GSTs), glutathione peroxidases (GPxs), heme oxygenase-1 (HO-1), NADPH quinone reductase 1 (NQO1), multidrug resistance protein transporters, thioredoxin (Trx) and thioredoxin reductase (TrxR). Additional genes that play a role in survival and

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stressed by NRF2 include chaperone, heat shock and Cyp/cytochrome P450 genes, UDP-glucuronosyl transferases, carbonyl reductase, lysozyme M, and peroxiredoxin genes [142,192].

Other NRF2-stimulated gene groups include those involved in the ubiquitin-proteasome system, cell growth, metabolism, apoptosis, protein translation, and cytoskeletal organization. Interestingly, the NRF2 gene itself contains AREs and is thus highly inducible by the protein it encodes [192].

NRF2 also plays a role in negative regulation of genes and/or pathways involved in longevity and stress resistance, for example, insulin and growth hormone (GH) / insulin-like growth factor (IGF) signaling. Reduced signaling through these pathways, mediated by reduced levels of peptide hormones or their receptors upon DR, correlates with both extended longevity and increased stress resistance [3,197,198]. Genes encoding both growth hormone receptor (GHR), which extends lifespan when genetically ablated in mice [198], and insulin-like growth factor 2 (IGF-2), which is similar to IGF-1, promote growth and acts as a hyperproliferative switch in the growth of tumors [199], are negatively regulated by NRF2 [142,192]. The gene encoding Insulin-Degrading Enzyme (IDE), which is responsible for the degradation of insulin and protection against hyperinsulinemia as well as degrading endogenous amyloid β-protein (Aβ) [200], is upregulated by NRF2 [142]. Thus, NRF2-based downregulation of insulin and GH/IGF signaling may contribute to longevity, stress resistance, and protection from age-related pathologies including type 2 diabetes and Alzheimer’s disease.

NRF2 Regulation

NRF2 is regulated at multiple levels, including transcription, translation, posttranslational modification and localization. The majority of studies have focused on negative regulation of NRF2 by KEAP1, which binds to and sequesters NRF2 in the cytoplasm, leading to its ubiquitination and proteasome-mediated degradation [201,202]. Obstruction of the NRF2-KEAP1 interaction allows for the release, nuclear translocation and activation of NRF2 [203]. Additional NRF2 regulation is KEAP1-independent [204-207], including transcriptional and translational mechanisms summarized in Figure 3 and described below.

Transcriptional Regulation of NRF2

Similar to other genes in the Phase II response, the NRF2 promoter contains two ARE-like sequences located at nucleotides -579 (GCCACAGTCGA) and -317 (TGACTCCGC) relative to its transcriptional start site [206,207]. Phase II response inducers such as D3T increase levels of NRF2 in the nucleus by up to 6-fold within twenty minutes and steady-state levels of NRF2 mRNA by 2-fold within six hours. Despite this positive feedback loop, NRF2 protein and mRNA levels decline within 24-hours of the initial NRF2 activation [207]. Several factors are capable of breaking this positive feedback loop. One is NRF3, another NF-E2 family member that can compete with NRF2 ARE binding and repress the transcription of ARE-containing genes [208]. Another is MiR-28, a small, non-coding microRNA that can bind to the 3' UTR of NRF2 mRNA and decrease its stability [204].

An additional mechanism for the induction of NRF2 transcription independent of NRF2 protein activity involves the aryl hydrocarbon receptor (AHR) and its interactions with xenobiotic response elements (XPE) in the NRF2 promoter region [206]. AHR is a transcription factor normally found in its inactive form in the cytoplasm. Upon binding to its many synthetic and naturally occurring ligands, including thiabendazole, guanabenz, lipoxin A4, bilirubin, prostaglandin G2, β-Naphthoflavone, tangeritin and dioxin, as well as DR mimetics like curcumin [209], AHR is imported into the nucleus, binds to the XPE in gene promoters and induces a variety of Phase I response drug metabolizing genes such as cytochrome p450s CYP1A1 and CYP1B1 [81,210]. Although necessary for proper drug metabolism, many Phase I response enzymes bioactivate their substrates into carcinogenic intermediates that can react with DNA and form adducts [81]. Because of the dangers posed by these bioactivated compounds, a swift induction of Phase II Response is required. This is mediated by AHR binding to three XPE domains in the NRF2 promoter located at -712 (GCCGTG), +755 (CACGCC) and +870 (CACGCC) [206]. Thus, NRF2 transcription is induced by NRF2/ARE and AHR/XPE, furthering the robustness of the Phase II response against reactive species and xenobiotics.

The decline in antioxidant system activities and gene expression with age [19,211] may be related to a decline in NRF2 activity and decreased expression of Phase II response genes in several tissues, including vascular endothelial and smooth muscle cells of the carotid arteries [212] and liver [213]. Epigenetic changes to the NRF2 promoter may underlie these age-related changes, as silencing of the NRF2 promoter by methylation of CpG islands has been reported in mouse models of prostate cancer [214,215]. Microarray analysis of human prostate cancer samples indicate a reduction in NRF2 mRNA [216]. Reversal of NRF2 deficiency in the prostate, possibly by prevention or removal of DNA methylation at the NRF2 promoter, may help to prevent the onset of prostate cancer. The DR mimetic curcumin, which was previously shown to inhibit the development of prostate cancer in mouse models [217], also acts as a hypomethylation agent and/or DNA methyltransferase inhibitor and restores the expression of NRF2 in prostate cells [215]. Increased levels of NRF2 mRNA, on the other hand, correlate with decreased ischemia reperfusion injury and increased organ functioning following liver transplantation [218]. Taken together, maintenance and/or stimulation of NRF2 transcriptional expression appears to be vital for cancer suppression, successful organ transplantation and overall defense against ROS-mediated damage.

Translational Regulation of NRF2

NRF2 protein translation can also be induced upon oxidative stress, for example in rat cardiomyocytes exposed to H₂O₂ [219]. The increased translation of NRF2 under cellular stress is attributed to an IRES element in the 5' untranslated region of human NRF2 mRNA [220], which allows for translational initiation when cap-dependent translation is repressed. Exogenous addition of the DR mimetic/phyto-oxidant sulphoraphane, but not that of the anti-oxidants GSH or n-acetylcysteine, greatly induces IRES-mediated NRF2 expression on polysomes. Simultaneously, stress, sulphoraphane, as well as the DR mimetic curcumin [221,222], induce the phosphorylation of eIF2a, the attenuation of global protein synthesis [220] and initiate translational derepression of genes with upstream ORFs or IRES in a eIF5B-dependent mode [223]. Thus, the production of ROS from DR-stimulated mitochondrial activity and/or xenobiotic stress as well as pharmacological perturbation of classical cap-dependent translational machinery can stimulate the expression of NRF2.

Posttranslational Modification and Localization of NRF2

NRF2 was first identified as a positive regulator of the Phase II Response by Itoh et al. [21]. In this study, treatment of cells with oxidative stress and electrophilic agents increased NRF2 nuclear protein levels as well as its DNA binding activity to AREs without increasing its mRNA levels [21,201], suggesting a cytoplasmic factor
**Figure 3: DR and DR Mimetics Activate NRF2**

Reduced food intake (DR), fasting or the use of DR mimetics stimulate a variety of cellular and endocrine responses that ultimately lead to NRF2 activation and the transcription of Phase II Antioxidant and Detoxification genes. Inside of the cell, growth-promoting signals are suppressed along with cap-dependent translation of mRNA into protein by inhibition of mTOR and eIF2α, while IRES cap-independent translation of NRF2 is stimulated. Mitochondrial oxidation of fatty acids is increased, which results in small amounts of ROS production. This ROS and/or Phase I metabolized DR mimetics (xenobiotics) enables for the oxidation of cysteine residues on KEAP1 and subsequent release and activation of NRF2.

Once in the nucleus, NRF2 associates with several other transcription factors and undergoes additional posttranslational modifications to execute its stimulatory and repressive activities. Recent evidence suggests acetylation of lysine residues 588 and 591 by p300/CBP enhances NRF2’s interaction with the ARE elements in gene promoters and stimulates their expression while under stress [236,237]. Deacetylation by Sir1 inhibits NRF2 activity and results in decreased nuclear and increased cytoplasmic pools of NRF2 [237]. On the contrary, histone deactylase 2 (HDAC2), but not HDAC1, aids in the transcriptional activity of NRF2, and blocking HDAC2’s enzymatic activity leads to decreased NRF2 activity [238]. Thus, both acetylation and deacetylation play important roles in NRF2 activity. These modifications and their modifiers will need to be studied more in depth to differentiate at what stages they impact or are required for NRF2 stability, localization and activity.

Small Maf transcription factors (MafG, MafK or MafL) form heterodimers with NRF2 that allows specific binding to the AREs of target genes (21; 239). Interestingly, the MafG gene itself is a target of NRF2/Small Maf-mediated transcription under electrophilic cellular stress [240], reinforcing the idea that a strong Phase II Response requires a positive feedback loop. An additional group of transcription factors, Jun proteins, have also been shown to associate with NRF2 in the upregulation of detoxifying enzymes containing AREs [241]. These Jun proteins include c-Jun, Jun-B and Jun-D. However, elevated levels of c-Jun actually decrease NRF2 mediated transcription. This is possibly responsible for NRF2 instability and inhibition. The half-life of NRF2 protein under normal physiological conditions is very short - only 10 to 40 minutes - further suggesting a powerful repressive mechanism that can also be turned off rapidly during oxidative and electrophilic stress [224,225]. This turns out to be a cytoplasmic inhibitor of NRF2 known as KEAP1, a homolog of the Drosophila actin cytoskeleton binding protein Kelch [201]. Mechanistically, each KEAP1 homodimer can bind a single NRF2 molecule and anchor it to the actin cytoskeleton in the cytoplasm [226,227]. KEAP1 not only blocks nuclear localization of NRF2, but also targets it for degradation by the E3/Cul3 mediated ubiquitin-proteasome pathway, hence the relatively short half-life of NRF2 [227,228]. NRF2’s escape from the grasp of KEAP1 and eventual activation primarily involves oxidation of and/or electrophilic binding to the many cysteine thiol groups present in KEAP1 [142,194,226,227]. By oxidation or attachment of electrophiles to these KEAP1 cysteine thiols, cross-linking and/or disruption of the KEAP1 homodimer occurs and results in the inability of KEAP1 to target NRF2 for degradation [227,229].

In addition to liberation from KEAP1, NRF2 undergoes additional posttranslational modifications before it enters the nucleus. For example, phosphorylation at serine 40 by PKC enhances NRF2 mediated transcription of the Phase II Response [230]. Other possible kinase candidates include the MAP kinases p38, PI3K, PKC, JNK and ERK [231,232]. However, whether this or other known serine/threonine phosphorylation events occur before or after release from KEAP1, and what exactly the roles these phosphates play in NRF2 stability and transcriptional activity remain largely unknown [231]. An additional factor that regulates NRF2 activity is the Parkinson’s associated protein DJ-1/PARK7. Mutations and/or deletions in DR-1/PARK7 are associated with homozygous recessive forms of Parkinson’s in humans as well as decreased ability to initiate an antioxidant stress response [233]. While the function, either structural or enzymatic, of DJ-1/PARK7 is not entirely known, it has been shown to increase the stability and/or inhibit the degradation of NRF2 by KEAP1/ E3/CUL3 mediated ubiquitin-proteasome pathway and aid its translocation into the nucleus [234,235].
by the formation of negative regulatory c-Jun+c-Fos complexes which interfere with the binding of the positive regulatory heterodimer Nrf2+c-Jun at the ARE binding site [241].

Balancing NRF2 Activation: Don’t leave the lights on

Although maintaining the ability to activate NRF2 is important for detoxification and prevention of aging-associated diseases, constitutive activation has dire consequences. This is seen in the deleterious and lethal phenotype of Keap1 knockout mice that die postnatally due to hyperkeratosis of the esophagus and forestomach [242]. This lends support to short-term rather than long-term use of DR and/or DR mimetics, as it may lessen negative side effects of long-term NRF2 activation while still offering health-promoting benefits. Additionally, oncogene-induced NRF2 transcription [243,244] or loss of function mutations in Keap1 [245] lead to overexpression and/or overactivation of NRF2 and directly increases tumor growth, chemoresistance and radioresistance. Due to DR eliciting a differential response between cancerous and non-cancerous tissue in that cancerous tissue becomes sensitized to chemotherapy and non-cancerous becomes more resistant [11,246], it seems prudent to give our normal, healthy tissue a fighting chance via DR against various cancer treatments. Doing so would hypothetically activate NRF2 and Phase II systems and increase resistance only in non-cancerous tissues, allowing for more aggressive treatments against endogenously resistance cancer cells.

Conclusions

In this review, we have touched upon several mechanistic topics related to stress resistance induced by DR and DR mimetics. Although not completely understood, these mechanisms shed light on a less appreciated side of DR quite apart from canonical aging-related GH, IGF1, AMPK and TOR pathways. These include: 1) The hormetic nature of small, transient amounts of ROS derived from mitochondria upon the switch to fatty acid oxidation (mitohormesis) and/or xenobiotic stress produced by the Phase I metabolism of DR mimetics (xenohormesis). Dietary restriction and its xenobiotic and exercise mimetics thus generate beneficial responses not passively but rather actively stimulate the hormetic response by generating mild amounts of reactive metabolites, oxygen species, and xenobiotic stress. 2) These small stressors produced by DR and DR mimetics activate the transcription factor NRF2 through transcriptional upregulation of NRF2 mRNA, the increased translation of NRF2 protein, and, most importantly, its release from KEAP1 in the cytoplasm and subsequent translocation and activation in the nucleus. Upon activation, NRF2 promotes the expression of over 200 potential cytoprotective genes and represses several pro-growth and pro-inflammation genes by binding to the ARE sequence in their gene promoters and may mediate many of the benefits of DR. 3) NRF2-mediated induction of Phase II Response genes may be crucial to stress resistance afforded by DR and DR mimetics. These Phase II Response genes are predominantly involved in maintaining the proper cellular redox state as well as detoxification of reactive xenobiotics and metabolites through the utilization of cellular reducing molecules GSH, Trx and NADPH. Interestingly, long-lived dwarf mice with defects in growth hormone releasing hormone also display constitutive increases in Phase II Response elements, particularly GSTs [247,248]. These mice are also more resistant to the stress of acetaminophen toxicity, although whether or not the Phase II response underlies their longevity or stress resistance phenotypes is not known. Similarly, further experiments are required to fully elucidate the role of the NRF2-mediated Phase II Response in DR benefits, as many of the existing studies are largely correlative in nature.

Besides reviewing the mechanisms of DR-derived benefits, we provided several examples of age-related reductions in cellular redox and detoxification defenses and how DR is capable of preventing and even reversing these deteriorations. Translation of DR benefits from preclinical models to the clinic is still rare, likely due to such reasons as unlikelihood of patient compliance and/or safety and efficacy of the dietary intervention prior to the stress of surgery and therapies. One such negative connotation of DR is its ability to decrease survival of aged mice in response to primary influenza infection [249], suggesting that DR use in the elderly or in relation to viral infection should be more closely examined. Nonetheless there are a few recent examples of studies examining the potential benefits of DR and DR mimetics in humans. These limited studies show that various forms of short-term DR can be employed as a means to increase favorable clinical outcomes in conjunction with surgery and chemotherapy [250-253]. In a different context, large populations voluntarily and routinely undergo various DR and fasting regimens, mostly for religious reasons. Favorable outcomes in these populations include lowered BMI, cholesterol, blood pressure as well as biomarkers of tissue damage and oxidative stress [254]. Impressively, fasting just one day per month in among Mormons is associated with decreased cardiac mortality, coronary artery disease and diabetes, even when all other risk and health factors are taken into consideration [255]. Could the opposite hold true for the phenomena termed “Merry Christmas Coronary” and “Happy New Year Heart Attack”? During the period between Thanksgiving and New Year’s Day in the U. S. A, a time frame associated with over indulgence in food and drink, there is a striking increase in deaths due to ischemic heart disease that is independent of the weather [256]. Could fasting and overeating have such opposite effects through a common pathway, namely activation and suppression of NRF2 and the Phase II Response, respectively, thus modulating vulnerability to ischemic heart disease-related mortality?

We foresee the use of short-term DR and DR mimetics in the clinic to enhance patient outcomes as a viable, readily available and inexpensive intervention with great potential. Optimization of dietary interventions in preclinical and clinical trials, a better mechanistic understanding of its stimulation of target genetic pathways as well as changes made in the current dogma of preoperative/pretreatment dietary intake may someday soon usher in a new era of preventative and palliative medicine.

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